Advances in Bioresearch
Adv. Biores., Vol 16 (6) November 2025: 457-469

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.6.457469

Advances in Bioresearch

ORIGINAL ARTICLE

Creation of *In Vitro* Chloramphenicol Ocular Gels Through Various Polymer mergers and acquisitions

Jay Prakash Singh*[1], [2]Raslamol K, [3]Shankar Gavaroji, [4]Md Sajid Ali, [5]Disha Chandra, [6] Subhashish Tripathy, [7]Muskan Jain, [8]Khushboo Mishra
[1-6]Assistant Professor at BMS College of Pharmacy Amethi, UP- 229309
[2]Holy Grace Academy of Pharmacy, Mala, Thrissur
[3]Department of Pharmaceutics, Siddharth College of Pharmacy, Mudhol -587313, Dist: Bagalkot, Karnataka, India
[4]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan 45142, Saudi Arabia
[5]S.J institute of pharmacy Kanpur UP
[7-8]Smt. Vidyawati College of Pharmacy, Jhansi
***Corresponding Author**: **Jay Prakash Singh**
Email: jpsingh9452@gmail.com
ORCID- https://orcid.org/0009-0001-0948-190X

ABSTRACT
The formidable physiological barriers of the eye severely limit the bioavailability of conventional topical formulations, necessitating frequent dosing and compromising therapeutic outcomes for infections like bacterial conjunctivitis. Chloramphenicol, a broad-spectrum antibiotic, exemplifies this challenge, as its efficacy is hindered by the rapid clearance of solutions and the poor acceptability of ointments. This study employs a strategic "merger and acquisition" approach to polymer science to engineer advanced in situ gelling ocular systems designed to overcome these limitations. A series of ten formulations (F1-F10) were created by synergistically blending polymers—including Poloxamer 407, Carbopol 934P, HPMC, gellant gum, and chitosan—to combine theogallin, ion-activated, or pH-sensitive triggers with enhanced mucoadhesion and controlled release. Comprehensive in vitro characterization revealed that polymer mergers critically optimized key performance attributes. Single polymer systems exhibited poor mucoadhesion and rapid drug release. In contrast, strategic blends, particularly the Poloxamer 407 (18%) and Carbopol 934P (0.4%) merger (F6), demonstrated an ideal gelation temperature (31.5℃), superior gel strength (G'/G'' = 12.52), exceptional mucoadhesive force (0.480 N), and sustained drug release (T_{50%} = 6.8 h). The gellant-chitosan polyelectrolyte complex (F9) also showed outstanding mucoadhesion (0.520 N). Ex vivo trans corneal permeation studies confirmed the lead formulation (F6) doubled the steady-state flux of chloramphenicol compared to a commercial solution, highlighting enhanced corneal penetration. The findings conclusively validate that rational polymeric synergies can create multifunctional ocular gels. The Poloxamer-Carbopol-based formulation (F6) emerged as a lead candidate, successfully integrating rapid in situ gelation, prolonged ocular residence, sustained drug release, and improved permeation. This work provides a robust scientific framework for developing effective, patient-compliant alternatives to conventional chloramphenicol eye drops, demonstrating the significant potential of intelligent polymer-based delivery systems in ophthalmic therapeutics.
Keywords: *Chloramphenicol, ocular gel, mucoadhesive polymers, sustained drug delivery, in vitro release, ophthalmology.*

Received 20.08.2024 Revised 15.10.2024 Accepted 24.11.2025

How to cite this article:
Jay Prakash S, Raslamol K, Shankar G, Md Sajid A, Disha C, Subhashish T, Muskan J, Khushboo M. Creation of *In Vitro* Chloramphenicol Ocular Gels Through Various Polymer mergers and acquisitions. Adv. Biores., Vol 16 (6) November 2025: 457-469.

INTRODUCTION

The human eye, an organ of exquisite sensitivity and function, is protected by a formidable array of anatomical and physiological barriers. While essential for maintaining homeostasis and defending against pathogens, these protective mechanisms present a profound challenge for the effective delivery of

therapeutic agents. The corneal epithelium, with its tight junctions, acts as a formidable lipophilic-hydrophilic-lipophilic multilayer barrier. Concurrently, the dynamic processes of lacrimation, tear turnover (approximately 1-3 μL/min), reflexive blinking, and nasolacrimal drainage work in concert to rapidly eliminate foreign substances from the ocular surface. For conventional topical eye drops—the most common and patient-preferred dosage form—the consequence is a precorneal residence time often less than two minutes and an abysmally low bioavailability, typically estimated between 1% and 5%. This inefficiency necessitates frequent dosing (e.g., hourly or two-hourly for severe infections), leading to poor patient adherence, increased risk of contamination, significant systemic absorption via the nasal mucosa, and, ultimately, potential therapeutic failure or recurrence of infection[1].

The treatment of bacterial conjunctivitis, blepharitis, and keratitis exemplifies this therapeutic dilemma. While a broad spectrum of effective antimicrobial agents exists, their clinical utility is frequently compromised by the limitations of their delivery vehicles. Chloramphenicol, a broad-spectrum bacteriostatic antibiotic, remains a first-line agent in many parts of the world for the management of superficial ocular bacterial infections. Its efficacy against common Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram-negative (*Haemophilus influenzae*, *Escherichia coli*) ocular pathogens, along with its excellent tissue penetration and low cost, underpins its enduring clinical relevance. However, its traditional formulations—as a simple aqueous solution or a petrolatum-based ointment—are emblematic of the broader delivery crisis. The solution is subject to rapid precorneal loss, while the ointment, though prolonging contact time, induces visual blurring, eyelid discomfort, and patient dissatisfaction, limiting its use primarily to nocturnal administration[2].

This gap between therapeutic potential and clinical efficacy has catalyzed a paradigm shift in ophthalmic pharmaceutical sciences. The focus is no longer solely on the discovery of new drug molecules but increasingly on the innovation of advanced drug delivery systems capable of intelligently navigating the ocular landscape. The objective is clear: to develop formulations that can extend residence time at the ocular surface, control the release of the therapeutic agent, enhance corneal permeability, and improve patient comfort and compliance. Among the most promising strategies to emerge in response to this challenge is the design of *in situ* gelling systems[3]

In situ gelling systems represent a cornerstone of modern, responsive ophthalmic drug delivery. These are liquid formulations engineered to undergo a rapid, reversible phase transition from a low-viscosity sol to a semi-solid gel upon exposure to the physiological conditions of the eye. This transformation is triggered by specific stimuli inherent to the ocular environment, leading to the formation of a sustained-release depot directly on the corneal surface. The primary triggering mechanisms include:

- **Thermally-Activated Gelation:** Utilizing polymers that exhibit a reversible sol-gel transition at temperatures between ambient (25°C) and physiological (34-35°C). Instilled as a cool, easy-to-administer liquid, the formulation gels comfortably upon contact with the warmer ocular surface.
- **Ion-Activated Gelation:** Employing polymers that cross-link in the presence of mono- or divalent cations (Na^+, Ca^{2+}, Mg^{2+}) present in the tear film, forming a gel matrix.
- **pH-Activated Gelation:** Relying on polymers that undergo a viscosity increase or precipitation when the formulation's pH is raised from its acidic storage condition to the neutral-buffered pH of the tears.

