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Formulation and Evaluation of Ophthalmic In Situ Gel of Antioxidant Pinobanksin

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ABSTRACT: This study centers on developing and evaluating an ophthalmic in situ gel containing the antioxidant Pinobanksin. The gel is designed to overcome limitations of conventional ocular drug delivery systems, such as rapid pre-corneal drug loss and low bioavailability. Leveraging in situ gel technology, the formulation transitions from a liquid state to a gel upon application, promoting extended retention on the eye surface and controlled drug release. The antioxidant properties of Pinobanksin help mitigate oxidative stress, thereby improving therapeutic outcomes. Evaluations, including physical characterization, stability tests, and drug release studies, validate the gel's effectiveness and its suitability as a user-friendly option for ocular treatments.

Keywords: Antioxidant, Pinobanksin, Gelling Capacity, Differential Scanning Calorimetry.

INTRODUCTION: The eye is an exceptional and invaluable organ. In ocular chemotherapy, topical drug administration is generally the preferred approach due to its convenience and safety. Numerous eye-related conditions can impact overall health and lead to vision loss. Consequently, drug delivery systems for ocular treatment are categorized into conventional systems and innovative systems. Topical application is the most common and efficient method for diagnosing and managing various ocular disorders. However, the unique anatomy, physiology, and biochemistry of the eye create significant resistance to foreign substances. These physiological barriers reduce drug absorption and result in a brief duration of therapeutic effects in ophthalmic drug delivery systems. Noncompliance is often linked to the frequent need for eye drop instillation. Conventional ophthalmic formulations, such as solutions, suspensions, and ointments, present several drawbacks, including inconsistent efficacy, blurred vision, and increased pre-corneal elimination, leading to poor drug bioavailability within the ocular cavity. The protective mechanisms of the eye pose a considerable challenge for formulation scientists, who must find ways to bypass these barriers without causing tissue damage.

The eye employs various defenses, such as blinking, baseline and reflex lachrymation, drainage mechanisms, and tear fluid with anti-infective components like lysozymes and immunoglobulins. These natural defenses contribute to the rapid and extensive loss of topically applied drugs from the eye's surface. Additionally, the nasolacrimal system facilitates the drainage of tear fluid. These protective measures are primarily responsible for the significant pre-corneal drug loss. The primary goal of ocular drug delivery systems is to achieve the optimal drug concentration at the target site for an adequate duration. Factors such as the physicochemical properties of the therapeutic agent and the

anatomical and physiological characteristics of the eye determine its disposition and elimination.

Importance of In-Situ Gelling Systems:

- The Sol-Gel transition exhibited by in-situ gels ensures controlled and sustained drug release after administration.
- Sustained drug release leads to reduced frequency of drug administration, enhancing convenience for patients.
- In-situ gels enable accurate dosing and controlled drug release, preventing drug accumulation and minimizing side effects.
- They significantly improve drug bioavailability while allowing for dose reduction.
- Gel formation increases drug residence time and enhances contact between the drug and tissue, optimizing therapeutic efficacy.
- Compared to pre-formed gels, in-situ gelling systems deliver precise doses at regular intervals.
- Their physical properties make them easier to administer, thereby enhancing patient comfort and compliance.

MATERIALS AND METHODS:

Preformulation Study:

Preformulation research serves as the foundational step in developing drug dosage forms. It is an exploratory phase conducted early in drug development, focusing on the analysis of the physical and chemical characteristics of the drug substance, both independently and in combination with excipients. The purpose of preformulation studies is to evaluate the compatibility of initial excipients with the active pharmaceutical ingredient (API), supporting experimental formulations through biopharmaceutical, physicochemical, and analytical investigations. Data from these studies provide essential insights for subsequent formulation efforts.

Selection of Drug and Polymer:

Natural polymers are often preferred over synthetic ones for preparing in situ gels due to their accessibility and compatibility. Examples include Sodium Alginate and Gellan Gum. Sodium alginate is a polysaccharide composed of D-mannuronic acid and L-glucuronic acid units linked via 1,4-glycosidic bonds. The arrangement and number of blocks in the alginate molecule vary depending on the algal source. In the presence of di- and trivalent metal ions, dilute aqueous alginate solutions form strong gels due to consecutive glucuronic residues in the L-glucuronic acid blocks. Sodium alginate also exhibits favorable viscosity-enhancing properties. HPMC K4M grade serves as an effective polymer for viscosity improvement in gel formulations.

