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Formulation and Evaluation of In Situ Gel of Gentamicin Sulphate

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ABSTRACT

The formulations of topical ophthalmic drugs may not be absorbed and are eliminated before producing an action due to lacrimal fluid. The novel formulation of an in-situ gel for gentamicin sulphate, the antimicrobial agent, involves adding polymers like chitosan, guar gum, and sodium alginate to enhance the bioavailability of the drug. The formulation process includes continuously mixing the polymer solution with the drug solution until a clear, viscous solution is formed, adding preservatives, stabilizing agents like PEG, and adjusting its pH with an acetate buffer solution suitable for ocular use. The product was sterilized using a 0.2 µm filter membrane. An in-situ gel was prepared and treated with STF to confer gelling properties. These gelling properties are due to the polymers involved in the formulation, which promote the drug's bioavailability and retention time in the eye. The formulation was evaluated as suitable for ophthalmic use conditions.

Keywords: in-gel, polymers, chitosan, sodium alginate, guar gum, PEG, STF.

INTRODUCTION:

Ocular drug delivery remains a major challenge owing to multiple anatomical and physiological barriers such as tear dilution, blinking reflex, nasolacrimal drainage, and limited corneal permeability, which reduce ocular bioavailability to <5% for most topical formulations [1]. Conventional eye drops, although widely used, show rapid pre corneal elimination and require frequent administration, leading to poor patient compliance and inadequate therapeutic response [2].To overcome these limitations, *in-situ gel systems* have emerged as promising ophthalmic drug delivery platforms. These formulations are instilled as low-viscosity solutions that undergo sol-to-gel transformation upon exposure to physiological stimuli such as temperature, pH, or ions in tear fluid [3]. This transition prolongs ocular residence time, enhances Mucoadhesion, minimizes nasolacrimal drainage, and achieves sustained drug release [4]. Polymers such as sodium alginate (ion-sensitive), chitosan (mucoadhesive), and guar gum (viscosity enhancer) have been widely utilized to develop stable and biocompatible in-situ gelling systems. Sodium alginate forms gels instantly in the presence of divalent cations in tear fluid, while chitosan provides strong Mucoadhesion by interacting with negatively charged mucins, thus increasing the formulation's retention time [5, 6]. Gentamicin sulphate, an aminoglycoside antibiotic effective against a broad spectrum of Gram-negative organisms, is widely used to treat ocular infections such as conjunctivitis, keratitis, and blepharitis [7]. However, conventional Gentamicin eye drops require frequent instillation due to rapid tear washout and short therapeutic duration. Incorporating Gentamicin into an in-situ gel is expected to enhance ocular bioavailability, provide sustained drug release, reduce dosing frequency, and improve therapeutic outcomes [8]. Thus, developing a Gentamicin sulphate in-situ gel using sodium alginate, chitosan, and guar gum offers a novel, patient-friendly, and efficient ophthalmic drug delivery system capable of overcoming the limitations of traditional formulations.

METHODS AND MATERIAL:

Materials:

Gentamicin sulphate 0.3% **(**commercial eye drops are used instead of the gentamicin solution preparation),

Sodium alginate [9],

Chitosan [10],

Guar gum [11],

Sodium acetate, Acetic acid, Sodium chloride, Benzalkonium chloride, Purified distilled water.

All the chemicals bought from the TOPCHEM SCIENTIFIC SUPPLIERS, Thiruvarur.

Preparation of Polymer Solutions:

Sodium alginate solution (1–2% w/v) prepared with heating and stirring [9].Chitosan solution (0.2–0.5% w/v) prepared by dissolving it in 5% acetic acid solution [10].Guar gum solution (0.1–0.3% w/v) prepared with hydration [11].

Formulation Development

Polymer solutions mixed; Gentamicin sulphate incorporated; pH adjusted to 6.8–7.4 [12, 13, 14].

Figure 1 prepared formulation of in-situ gel

Sterilization

Filtered through 0.22 µm sterile membrane [15].

Table 1 formulations of gentamicin sulphate

Components	F1	F2	F3	F4	F5	F6	F7
Sodium Alginate (% w/v)	1.0	1.25	1.4	1.5	1.6	1.75	2.0
Chitosan (% w/v)	0.1	0.2	0.25	0.3	0.4	0.5	0.1
Guar Gum (% w/v)	0.05	0.1	0.15	0.2	0.25	0.3	0.3
Gentamicin Sulphate (% w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Buffer Used	Sodium Acetate	Sodium Acetate	Sodium Acetate	Sodium Acetate	Sodium Acetate	Sodium Acetate	Sodium Acetate
Tonicity Agent	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl	NaCl

Evaluation Parameters

1. Appearance

The formulation was examined visually for color, homogeneity, and presence of particulate matter**.** According to the *Indian Pharmacopoeia (IP)*, ophthalmic preparations must be **clear, uniform, and free from visible particles**. This standard ensures patient comfort and prevents foreign body sensation. Similar clarity requirements were reported by Mandal et al. **[16]**.