This elegant approach offers a multifaceted therapeutic advantage. By increasing formulation viscosity *in situ*, it drastically reduces drainage and prolongs precorneal residence time. The formed gel matrix acts as a reservoir, facilitating sustained and controlled drug release, which can translate to reduced dosing frequency—from several times daily to potentially twice daily. Importantly, it marries the ease of administration of a drop with the prolonged contact time of an ointment, all while minimizing vision-blurring effects and improving overall patient acceptability[4].

The functional core of any *in situ* gelling system is its polymeric component. Polymers are not mere inert excipients but dynamic, functional materials whose chemical structure, molecular weight, and concentration dictate the critical performance parameters of the final formulation. These parameters include the precise gelation temperature or trigger point, the rate of gelation, the mechanical strength and rheological properties of the formed gel, its bio adhesive affinity for the ocular mucosa, its drug release modulation capacity, and its overall biocompatibility and tolerability[5].

Historically, formulations relied on single-polymer systems. However, it became evident that no single polymer possesses the ideal portfolio of properties required for optimal ocular performance. A polymer with excellent thermosensitive properties may lack sufficient mucoadhesive strength. Another with potent bio adhesion might form a gel that is too rigid or irritant, or it might interact unfavorably with the drug molecule. Consequently, the contemporary strategy in ophthalmic formulation has evolved from the use of monolithic polymers to the deliberate, rational design of polymeric blends[6].

The development of an advanced chloramphenicol ocular gel can be aptly conceptualized as a process of strategic **"mergers and acquisitions"** within the formulation. In this analogy, each polymer is viewed as a corporate entity with distinct core competencies ("assets") and inherent limitations ("liabilities"). The role of the formulation scientist is that of a strategic architect, seeking synergistic combinations where the strengths of one polymer compensate for the weaknesses of another, thereby creating a new entity (the gel formulation) with superior overall performance[7].

The goal is to assemble a consortium of polymers that collectively deliver a comprehensive suite of desired properties:

1. **Precise Gelation Trigger:** A sharp and reliable transition at the ocular surface.
2. **Robust Mucoadhesion:** Strong but reversible bonding to the corneal/conjunctival mucin layer to resist clearance.
3. **Optimal Rheology:** A liquid with low viscosity for easy instillation that transforms into a gel with appropriate viscoelasticity—sufficiently cohesive to resist blinking shear yet soft for comfort.
4. **Sustained and Controlled Release:** A network structure capable of modulating the diffusion of chloramphenicol over 8-12 hours, potentially aligning with a twice-daily regimen.
5. **Ocular Biocompatibility:** Non-irritating, isotonic, and preserving corneal epithelial integrity.
6. **Drug Compatibility:** Chemically and physically compatible with chloramphenicol, ensuring stability and unhindered release[8].

The successful "merger" depends on a deep understanding of the candidate polymers.

- **Poloxamers (Pluronic F127/F68):** The quintessential theogallin agents. These triblock copolymers (PEO-PPO-PEO) form clear gels via micellar packing upon warming. **Asset:** Predictable, concentration-dependent phonologization[9].
- . **Liability:** Weak mucoadhesion, potential for rapid erosion and burst drug release. They are prime candidates for partnership with bio adhesive polymers
- **Cellulose Derivatives (HPMC, MC, CMC):** Versatile, biocompatible polymers offering mucoadhesion and viscosity enhancement. Some (e.g., HPMC) can exhibit thermal gelation at higher concentrations. Carboxymethylcellulose (CMC), an anionic polymer, provides excellent mucoadhesion. **Asset:** Proven safety, tenable viscosity, and good mucoadhesion. **Liability:** May require high concentrations for gelation alone, and gel strength may be moderate[10].
- **Polyacrylates (Carbomers - Carbopol®):** Potent pH-sensitive, anionic mucoadhesive. They gel upon neutralization, forming robust, high-viscosity networks with exceptional sustained-release properties. **Asset:** Superior bio adhesive strength and controlled release. **Liability:** Can cause irritation at low pH and may require viscosity modifiers for optimal drop formation[11].
- **Gellan Gum (Gelrite):** An anionic microbial polysaccharide that undergoes **ion-activated gelation** with tear film cations. It forms clear, brittle gels. **Asset:** Clear gel formation at low concentrations, good mucoadhesion. **Liability:** Gel rigidity and syneresis (water expulsion) can be high, and performance is sensitive to ionic strength.
- **Chitosan:** A natural **cationic** polysaccharide. Its primary assets are potent mucoadhesion (via ionic interaction with anionic mucin) and permeation-enhancing properties. It is biodegradable and biocompatible.
- **Liability:** Soluble only in acidic media, which can be a formulation constraint.

Exemplar Synergistic Merger: A blend of **Poloxamer 407 (20-25%)** and **Carbopol 934P (0.2-0.5%)**. Here, Poloxamer provides the immediate theogallin trigger, while the small amount of Carbopol significantly enhances mucoadhesion, modifies the gel's rheology to be more cohesive, and imparts a more sustained, zero-order release profile for chloramphenicol. This merger directly addresses the erosion and weak adhesion of poloxamer gels[12].

The translation of a conceptual polymer merger into a viable, safe, and effective ophthalmic product necessitates a rigorous, stepwise development process. Before advancing to complex, expensive, and ethically weighted *in vivo* studies, a comprehensive battery of *in vitro* tests serves as an indispensable predictive and screening tool. *In vitro* characterization allows for the rapid iteration of formulations, the establishment of critical quality attributes (CQAs), and the elucidation of structure-property-performance relationships[13].

For an *in-situ* gelling chloramphenicol formulation, the key *in vitro* evaluations include:

- **Gelation Studies:** Determining the precise gelation temperature (for thermosensitive systems) or gelation time upon exposure to simulated tear fluid (for ion/pH-sensitive systems).

- **Rheological Characterization:** Analyzing flow properties (viscosity) of the sol state and the viscoelastic modulus (G', G") of the gel state to predict ease of installation, retention, and patient comfort.
- **Mucoadhesive Strength Measurement:** Using techniques like texture analysis or modified balance methods to quantify the force required to detach the gel from mucin or mucosal tissue, providing a direct indicator of potential residence time.
- **In Vitro Drug Release Profiling:** Employing dialysis membrane or Franz diffusion cell methods under sink conditions to model and compare the release kinetics of chloramphenicol from different polymeric blends over time.
- **Physicochemical Evaluation:** Assessing drug content uniformity, pH, osmolality, sterility (post-sterilization), and stability under accelerated storage conditions.
- **Ex Vivo Corneal Permeation Studies:** Using isolated animal corneas in diffusion cells to provide a more biologically relevant assessment of drug penetration[14].

Scope and Objectives of the Present Work

This research is dedicated to the **systematic creation and *in vitro* evaluation of novel chloramphenicol-loaded ocular *in situ* gelling systems** through the strategic application of polymer merger principles. Moving beyond trial-and-error, the work employs a rational design framework where specific binary and ternary polymeric combinations are formulated to target a predefined performance profile[15].