Drug Properties Investigated:

Authentication of Drug (Pinobanksin):

I. Organoleptic Properties:

- Appearance: Observed visually.
- Color: A small quantity was viewed under appropriate lighting conditions.
- Odor: A minimal sample was used for olfactory assessment.

II. Melting Point Determination:

The melting point was determined using the capillary method with a Thiele tube apparatus. A drug-filled capillary tube was sealed, tied to a thermometer, and submerged in liquid paraffin within the Thiele tube. Heat was applied, and the temperature at which the drug melted was recorded.

III. Authentication of Excipients:

- Appearance: Observed visually.
- Color: Examined under adequate illumination.
- Odor: A small sample was assessed.

Compatibility Study:

Compatibility studies were conducted to ensure the drug's stability with selected excipients under experimental conditions. FTIR analysis was used to evaluate interactions between the drug and excipients.

FTIR Analysis:

A dry sample of the drug and a polymer blend was applied directly to the transparent surface of the FTIR instrument. The IR spectrum was recorded over 24 cycles in the range of 4000–400 cm^{-1} using a diffuse reflectance method.

DSC Study:

Differential Scanning Calorimetry (DSC) analyzed the heat required to increase the temperature of a drug sample compared to a reference sample, providing thermal characteristics of the drug.

Preparation of Standard Curves:

I. Calibration Curve in Phosphate Buffer (pH 7.4):

A stock solution of 100 µg/ml was prepared by dissolving 10 mg of the drug in 100 ml of phosphate buffer. Aliquots of 1–6 ml were diluted to 10 ml to achieve concentrations of 10–60 µg/ml. Absorbance was recorded at 296 nm using a UV-visible spectrophotometer.

II. Calibration Curve in Distilled Water:

A stock solution of 100 µg/ml was prepared by dissolving 10 mg of the drug in 100 ml of distilled water. Aliquots of 1–6 ml were diluted to 10 ml to produce concentrations of 10–60 µg/ml. Absorbance was recorded at 269 nm using a UV-visible spectrophotometer.

Development of Formulations:

Ion sensitive in situ ocular gel of Pinobanksin formulation development

Table 1: Development of Formulations

Sr. no	Name of Ingredients	F1.	F2.	F3.	F4.	F5.	F6.	F7.	F8.	F9.
1	Pinobanksin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	Sodium Alginate.	0.5	0.5	0.5	1.0	1.0	1.0	1.5	1.5	1.5
3	HPMC K4M	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75
4	Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
5	Deionized water q.s.to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation and Characterization:

Determination of Visual Appearance and Clarity:

Clarity is one of the critical parameters for evaluating formulations. Visual inspection was conducted against both white and black backgrounds to identify the presence of any particulate matter.

pH Determination:

The pH of the nasal formulation is a key consideration to ensure stability and patient comfort. The formulation's pH should remain stable without causing irritation. Ocular formulations typically require a pH range of 5 to 7.4. A digital pH meter was used to determine the pH of the prepared formulations.

Drug Content:

To analyze drug content, 10 mg of the formulation was diluted with water. The drug concentration was measured at 296 nm using a UV-visible spectrophotometer after appropriate dilutions, and the drug concentration was calculated.

Determination of Gelling Capacity:

The gelling capacity was assessed by observing formulation behavior, including gelling time and erosion time, under varying environmental conditions:

- **(+)**: Gelled within minutes but dissolved quickly.
- **(++)**: Gelled within minutes and remained intact for a few hours.
- **(+++)**: Gelled immediately and remained stable for an extended period.

Viscosity of Formulation in Solution and Gel States:

The viscosity was measured using a Brookfield viscometer with spindle number 7, operating at 50 rpm. Initially, the viscosity of the gel solution was recorded. The solution was then allowed to gelify by increasing the sodium alginate concentration using a water bath, and the viscosity of the gel was measured.