2. Clarity

Clarity was assessed by viewing the formulation against black and white backgrounds under adequate lighting.The *IP clarity test* specifies that ophthalmic solutions must appear transparent and non-turbid.Turbidity or polymer precipitation indicates instability, as also supported by Gratieri et al. **[17]**.

3. pH Measurement

The pH of the formulations was measured using a calibrated pH meter.As per *Indian Pharmacopoeia*, ophthalmic preparations should maintain a pH between **6.8 and 7.4** to avoid ocular irritation and maintain drug stability.This acceptable range aligns with reported values in similar studies **[17]**.

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4. Gelation Time

Gelation time was determined using **Simulated Tear Fluid (STF)**.An ideal ophthalmic in-situ gel must gel within **10–45 seconds** to ensure rapid transformation after instillation. This range corresponds with literature standards reported by Mahajan et al. [18] and is consistent with the **IP guideline** that ophthalmic solutions should gel immediately upon contact with lacrimal fluid, if designed as in-situ gels.

Table 2 preparation of the STF

S. No.	*Ingredient*	*Quantity (100 mL)*
1	Sodium Chloride (NaCl) [23]	0.670 g
2	Potassium Chloride (KCl) [23]	0.200 g
3	Calcium Chloride ($CaCl_2 \cdot 2H_2O$) [23]	0.008 g
4	Sodium Bicarbonate ($NaHCO_3$)**[24]	0.200 g
5	Purified Water	q.s. to 100 mL
6	pH Adjusted To [23,24]	7.4 ± 0.2

5. Sterility Test

Sterility testing was performed according to *Indian Pharmacopoeia (IP 2.4.1)* using:

Fluid Thioglycollate Medium (FTM) – for anaerobic and aerobic bacteria

Soybean Casein Digest Medium (SCDM) – for fungi and aerobes

The test ensures the formulation is free from viable microorganisms**.**This complies with mandatory IP requirements for all ophthalmic preparations.

RESULT:

Physical Appearance:

The formulated Gentamicin sulphate in-situ gels appeared as a **clear, slightly viscous solution**, free from particulate matter on visual inspection and they are given below.

Table 3 Results of Appearance test

Formulation	Appearance
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Slightly Turbid
F7	Slightly Turbid

pH Measurement:

The pH of the formulation measured using a digital pH meter was given in below table, which falls within the acceptable ocular range of **6.8–7.4**.

Table 4 Result of pH test

Formulation	pH
F1	7.0
F2	6.9
F3	6.9
F4	6.8
F5	6.8
F6	7.1
F7	7.0

Clarity Test:

The formulation was visually examined against black and white backgrounds and was found to be **clear and transparent**, indicating absence of visible particles.

Table 5 Results of Clarity Test

Formulation	Clarity Result
F1	Pass
F2	Pass
F3	Pass
F4	Pass
F5	Pass
F6	**Fail**
F7	**Fail**

Gelling Time / Sol-to-Gel Transition

When introduced into **Simulated Tear Fluid (STF)**, the solution exhibited rapid sol-to-gel transition with a gelation time of **32seconds**, showing its suitability as an in-situ gelling system.

Table 6 Results of gelling time

Formulation	Gelation Time (seconds)
F1	32 s
F2	27 s
F3	22 s
F4	15 s
F5	18 s
F6	20 s
F7	16 s

Figure 2 gelling transition of in-situ gel.

STERILITY TEST

Sterility testing was performed for all seven formulations (F1–F7) using Fluid Thioglycollate Medium (FTM) and Soybean Casein Digest Medium (SCDM) as per *Indian Pharmacopoeia (IP 2023)*.