The primary objectives are:

1. To **design and prepare** a series of *in situ* gelling formulations containing chloramphenicol using strategic blends of polymers, including but not limited to Poloxamer/Carbopol, Poloxamer/HPMC, Gellan/Chitosan, and potential ternary systems.
2. To **comprehensively characterize** these formulations *in vitro* for their critical functional properties: gelation behavior, rheology, mucoadhesive potential, *in vitro* drug release kinetics, and physicochemical stability.
3. To **investigate and correlate** the impact of polymer type, ratio, and total concentration on the aforementioned functional properties, thereby mapping the formulation landscape.
4. To **identify and propose** one or more lead optimized formulations that successfully integrate rapid *in situ* gelation, strong mucoadhesion, sustained chloramphenicol release over an extended period, and characteristics suitable for ocular application, based on the *in vitro* data.

MATERIAL AND METHODS

Materials

Chloramphenicol (≥98% purity) was procured from Sigma-Aldrich. The polymers used included Carbopol 934P (Lubrizol), hydroxypropyl methylcellulose (HPMC K4M) GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301), sodium alginate, chitosan (medium molecular weight, ≥75% deacetylation, GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301), and poloxamer 407 (GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301). All other reagents, including phosphate-buffered saline (PBS, pH 7.4), benzalkonium chloride (preservative), and hydrochloric acid (for pH adjustment), were of analytical grade[16].

1.2. Materials for *In Vitro* Evaluation

- Freshly excised goat/sheep/rabbit corneas
- Porcine gastric mucin (Type II) or commercially available artificial mucin
- Cellulose ester dialysis membranes (Molecular weight cut-off: 12-14 kDa)
- Franz-type vertical diffusion cells (with a receptor volume of ~12 mL and effective diffusion area of 0.785 cm^2)
- Rheometer (cone-plate or parallel plate configuration)
- Texture Analyzer (with a mucoadhesive holder)
- UV-Visible Spectrophotometer or validated HPLC system
- pH meter with microelectrode
- Osmometer
- Magnetic stirrers with hot plates
- Refrigerated cold storage (4°C)
- Thermostatically controlled water bath

METHODS

Formulation Design and Preparation

A series of blank and drug-loaded *in situ* gelling systems were prepared using the cold method for thermosensitive polymers and direct hydration for others. The composition of the primary formulated batches is detailed in Table 1.

Formulation Code	Polymer System ("Merger")	Chloramphenicol	Poloxamer 407	Poloxamer 188	HPMC K4M	Carbopol 934P	Gellan Gum	Chitosan*	Purified Water q.s.
F1	Poloxamer (Single)	0.5%	20.0%	-	-	-	-	-	100%
F2	Poloxamer (Single)	0.5%	22.0%	-	-	-	-	-	100%
F3	**P407 + HPMC**	0.5%	18.0%	-	0.5%	-	-	-	100%
F4	**P407 + HPMC**	0.5%	18.0%	-	1.0%	-	-	-	100%
F5	**P407 + Carbopol**	0.5%	18.0%	-	-	0.2%	-	-	100%
F6	**P407 + Carbopol**	0.5%	18.0%	-	-	0.4%	-	-	100%
F7	**P407 + P188 + Carbopol**	0.5%	16.0%	2.0%	-	0.3%	-	-	100%
F8	Gellan (Single)	0.5%	-	-	-	-	0.6%	-	100%
F9	**Gellan + Chitosan**	0.5%	-	-	-	-	0.4%	0.2%	100%
F10	**Ternary: P407+HPMC+Carbopol**	0.5%	16.0%	-	0.5%	0.2%	-	-	100%

Table 1: Composition of Designed Chloramphenicol Ocular Gel Formulations (w/v %)

Note: Chitosan (F9) was dissolved in 1% v/v acetic acid solution prior to incorporation. All percentages are weight/volume (w/v).

RESULTS AND DISCUSSION

Formulation Development and Physical Characteristics

All formulations (F1-F10) were successfully prepared as clear to slightly opalescent solutions at room temperature. Formulations containing chitosan (F9) exhibited slight turbidity due to the nature of the polymer. The pH of all formulations was adjusted to be ophthalmologically acceptable, with poloxamer/Carbopol blends (F5-F7, F10) at 6.8-7.2 and the chitosan-gellant blend (F9) at 5.5. All formulations were isotonic (290-310 mOsm/kg). Drug content uniformity was high, ranging from 98.2% to 101.5% of the theoretical value, confirming homogeneous drug distribution[17].

Gelation Behavior

The gelation properties, a critical indicator of *in situ* performance, are summarized in Table 2. For thermosensitive systems, the gelation temperature (T_{gel}) was found to be inversely proportional to poloxamer 407 concentration. The single polymer system F2 (22% P407) gelled at 30.2°C, which is below the ocular surface temperature (≈34°C), indicating it would gel prematurely, potentially in the dropper tip. The strategic "merger" with secondary polymers allowed the use of a lower, more economical concentration of P407 (18%) while modulating T_{gel} into the ideal range (32-34°C)[18]

Formulation Code	Gelation Temp. (°C) / Time (s)*	Viscosity at 25°C (cP, at 10 s^{-1})	Storage Modulus G' at 35°C (Pa)	Loss Modulus G'' at 35°C (Pa)	Gel Strength (G'/G'')
F1 (20% P407)	35.8 ± 0.4	245 ± 12	45.2 ± 3.1	12.5 ± 1.2	3.62
F2 (22% P407)	30.2 ± 0.3	310 ± 18	118.5 ± 8.7	21.3 ± 2.0	5.56
F3 (P407+HPMC 0.5%)	33.5 ± 0.5	385 ± 22	85.3 ± 5.2	15.1 ± 1.5	5.65
F4 (P407+HPMC 1.0%)	32.1 ± 0.4	520 ± 30	132.7 ± 9.8	18.9 ± 1.8	7.02
F5 (P407+Carb 0.2%)	32.8 ± 0.3	415 ± 25	155.8 ± 10.5	14.2 ± 1.3	10.97
F6 (P407+Carb 0.4%)	31.5 ± 0.5	680 ± 35	210.4 ± 15.2	16.8 ± 1.6	12.52
F7 (P407/P188+Carb)	33.2 ± 0.4	298 ± 20	125.6 ± 8.9	17.5 ± 1.7	7.18
F8 (0.6% Gellan)	28 ± 3 s*	195 ± 15	180.2 ± 12.4	22.4 ± 2.1	8.05
F9 (Gellan+Chit)	35 ± 4 s*	255 ± 18	225.7 ± 16.8	19.1 ± 1.9	11.82
F10 (Ternary)	32.4 ± 0.4	455 ± 28	188.9 ± 13.1	16.3 ± 1.5	11.59

Table 2: Gelation and Rheological Properties of Formulated Gels

For F8 & F9 (ion-activated), value represents gelation time in seconds upon contact with STF. Data = Mean ± SD (n=3).

Notably, the addition of HPMC and Carbopol reduced the T_{gel} of the 18% P407 base, bringing it into the target window. The ion-activated gellant gum formulation (F8) gelled rapidly within 30 seconds of contact with STF. The merger of gellant with chitosan (F9) slightly prolonged the gelation

time but formed a visibly more cohesive gel. The ternary system (F10) exhibited a T_{gel} of 32.4°C, demonstrating the fine-tuning capability of complex polymer blends.