In Vitro Drug Release Studies:

Diffusion studies were conducted using a Franz diffusion cell with a dialysis membrane separating donor and receptor compartments. The gel was evenly applied to the membrane, and the receptor compartment was filled with phosphate buffer saline (pH 7.4) maintained at 37°C. Samples of 1 mL were taken at specified intervals and replaced with fresh buffer. Drug release was analyzed spectrophotometrically at 296 nm, and the release rate was determined by plotting the amount of drug permeated against the square root of time.

Stability Studies:

The in situ gelling formulations of Pinobanksin were stored in aluminum foil-sealed glass vials at 25°C for two months. Samples were withdrawn after 15, 30, 45, and 60 days, and parameters such as appearance, pH, gelling capacity, drug content, and in vitro release were assessed.

RESULTS AND DISCUSSION

Characterization of Pinobanksin:

Organoleptic properties:

Table 2: Organoleptic Properties of Pinobanksin

Sr. No.	Parameter	Reported	Observed	Conclusion
1	Appearance	Crystalline	Crystalline	Complies with standard
2	Color	White	White	
3	Odor	Odorless	Odorless	

Melting point Pinobanksin:

Table 3: Melting point of Pinobanksin

Sample	Reported	Observed	Conclusion
Ketorolac Tromethamine	165-167 ℃	167 ℃	Complies with standard

Authentication of polymer:
Sodium Alginate:

a. Organoleptic properties:

Table 4: Organoleptic properties of Sodium Alginate

Sr.no.	Parameter	Reported	Observed	Conclusion/Comment
1.	Nature	Crystalline	Crystalline	Complies with Standard
2.	Color	Yellowish	Yellowish	
3.	Odor	Odorless	Odorless	

b. Melting point determination:

The melting point of polymer was determined by digital melting point apparatus Using Capillary meter

Table 5: Melting Point of Sodium Alginate

Sample	Reported	Observed	Conclusion
Sodium Alginate	85-900 C	90 °C	Complies with standard

8.1.6 FT-IR spectroscopy study:

The IR spectra of pure drug, excipients and mixture was obtained by using FTIR spectrophotometer.

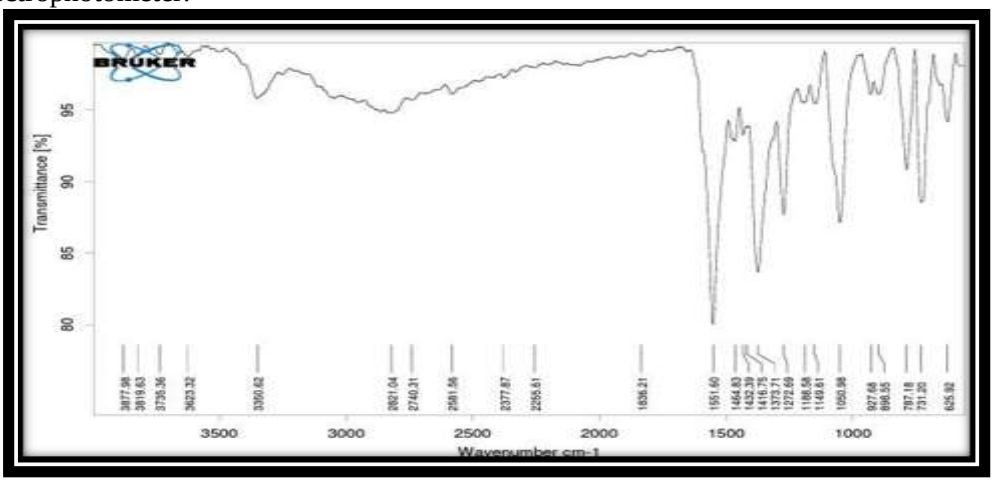

Figure 1: IR spectra of pure drug

Compatibility study:

The compatibility study determines stability of drug with excipients under experimental conditions.