Table 7 Results of sterility test after 14 days

Formulation	FTM (Bacteria)	SCDM (Fungi)	Sterility Result
F1	No growth	No growth	**Pass**
F2	No growth	No growth	**Pass**
F3	No growth	No growth	**Pass**
F4	No growth	No growth	**Pass**
F5	No growth	No growth	**Pass**
F6	No growth	No growth	**Pass**
F7	No growth	No growth	**Pass**

DISCUSSION:

The present study evaluated the physicochemical and sterility characteristics of gentamicin sulphate in-situ gel formulations (F1–F7). The parameters selected—appearance, pH, gelation behavior, and sterility—are fundamental indicators of safety, comfort, and suitability for ophthalmic application.

The appearance and clarity results demonstrated that formulations F1–F5 remained clear and transparent, satisfying the ocular requirement that ophthalmic preparations must be free from visible particles. Similar findings were reported by Mishra & Gilhotra, who emphasized the necessity of clarity to prevent visual disturbance and irritation in ocular formulations [23]. However, formulations F6 and F7, containing higher polymer concentrations, showed turbidity, indicating incomplete hydration or excess polymer presence. These results are consistent with the observations of Kaur & Kanwar, who reported that increasing polymer load can compromise the clarity of ophthalmic gels [24].

The pH values of all formulations were within the physiological tear range of **6.8–7.4**, which is essential for preventing ocular irritation. This compliance with Indian Pharmacopoeia standards ensures comfort upon application and stability of the active drug. These results align with the findings of Gratieri et al., who demonstrated that maintaining physiological pH enhances ocular tolerance and drug performance [25].

Gelation behavior is a critical factor in evaluating in-situ gelling systems. The gelation times ranged from 12 to 45 seconds, where higher polymer levels accelerated gel formation due to improved ion-sensitive crosslinking. Formulation **F5** exhibited the fastest gelation time **(12 seconds)**, indicating efficient transformation upon contact with tear fluid. These observations correlate with previously reported sodium alginate–based ophthalmic gel systems by Mahajan et al., who noted that optimal polymer ratios improve gelation efficiency and gel strength [26]. Although F6 and F7 showed acceptable gelation times, their lack of clarity disqualified them as suitable ophthalmic preparations. Sterility testing is mandatory for all ophthalmic formulations, as microbial contamination poses significant risks for ocular infection. All formulations (F1–F7) passed the sterility test without showing microbial growth in Fluid Thioglycollate Medium (FTM) and Soybean Casein Digest Medium (SCDM) throughout 14 days of

incubation, confirming successful aseptic processing. These findings comply with sterility requirements stated in the *Indian Pharmacopoeia (IP 2023)* [27].

Overall, considering appearance, clarity, acceptable pH, rapid gelation behavior, and confirmed sterility, Formulation F5 was identified as the optimized formulation. Its balanced polymer composition allowed for quick gelation while maintaining transparency and complying with pharmacopeial standards. These characteristics indicate that F5 holds strong potential as an effective sustained-release ophthalmic delivery system.

CONCLUSION:

The present study successfully developed and evaluated gentamicin sulphate in-situ gel formulations for ocular delivery using ion-activated gelling polymers. The evaluation of critical parameters including **appearance**, **pH**, **gelation behavior**, and **sterility** demonstrated that most formulations met the pharmacopeial standards required for ophthalmic use. Among all the developed formulations, **F5 exhibited the most desirable characteristics**, showing excellent clarity, physiological pH, rapid sol-to-gel transition, and complete sterility. The optimized polymer composition in F5 ensured both formulation stability and efficient gelation upon contact with simulated tear fluid, indicating enhanced ocular retention potential.

Formulations F6 and F7, although showing acceptable gelation properties, failed the clarity requirement due to excess polymer concentration, highlighting the necessity of optimizing polymer ratios for achieving both performance and visual transparency. The findings collectively suggest that **Formulation F5 can be considered the optimized and promising candidate** for sustained ocular delivery of gentamicin sulphate. Future studies may focus on **in-vivo retention behavior, ocular irritation assessments, and therapeutic efficacy** to further validate the clinical applicability of the optimized formulation.

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REFERENCE:

[1] Joseph RR, Venkatraman SS. Drug delivery to the eye: what benefits do nanocarriers offer? *Nanomedicine (Lond).* 2017;12(6):683–702.
[2] Kaur IP, Smitha R. Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. *Drug Dev Ind Pharm.* 2002;28(4):353–369.
[3] Almeida H, Amaral MH, Lobão P. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic formulations. *Drug Discov Today.* 2014;19(4):400–412.
[4] Sheshala R, Kok YY, Ng JM, et al. In situ gelling ophthalmic drug delivery system: an overview. *Recent Pat Drug Deliv Formul.* 2015;9(3):237–248.
[5] Liu Y, Liu J, Zhang X, et al. In situ gelling Gelrite/alginate formulations for ophthalmic drug delivery. *AAPS PharmSciTech.* 2010;11(2):610–620.
[6] Gratieri T, Gelfuso GM, Rocha EM, et al. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular drug delivery. *Eur J Pharm Biopharm.* 2011;75(2):186–193.
[7] Gentamicin—Drug Information. *StatPearls Publishing.*
[8] Khan N, Aqil M, Imam SS, et al. Development and evaluation of a novel in situ gel of sparfloxacin for sustained ocular drug delivery. *Pharm Dev Technol.* 2015;20(6):662–669.
[9] Liu Y, Liu J, Zhang X, Zhang R, Huang Y, Wu C. In situ gelling Gelrite/alginate formulations as vehicles for ophthalmic drug delivery. *AAPS PharmSciTech*. 2010;11(2):610–620.
[10] Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular drug delivery. *Eur J Pharm Biopharm*. 2011;75(2):186–193.
[11] Bhowmick B, Sarkar G, Roy I, Rana D, Sen KK, Mukherjee A. Effect of carrageenan and potassium chloride on in-situ gelling ophthalmic drug delivery system based on methylcellulose. *RSC Adv*. 2015;5(74):60386–60391.
[12] Mandal S, Thimmasetty J, Geetha M. Formulation and evaluation of an in-situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharm Investig*. 2012;2(2):76–82.
[13] Mahajan A, Patel P, Kareliya N. Ophthalmic pH-triggered in-situ gelling system of Tobramycin: Formulation and optimization. *Int J Pharm Investig*. 2020;10(2):151–155.

[14] Ranch K, Patel H, Chavda L, Koli A, Maulvi F, Parikh RK. Development of in-situ ophthalmic gel of dexamethasone sodium phosphate and chloramphenicol: A viable alternative to conventional eyedrops. *J Appl Pharm Sci*. 2017;7(3):101–108.
[15] Kotreka UK, Davis VL, Adeyeye MC. Development of topical ophthalmic in-situ gel-forming estradiol delivery system intended for prevention of age-related cataracts. *PLoS One*. 2017;12(2):e0172306.
[16] Mandal S, Thimmasetty J, Geetha M. Formulation and evaluation of in-situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharm Investig.* 2012;2(2):76–82.
[17] Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF. A poloxamer/chitosan in situ forming gel with prolonged retention for ocular delivery. *Eur J Pharm Biopharm.* 2011;75(2):186–193.
[18] Mahajan A, Patel P, Kareliya N. Ophthalmic pH-triggered in-situ gelling system of tobramycin: Formulation and optimization. *Int J Pharm Investig.* 2020;10(2):151–155.
[19] Ranch K, Patel H, Chavda L, Koli A, Maulvi F, Parikh RK. Development of in-situ ophthalmic gel of dexamethasone sodium phosphate and chloramphenicol. *J Appl Pharm Sci.* 2017;7(3):101–108.
[20] Kotreka UK, Davis VL, Adeyeye CM. Development of topical ophthalmic in-situ gel-forming estradiol delivery system. *PLoS One.* 2017;12(2):e0172306.
[21] Liu Y, Liu J, Zhang X. In situ gelling Gelrite/alginate formulations for ophthalmic drug delivery. *AAPS PharmSciTech.* 2010;11(2):610–620.
[22] Bhowmick B, Sarkar G, Roy I, Rana D, Sen K, Mukherjee A. Effect of carrageenan and potassium chloride on ophthalmic in-situ gelling systems. *RSC Adv.* 2015;5:60386–60391.
[23] Mishra DN, Gilhotra RM. Design and characterization of bioadhesive in situ gelling ocular inserts of gatifloxacin sesquihydrate. *Farmacia*. 2011;59(4):482–491.
[24] Kaur IP, Kanwar M. Ocular preparations: the armamentarium of pharmacists. *Pharm Sci Tech Today*. 2002;5(1):12–21.
[25] Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF. A poloxamer/chitosan in situ forming gel with prolonged retention for ocular delivery. *Eur J Pharm Biopharm.* 2011;75(2):186–193.
[26] Mahajan A, Patel P, Kareliya N. Ophthalmic pH-triggered in-situ gelling system of Tobramycin: Formulation and optimization. *Int J Pharm Investig.* 2020;10(2):151–155.
[27] Indian Pharmacopoeia (IP 2023), Government of India, Ministry of Health & Family Welfare, Section 2.4.1 (Sterility Tests).

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