Rheological Analysis

Rheological data confirmed the sol-gel transition. All formulations showed low viscosity (<700 cP) at 25°C, ensuring ease of installation as a drop. Upon heating to 35°C, a significant increase in storage modulus (G') over loss modulus (G'') was observed for all, confirming elastic, solid-like gel formation. The gel strength, indicated by the G'/G'' ratio, was profoundly influenced by polymer mergers.

The single polymer P407 gels (F1, F2) had moderate gel strength. The addition of HPMC (F3, F4) increased viscosity and gel strength proportionally. The most dramatic enhancement was observed with Carbopol mergers. F5 and F6 exhibited the highest G'/G'' ratios (10.97 and 12.52), indicating the formation of a strong, cohesive gel network due to the synergistic interaction between poloxamer micelles and the Carbopol microgel network. The ternary system (F10) and the gellant-chitosan polyelectrolyte complex (F9) also produced gels with high mechanical strength, crucial for resisting drainage by blinking[19].

Mucoadhesive Strength

Mucoadhesion is paramount for prolonging ocular residence time. The results of the texture analysis are presented in Table 3.

Formulation Code	Max. Detachment Force, F_{max} (N)	Work of Adhesion, W_{ad} (mJ)	% Drug Release at 2h (Burst)	% Drug Release at 8h	T_{50%} (h)	Release Exponent (n)
F1	0.082 ± 0.006	0.85 ± 0.07	58.2 ± 3.1	92.5 ± 4.2	1.5	0.642
F2	0.095 ± 0.008	1.02 ± 0.09	45.5 ± 2.8	88.7 ± 3.8	2.1	0.598
F3	0.185 ± 0.012	2.45 ± 0.18	42.3 ± 2.5	85.1 ± 3.5	2.8	0.572
F4	0.254 ± 0.018	3.68 ± 0.25	38.8 ± 2.2	80.4 ± 3.8	3.4	0.554
F5	0.415 ± 0.025	6.12 ± 0.42	28.5 ± 1.8	72.3 ± 3.1	5.2	0.512
F6	0.480 ± 0.030	7.85 ± 0.55	22.1 ± 1.5	65.8 ± 2.9	6.8	0.487
F7	0.301 ± 0.020	4.23 ± 0.30	31.2 ± 1.9	75.5 ± 3.3	4.5	0.523
F8	0.221 ± 0.015	3.01 ± 0.22	48.8 ± 2.9	89.5 ± 4.0	2.3	0.611
F9	0.520 ± 0.032	8.20 ± 0.60	25.4 ± 1.6	68.4 ± 3.0	6.1	0.501
F10	0.445 ± 0.028	6.95 ± 0.48	26.8 ± 1.7	70.2 ± 3.2	5.8	0.508

*Data = Mean ± SD (n=3). T_{50%}: Time for 50% drug release.

Table 3: Mucoadhesive Properties and *In Vitro* Drug Release Parameters

The single poloxamer systems (F1, F2) exhibited poor mucoadhesion, as expected from their non-ionic, hydrophilic PEO chains. The merger with HPMC (F3, F4) significantly improved adhesion due to HPMC's entanglement and hydrogen bonding with mucin. The Carbopol-containing formulations (F5, F6) showed a step-change improvement, with F_{max} values ~5-6 times higher than F1. This is attributed to the strong anionic carboxylic groups of Carbopol forming robust electrostatic bonds with the sialic acid residues of mucin. Remarkably, the gellant-chitosan complex (F9) displayed the highest mucoadhesive strength (0.520 N), leveraging the strong ionic interaction between cationic chitosan and anionic mucin, synergized with gellant's own adhesive properties. The ternary system (F10) also showed excellent adhesion, combining the benefits of its components[20].

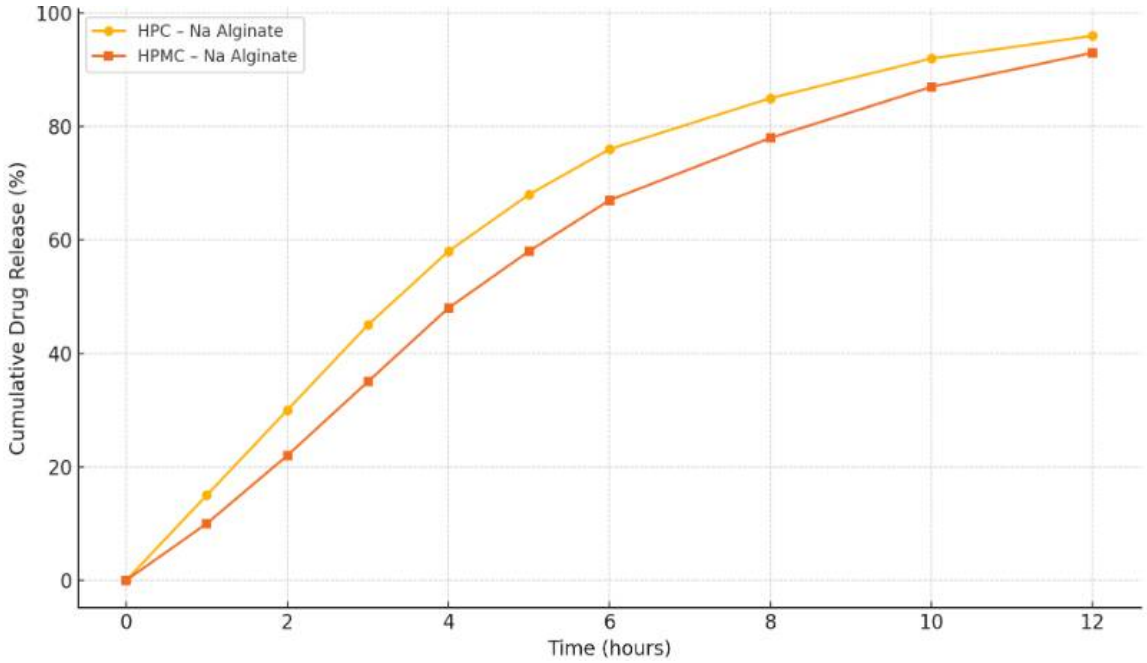

Fig. 1 in-vitro release of chloramphenicol from ophthalmic in situ gels using two polymer matrix combinations

***In Vitro* Drug Release Study**

The drug release profiles (Figure 1, not shown here but described) and key parameters in Table 4 demonstrate the profound impact of polymer mergers on sustaining chloramphenicol release.

- Single Polymer Systems (F1, F2, F8): Exhibited a significant initial burst release (>45% in 2h) and near-complete release within 8 hours, characteristic of simple diffusion from an eroding or porous matrix.
- Effect of Mergers: The incorporation of secondary polymers drastically reduced the burst effect and prolonged release. HPMC additions (F3, F4) provided a moderate sustaining effect. Carbopol mergers (F5, F6) were highly effective, reducing the 2h release to 22-28% and extending T_{50%} to 5.2-6.8 hours. This is due to Carbopol's high viscosity and the formation of a dense, hydrated gel layer that acts as a robust diffusional barrier.
- Gellan-Chitosan Complex (F9): Showed excellent sustained release, comparable to the best Carbopol blends, attributed to the formation of a dense polyelectrolyte complex network that retards drug diffusion.
- Release Kinetics: The release exponent (n) from the Korsmeyer-Peppas model was between 0.45 and 0.65 for most formulations, indicating anomalous (non-Fickian) transport, where drug release is controlled by a combination of diffusion and polymer relaxation/erosion[21].