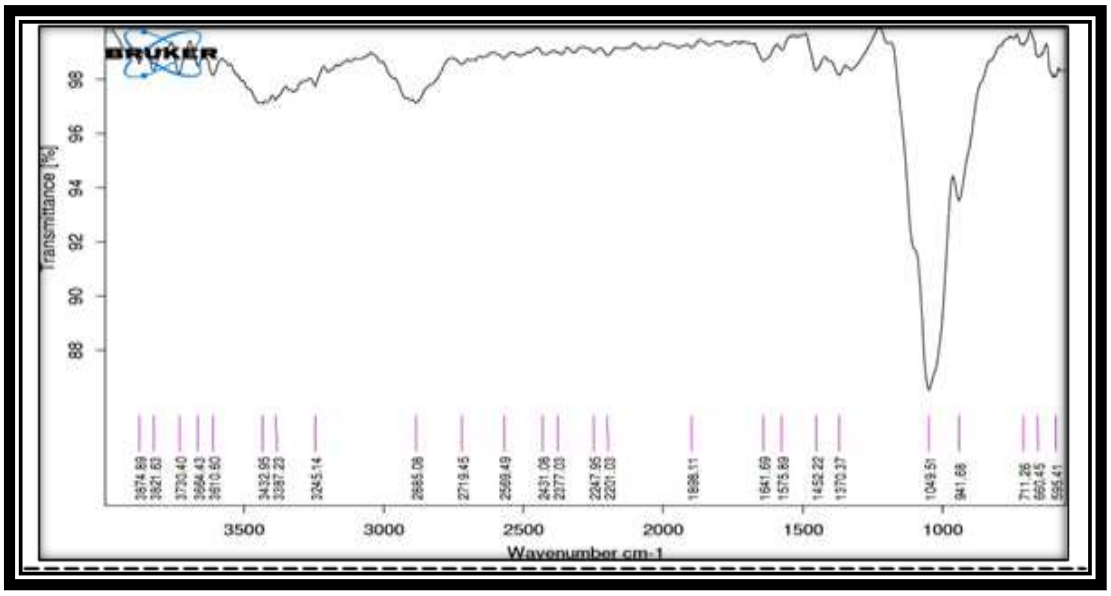

Figure 2: Compatibility study

DSC study:

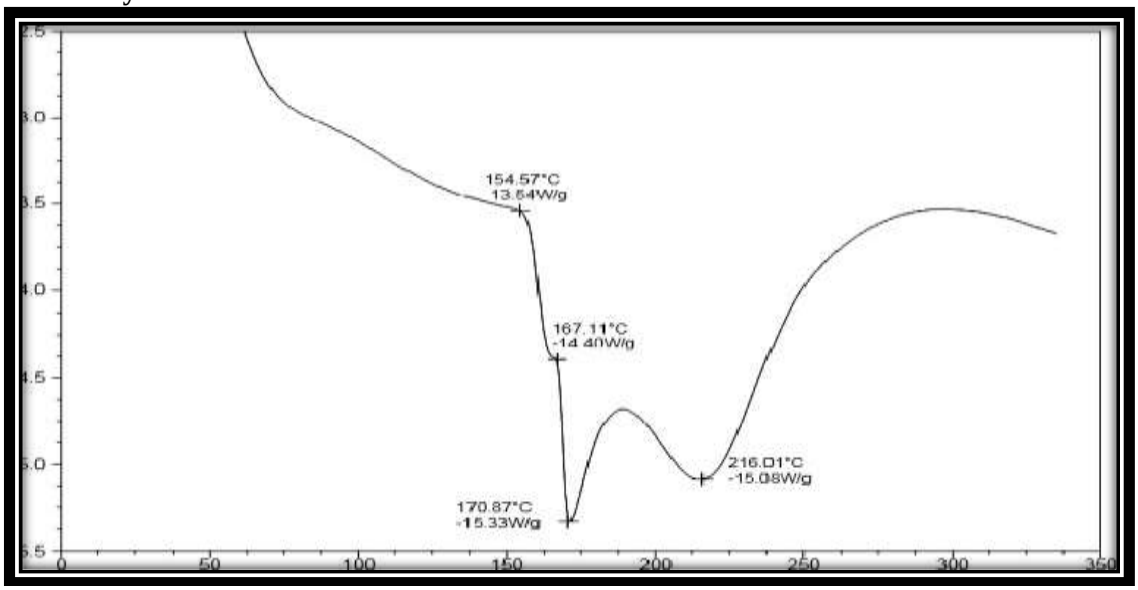

Figure 3: DSC thermogram of the pure drug

Evaluation of in Situ Gel:

Determination of visual appearance and clarity:

Table 6: Determination of visual appearance and clarity

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance	++	++	++	++	++	++	++	++	++

The batches F1 to F9 shows that no particulate matters in formulation

Table 7: Particulate matters in formulation

Sr.no	Formulation	Appearance of solution	Appearance of gel
1	F1	Transparent	Transparent
2	F2	Transparent	Transparent
3	F3	Transparent	Transparent
4	F4	Transparent	Transparent
5	F5	Transparent	Transparent
6	F6	Transparent	Transparent
7	F7	Transparent	Transparent
8	F8	Transparent	Transparent
9	F9	Transparent	Transparent

Determination pH of gel: pH of the all formulation was measured by using digital pH meter at room temperature.

Table 8: Determination pH of gel

Batches	F1.	F2.	F3.	F4.	F5.	F6.	F7.	F8.	F9.
pH	6.92	7.2	7.1	7.21	7.32	7.13	7.3	7.2	7.21

Drug content:

Table 9: Drug content of in situ gel

Batches	F1.	F2.	F3.	F4.	F5.	F6.	F7.	F8.	F9.
Drug content%	93.3	94.5	92.6	95.5	96.2	96.28	97.28	97.28	97.40

A spectrophotometer was used to determine the amount of Pinobanksin in each batch.

Gelling Capacity:

+- Gelled in a few minutes and dissolves quickly (within minutes),
++- Gelled in a few minutes and remains intact for a few hours,
+++- Immediately gelled and remained intact for an extended period of time.

Table 10: Gelling Capacity of in situ gel

Formulations	F1.	F2.	F3.	F4.	F5.	F6.	F7.	F8.	F9.
Gelling capacity	++	++	++	++	++	++	++	++	++

Viscosity determination:

Table 11: Viscosity before gelling and after gelling determination

Formulation	**Before gelling Viscosity(Pa s)**	**After gelling Viscosity(Pa s)**
F1	55.33	63.63
F2	103.30	110.83
F3	148.3	168.49
F4	150.3	169.45
F5	160.3	172.3
F6	166.2	174.3
F7	168.4	176.3
F8	170.2	178.6
F9	172.3	182.2

The viscosity measurements were performed with a Brookfield viscometer model LVDV-E model.

In vitro permeation study:

Results obtained are show in following table

Table 12: In vitro drug release study

Time	**% Cumulative Drug Release**								
0	0	0	0	0	0	0	0	0	0
5	17.88	22	17.82	18.70	17.94	18.05	18.17	26.29	17.70
15	27.65	23.70	36.06	28.74	26.36	25.60	31.49	31.81	32.06
30	35.26	30.08	38.48	36.51	31.56	39.01	39.25	39.57	40.43
60	42.07	31.39	40.22	47.78	37.52	45.85	48.44	48.77	49.08
120	48.04	37.94	48.45	51.62	55.32	54.15	51.54	51.86	52.20
180	54.95	51.30	49.90	54.95	57.71	56.17	54.04	55.60	57.14

240	58.42	56.43	53.39	57.76	60.47	60.56	57.74	61.64	62.08
300	62.77	59.51	60.18	61.19	63.02	62.33	60.57	66.73	66.84
360	64.75	60.29	62.94	64.32	65.36	65.45	63.62	70.24	71.29
420	64.17	64.25	65.56	66.26	69.76	69.60	70.41	73.74	72.13