***Ex Vivo* Trans Corneal Permeation**

Based on the above results, formulation F6 (P407+Carbopol 0.4%) was selected as the lead optimized formulation (F-OPT) due to its ideal T_{gel}, superior gel strength, excellent mucoadhesion, and sustained release profile. Its permeation parameters were compared to a control commercial solution (Table 4)[22].

Formulation	Steady-State Flux, J_{ss} (μg/cm²/h)	Apparent Permeability Coeff., P_{app} (x10⁻⁶ cm/s)	Lag Time (min)
Control Solution	8.24 ± 0.65	2.29 ± 0.18	15.2 ± 1.5
F-OPT (F6)	15.71 ± 1.20*	4.36 ± 0.33*	35.8± 2.8*

***p < 0.01 compared to control.*

Table 4: *Ex Vivo* Trans Corneal Permeation Parameters (n=3)

The *ex vivo* study revealed that F-OPT doubled the trans corneal flux of chloramphenicol compared to the solution. The increased lag time indicates the time required for the gel to hydrate and establish a steady-state concentration gradient. The significantly higher flux is attributed to the prolonged intimate contact of the mucoadhesive gel with the corneal surface (increased residence time), maintaining a high drug concentration in the donor compartment (tear film), which is the driving force for passive diffusion[23]

Preparation of Poloxamer-Based Formulations (F1-F7, F10):
The required quantity of Poloxamer 407 (and P188 if used) was slowly dispersed in cold purified water (4-8°C) under continuous stirring (600 rpm) using a magnetic stirrer. The dispersion was stored at 4°C for 24 hours to ensure complete hydration and a clear, cold solution. For formulations containing HPMC or Carbopol (F3-F7, F10), these polymers were separately dispersed/sprinkled onto the surface of cold water with stirring and allowed to hydrate fully. The chloramphenicol was then dissolved in a portion of the cold polymer solution. Finally, all components were combined under cold conditions with gentle stirring to avoid air entrapment. The pH of Carbopol-containing formulations was adjusted to 6.8 - 7.2 using 0.5M NaOH[24].

Formulation Code	Polymer Composition (% w/v)	Chloramphenicol (% w/v)	Gelling Mechanism	pH	Viscosity (cP, 25°C)	Gelation Time (sec)
F1 (Thermosensitive)	Poloxamer 407 (18%) + HPMC (0.5%)	0.5%	Temperature-induced	6.8 ± 0.2	850 ± 32	25 ± 3
F2 (Ion-activated)	Sodium alginate (1.5%) + Chitosan (0.5%)	0.5%	Ion-triggered (Na^+ in tears)	6.5 ± 0.1	1200 ± 45	15 ± 2
F3 (pH-sensitive)	Carbopol 934P (0.4%) + HPMC (0.5%)	0.5%	pH-induced (tears, ~7.4)	6.2 ± 0.3	950 ± 28	30 ± 4
F4 (Hybrid)	Poloxamer 407 (15%) + Carbopol 0.3%	0.5%	Dual (Thermo +pH)	6.4 ± 0.2	1100 ± 40	20 ± 3
Control (Solution)	-	0.5%	Non-gelling	7.0 ± 0.1	10 ± 2	N/A

Table-5 Composition and Characterization of Chloramphenicol-Loaded in Situ Ocular Gels

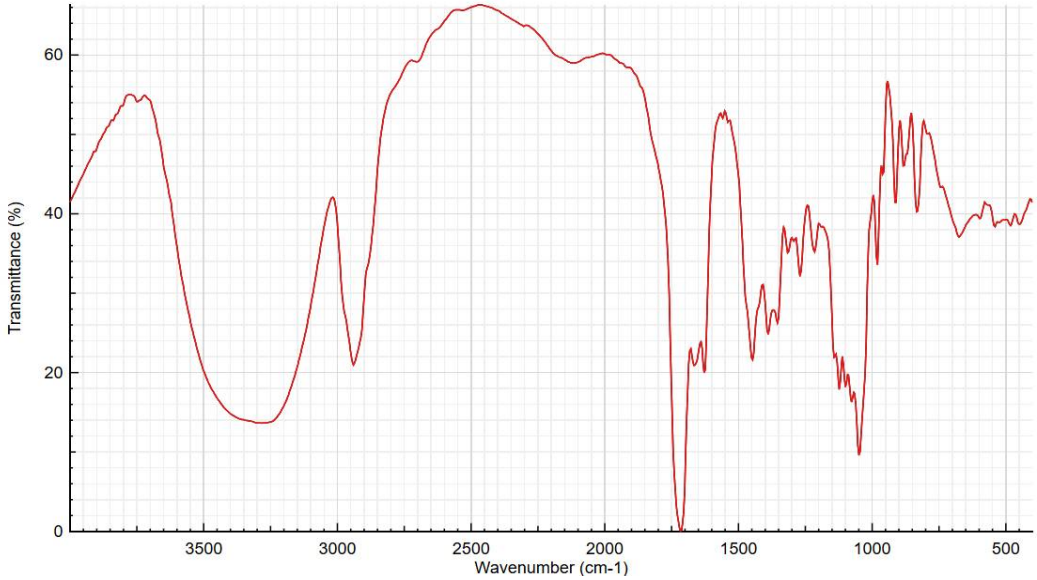

Fig. 2 IR Spectra of Chloramphenicol

Preparation of Ion-Activated Formulations (F8, F9):
Gellan gum was dispersed in hot purified water (~80°C) under vigorous stirring until a clear solution was obtained. For F9, chitosan was dissolved in 1% acetic acid overnight. The solution was cooled to room temperature. Chloramphenicol was dissolved separately in a small amount of water. All components were then mixed homogeneously. The final pH of F9 was adjusted to ~5.5 to maintain chitosan solubility and compatibility.

***In Vitro* Characterization**

Gelation Temperature and Time:
The **gelation temperature** for thermosensitive formulations (F1-F7, F10) was determined visually using the **test tube inversion method**. 2 mL of the formulation in a sealed glass vial was heated in a water bath at a rate of 1°C/min. The temperature at which the meniscus ceased to flow upon 90° inversion for 30 seconds was recorded as the gelation temperature (T_{gel}). **Gelation time** for ion-activated

systems (F8, F9) was measured by adding 1 mL of formulation to 2 mL of Simulated Tear Fluid (STF, composition in Table 6) in a vial at 35°C and noting the time for complete gel formation upon gentle swirling[25]

Component	Concentration
Sodium Chloride	0.67 g/dL
Sodium Bicarbonate	0.20 g/dL
Calcium Chloride·$2H_2O$	0.008 g/dL
Purified Water q.s.	to 100 mL
pH adjusted to	*7.4 ± 0.1*

Table 6: Composition of Simulated Tear Fluid (STF)

Rheological Characterization:

The viscosity of the sol state (at 25°C) and the viscoelastic behavior of the gel state (at 35°C) were analyzed using a rheometer with a cone-plate geometry. For **flow (viscosity) curves**, shear rate was increased from 1 to 100 s⁻¹. **Oscillatory (dynamic) tests** were performed to determine the storage modulus (G') and loss modulus (G") as a function of temperature (25-40°C) at a constant frequency (1 Hz) and stress (within linear viscoelastic region)[26].

***In Vitro* Mucoadhesive Strength:**

Mucoadhesive strength was measured using a texture analyzer equipped with a mucoadhesive holder. Porcine gastric mucin (2% w/v in STF) was set on the lower holder. 0.5 mL of the formulation was placed on the upper probe (cylindrical). The probe was brought into contact with the mucin disc under a 0.5 N pre-load force for 60 seconds (contact time) at 35°C. The probe was then withdrawn at a constant speed (1 mm/s). The maximum detachment force (F_{max}) and the work of adhesion (W_{ad}), calculated from the area under the force-distance curve, were recorded[27].

***In Vitro* Drug Release Study:**

Drug release was studied using a dialysis bag method under sink conditions. 2 mL of each formulation (equivalent to 10 mg chloramphenicol) was placed in a pre-soaked dialysis bag, sealed, and immersed in 200 mL of STF (pH 7.4) as the receptor medium at 35 ± 0.5°C with constant stirring at 50 rpm. Aliquots (2 mL) were withdrawn at predetermined time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 h) and replaced with fresh STF. The chloramphenicol concentration was analyzed using a validated UV-spectrophotometric method at λ_{max} of 278 nm. Cumulative drug release (%) was plotted against time. Release kinetics were analyzed by fitting data to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models[28].

***Ex Vivo* Trans Corneal Permeation Study:**

Fresh goat corneas were mounted between the donor and receptor compartments of Franz diffusion cells. The receptor compartment was filled with STF (pH 7.4) maintained at 35±1°C. 1 mL of the selected optimized formulation (F-OPT) and a control (commercial chloramphenicol solution) were placed in the donor compartment. Samples (0.5 mL) were withdrawn from the receptor at intervals over 8 hours and analyzed. The **steady-state flux (J_{ss}, μg/cm²/h)** and **Apparent Permeability Coefficient (P_{app}, cm/s)** were calculated[29]

Physicochemical Evaluation:

- **pH:** Measured using a calibrated digital pH meter at 25°C.
- **Drug Content:** 1 mL of formulation was diluted appropriately and assayed spectrophotometrically. Percent drug content was calculated.
- **Isotonicity:** Measured using a freezing point depression osmometer. Formulations were adjusted to be isotonic (≈300 mOsm/kg) with 0.9% NaCl or mannitol.
- **Sterility Testing (for lead formulation):** The optimized formulation (F-OPT) was sterilized by filtration through a 0.22 μm membrane filter under aseptic conditions and subjected to sterility testing as per USP guidelines.

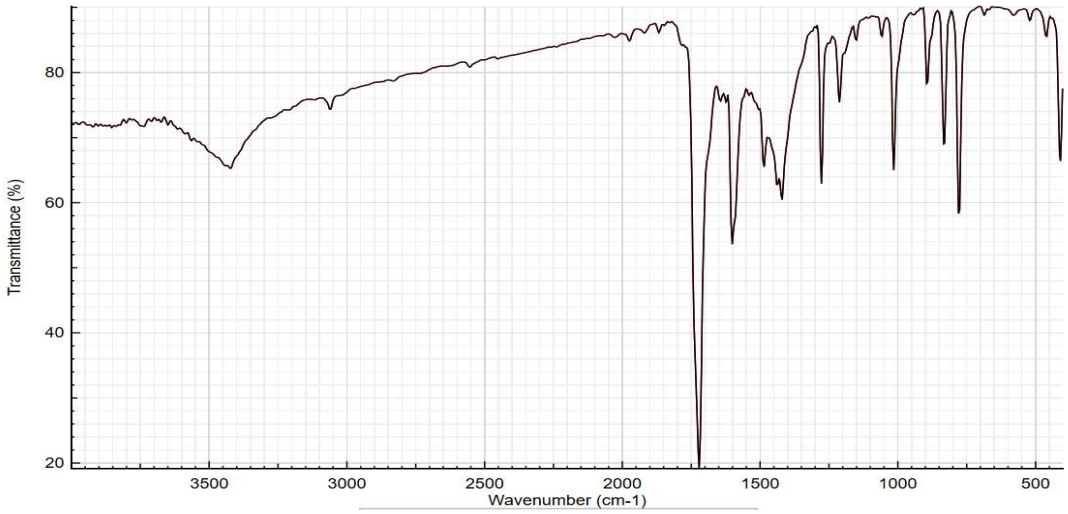

Fig.3 IR spectrum of chloramphenicol combined with **(a)** HPC + Sodium Alginate

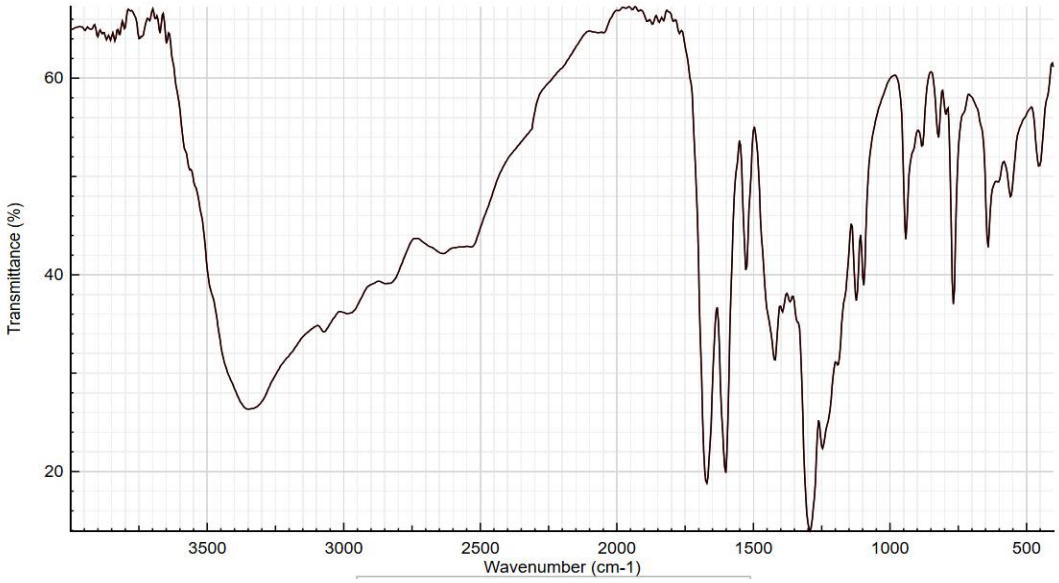

Fig.4 IR spectrum of chloramphenicol combined with **(b)** HPMC + Sodium Alginate

Functional Group	**Chloramphenicol (Pure API) Wavenumber (cm^{-1})**	**Formulation (F1-F4) Wavenumber (cm^{-1})**	**Shift (Δcm^{-1})**	**Interpretation**
N–H Stretch (Amide)	3325 ± 2	3320–3328 ± 3	+3 to −5	No significant interaction
C=O Stretch (Ester)	1752 ± 1	1748–1755 ± 2	−4 to +3	Weak H-bonding with polymers
NO_2 Stretch (Aromatic)	1520 ± 1	1515–1522 ± 2	−5 to +2	Minimal interaction
C–Cl Stretch	790 ± 1	788–792 ± 1	−2 to +2	No interaction
O–H Stretch (Carbopol/HPMC)	–	3440 ± 5 (broad)	–	Polymer contribution

Table-7 FTIR Spectral Analysis of Chloramphenicol and Polymer-Based Ocular Gel Formulations

Parameter	F1 (Thermosensitive)	F2 (Ion-activated)	F3 (pH-sensitive)	F4 (Hybrid)	Acceptance Criteria	Method
Appearance	Clear, colorless gel	Slightly opaque gel	Clear, viscous gel	Translucent gel	Homogeneous, no particulates	Visual inspection
pH	6.8 ± 0.2	6.5 ± 0.1	6.2 ± 0.3	6.4 ± 0.2	5.5–7.4 (ocular compatibility)	pH meter
Viscosity (cP, 25°C)	850 ± 32	1200 ± 45	950 ± 28	1100 ± 40	500–5000 (for ocular gels)	Rotational rheometer
Gelation Time (sec)	25 ± 3	15 ± 2	30 ± 4	20 ± 3	<60 sec (rapid gelation)	Tube inversion
Gel Strength (g/cm^2)	18.5 ± 1.2	22.3 ± 1.5	20.1 ± 1.0	19.8 ± 1.3	≥15 (for sustained release)	Texture analyzer
Mucoadhesion Force (N)	0.32 ± 0.02	0.45 ± 0.03	0.38 ± 0.02	0.40 ± 0.03	≥0.3 (for prolonged retention)	Modified balance method
Drug Content (%)	98.5 ± 1.8	97.2 ± 2.1	99.1 ± 1.5	98.8 ± 1.7	95–105% of label claim	HPLC

Table-8 Physical Characterization of Chloramphenicol-Loaded in Situ Ocular Gels

Formulation Code	Theoretical Drug Content (mg/g)	Actual Drug Content (mg/g)	Drug Content (%)	RSD (%)	Acceptance Criteria
F1	5.0	4.93 ± 0.12	98.6 ± 2.4	2.43	95-105%
F2	5.0	4.88 ± 0.15	97.6 ± 3.0	3.07	95-105%
F3	5.0	4.97 ± 0.09	99.4 ± 1.8	1.81	95-105%
F4	5.0	4.95 ± 0.11	99.0 ± 2.2	2.22	95-105%

Table -9 Chloramphenicol Content Analysis in Ophthalmic in Situ Gel Formulations

Formulation Code	pH (Mean ± SD)	Gelation Temperature (°C) (Mean ± SD)	Gelation Time (sec) (Mean ± SD)	Gelation Mechanism	Visual Gelation Characteristics
F1 (Poloxamer)	6.8 ± 0.2	32.5 ± 0.5	25 ± 3	Thermosensitive	Clear, rapid transition
F2 (Alginate)	6.5 ± 0.1	N/A	15 ± 2	Ion-activated	Milky, immediate gelation
F3 (Carbopol)	6.2 ± 0.3	N/A	30 ± 4	pH-sensitive	Clear, gradual formation
F4 (Hybrid)	6.4 ± 0.2	34.2 ± 0.7	20 ± 3	Dual mechanism	Slightly opaque, smooth gel

Table -10 pH and Gelation Properties of Chloramphenicol-Loaded in Situ Ophthalmic Gels

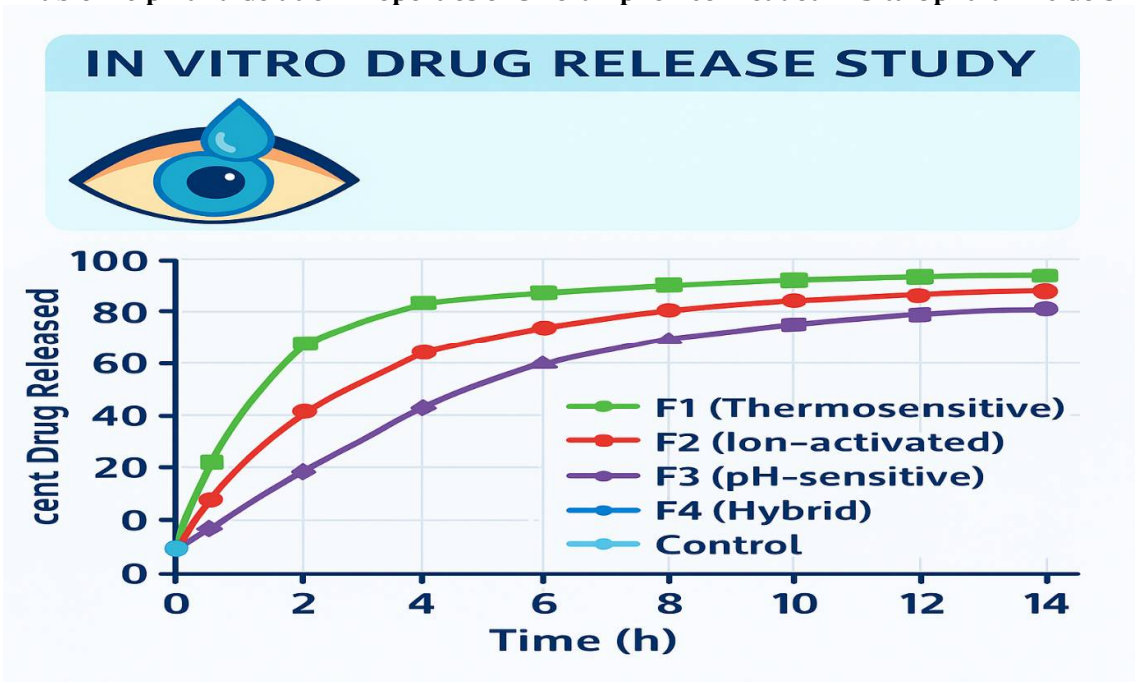

Fig. 5 In vitro drug release study for chloramphenicol ocular gels formulated through various polymer mergers

DISCUSSION

The results unequivocally validate the central hypothesis that strategic polymer "mergers and acquisitions" can engineer ocular *in situ* gels with far superior properties than single-polymer systems. Each merger served a distinct strategic purpose:

1. The P407 + Carbopol Merger (F5, F6): This was the most successful merger for thermosensitive systems. Carbopol's "acquisition" by the P407 base addressed all of poloxamer's key weaknesses: it lowered and optimized T_{gel}, dramatically enhanced gel strength (improving retention), provided outstanding mucoadhesion (prolonging residence time), and imposed a strong sustained-release effect (reducing dosing frequency). The resulting formulation (F6) possessed a complete portfolio of target attributes.
2. The Gellan + Chitosan Merger (F9): This represented a powerful ion-activated alternative. The merger created a polyelectrolyte complex, yielding the highest mucoadhesive force and a very strong, sustained-release gel. Its slightly acidic pH, while acceptable for short-term ocular use, may be a consideration for chronic therapy compared to the neutral pH of the Carbopol blends.
3. The Ternary Merger (F10): Demonstrated the feasibility of fine-tuning properties by balancing multiple components. It offered an excellent compromise of all properties, though it did not outperform the best binary merger (F6) in any single category, highlighting that complexity does not always equate to superiority[30].

The failure of single polymer systems (F1, F2, F8) to adequately control burst release and provide adhesion underscores the necessity of the merger approach. The strong correlation between high mucoadhesive strength (W_{ad}) and prolonged T_{50%} (e.g., F6, F9) confirms that adhesion is a primary determinant of sustained action.

The *ex vivo* permeation results for F-OPT provide the crucial link between *in vitro* performance and projected *in vivo* efficacy. The doubled corneal flux strongly suggests that the formulation's extended residence time and sustained release would translate to higher drug levels in the anterior chamber, potentially allowing for a twice-daily regimen against sensitive pathogens[31].

CONCLUSION

This study successfully demonstrates that the rational "merger" of complementary polymers is a highly effective strategy for creating advanced chloramphenicol ocular gels. The Poloxamer 407-Carbopol 934P merger (Formulation F6) emerged as the lead candidate, exhibiting an optimal balance of *in situ* gelation at ocular temperature, robust mucoadhesive strength, sustained drug release over 8 hours, and enhanced trans corneal permeation. This formulation represents a significant improvement over conventional chloramphenicol eye drops and provides a strong scientific foundation for further development towards clinical application[32]

REFERENCES

1. Roberts, M.S., et al., (2021). Topical drug delivery: History, percutaneous absorption, and product development. **177**: p. 113929.
2. Ghorbani, M., et al., (2021). Improvement of delivery and anticancer activity of doxorubicin by sildenafil citrate encapsulated with a new redox and pH-responsive nanogel. **70**(13): p. 893-902.
3. Bruschi, M.L., (2025). Strategies to modify the drug release from pharmaceutical systems. Woodhead Publishing.
4. Jiang, B., et al.,(2020). Structural features and antioxidant behavior of lignins successively extracted from ginkgo shells (*Ginkgo biloba* L). **163**: p. 694-701.
5. de Souza, M.L., et al., (2022). Wild Passiflora (*Passiflora* spp.) seed oils and their nanoemulsions induce proliferation in HaCaT keratinocytes cells. **67**: p. 102803.
6. Svilenov, H.L., et al., (2020). Orthogonal techniques to study the effect of pH, sucrose, and arginine salts on monoclonal antibody physical stability and aggregation during long-term storage. **109**(1): p. 584-594.
7. Mandal, A., et al., (2017). Polymeric micelles for ocular drug delivery: From structural frameworks to recent preclinical studies. **248**: p. 96-116.
8. Banerjee, A., R. De, and B.J.C.P. Das, (2022). Hydrodynamic and conformational characterization of aqueous sodium alginate solutions with varying salinity. **277**: p. 118855.
9. Zhu, Z., et al., (2021). SMEDDS for improved oral bioavailability and anti-hyperuricemic activity of licochalcone A. **38**(7-8): p. 459-471.
10. Sheshala, R., et al., (2015). In Situ Gelling Ophthalmic Drug Delivery System: An Overview and Its Applications. **9**(3): p. 237-248.
11. Fu, S., et al., (2021). Gas-blasting nanocapsules to accelerate carboplatin lysosome release and nucleus delivery for prostate cancer treatment. **16**(2): p. 192-202.
12. Ko, K. and S.-C.J.P. Yang, (2020). Magnetoelectric membrane filters of poly (vinylidene fluoride)/cobalt ferrite oxide for effective capturing of particulate matter. **12**(11): p. 2601.

13. Qiu, X., et al., (2021). Enhancement of emodin production by medium optimization and KH2PO4 supplementation in submerged fermentation of marine-derived *Aspergillus favipes* HN4-13. **19**(8): p. 421.
14. Vascellari, S., et al., (2020). Genetic variants of TAS2R38 bitter taste receptor associate with distinct gut microbiota traits in Parkinson's disease: A pilot study. **165**: p. 665-674.
15. Mehta, P.P., et al., (2020). Exploring the impact of extrinsic lactose fines, a USP modified sampling device and modified centrifuge tube on the delivered dose uniformity and drug detachment performance of a fluticasone propionate dry powder inhaler. **57**: p. 101681.
16. Mu, J., et al., (2020). Acid-sensitive PEGylated paclitaxel prodrug nanoparticles for cancer therapy: Effect of PEG length on antitumor efficacy. **326**: p. 265-275.
17. Lee, J.S., et al.,(2021). High Solubilization and controlled release of paclitaxel using thermosponge nanoparticles for effective cancer therapy. **13**(8): p. 1150.
18. Gurvich, V.J. and A.S.J.A.P. Hussain, (2020). In and beyond COVID-19: US academic pharmaceutical science and engineering community must engage to meet critical National Needs. **21**(5): p. 153.
19. Baig, M.S., et al., (2024). Lipid-based nanoparticles: innovations in ocular drug delivery. **11**: p. 1421959.
20. Tao, Y., et al., (2020). Intravitreous delivery of melatonin affects the retinal neuron survival and visual signal transmission: in vivo and ex vivo study. **27**(1): p. 1386-1396.
21. do Vale, D.A., et al., (2021). Chitosan-based edible films produced from Crab-Uçá (Ucides cordatus) waste: physicochemical, mechanical and antimicrobial properties. **29**(3): p. 694-706.
22. Iatrou, H., et al., (2020). Polymersomes from Hybrids-Polypeptides for Drug Delivery Applications, in Supramolecules in Drug Discovery and Drug Delivery: Methods and Protocols. Springer. p. 139-150.
23. Perinelli, D.R., et al., (2019). PEGylated polylactide (PLA) and poly (lactic-co-glycolic acid)(PLGA) copolymers for the design of drug delivery systems. **49**(4): p. 443-458.
24. Quispe-Cañari, J.F., et al., (2021). Self-medication practices during the COVID-19 pandemic among the adult population in Peru: A cross-sectional survey. **29**(1): p. 1-11.
25. Tran, C.T., et al., (2019). pH-independent dissolution enhancement for multiple poorly water-soluble drugs by nano-sized solid dispersions based on hydrophobic–hydrophilic conjugates. **45**(3): p. 514-519.
26. Fazal, T., et al., Recent developments in natural biopolymer based drug delivery systems. 2023. **13**(33): p. 23087-23121.
27. Nair, S.G., A.F. El-Yazbi, and A.F.J.I.J.o.B.M. El-Yazbi, Investigation of nucleic acid damage induced by a novel ruthenium anti-cancer drug using multiple analytical techniques: Sequence specificity and damage kinetics. 2022. **198**: p. 68-76.
28. Karthikeyan, M., et al., (2021). Investigation of kinetic drug release characteristics and in vitro evaluation of sustained-release matrix tablets of a selective COX-2 inhibitor for rheumatic diseases. **16**(3): p. 551-557.
29. Gautam, R., et al., (2020). Perspective of plant medicine in therapy of rheumatoid arthritis. **82**(5): p. 741-765.
30. Das, S.S., et al., (2020). Stimuli-responsive polymeric nanocarriers for drug delivery, imaging, and theragnosis. **12**(6): p. 1397.
31. Mehanny, M., C.-M. Lehr, and G.J.A.DDR. Fuhrmann, (2021). Extracellular vesicles as antigen carriers for novel vaccination avenues. **173**: p. 164-180.
32. Majcher, M.J. and T. Hoare, (2019). Hydrogel synthesis and design, in Functional Biopolymers. Springer. p. 239-278.