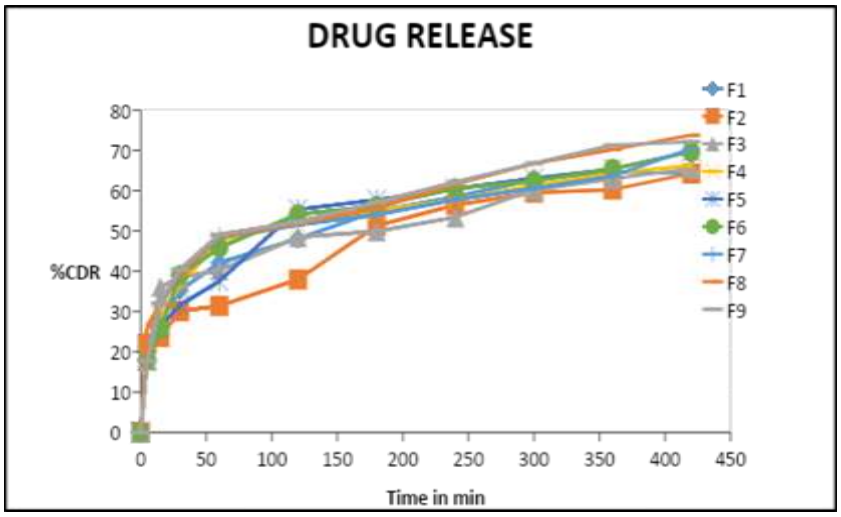

Figure 4: Drug release

Stability: Study of Optimized Batch Stability

The results of the optimized batch stored at room temperature are shown below

Table 13: Stability study of Optimized Batch

Sr. No	Time (day)	Appearance	pH	Gelation Capacity	Drug content (%)	In vitro release (%)
1	15	Transparent	7.3	++	98.35	68.84
2	30	Transparent	7.4	++	98.25	67.23
3	45	Transparent	7.4	++	97.28	66.02
4	60	Cloudy	6.2	++	96.78	65.45

CONCLUSION:

The ion-sensitive ophthalmic in situ gelling drug delivery system was successfully developed using Pinobanksin along with sodium alginate and HPMC K4M as polymers. The system was thoroughly characterized for its appearance, clarity, pH, gelation temperature, gelation capacity after dilution with STF, rheological properties, in vitro release in simulated tear fluid, and ex vivo diffusion studies using goat cornea. The ex vivo drug release closely matched the results obtained from in vitro studies conducted in simulated tear fluid. At room temperature, the formulation remained in liquid form and transformed into a gel rapidly upon contact with tear fluid.

The results demonstrated that the combination of sodium alginate and HPMC K4M as viscosity-enhancing agents produced optimal outcomes at concentrations optimized through the DESIGN EXPERT tool. It was concluded that increasing the concentrations of these polymers contributed to sustained drug release. Stability testing revealed that the Pinobanksin in situ gel maintained good stability at room temperature (25°C), with no observable physical changes during storage. Evaluations of viscosity, pH, drug content, and gelation temperature were conducted at 15-day intervals over a period of 60 days, and no significant changes were recorded at 25°C.

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REFERENCES:

1. Abaram S.,Furtados., Bharti S.,Basavaraj B.V., Devenshwarn R. AND Mahadevan V: Sustained Ophthalmic Delivery of ofloxacin from an Ion activated Insitu Gelling System, Pak. J. Pharm. Sci., 2009: Vol 22(2).175-179.

2. Azmat Shaikh , Talat Farheen, Sadhana Shahi; Formulation Development And Evaluation Of Ph Triggered In Situ Ophthalmic Gel Of Epinastine Hydrochloride; IJPSR, 2016; Vol. 7(3): 1131-1138.

3. Bochot A, Fattal E. Liposomes for intravitreal drug delivery: a state of the art. J Control Release. 2012;161:628–634. doi: 10.1016/j.jconrel.2012.01.019.

4. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems--recent advances. Prog Retin Eye Res. 1998;17:33–58. doi: 10.1016/S1350-9462(97)00002-5.

5. Burgalassi S, Chetoni P, Monti D, Saettone MF. Cytotoxicity of potential ocular permeation enhancers evaluated on rabbit and human corneal epithelial cell lines. Toxicol Lett. 2001;122:1–8. doi: 10.1016/S0378-4274(01)00261-2.

6. C. Vijaya, K.S. Goud, Ion-activated in situ gelling ophthalmic delivery systems of azithromycin, Indian J. Pharm. Sci. 73 (6) (2011) 615–620, http://dx.doi.org/ 10.4103/0250-474X.100234 23112394.

7. Carlfors J, Edsman K, Petersson R, Jörnving K. Rheological evaluation of Gelrite® in situ gels for ophthalmic use. European journal of pharmaceutical sciences. 1998 Apr 1;6(2):113-9.

8. Cholkar K, Patel SP, Vadlapudi AD, Mitra AK. Novel strategies for anterior segment ocular drug delivery. J Ocul Pharmacol Ther. 2013;29:106–123. doi: 10.1089/jop.2012.0200.

9. Cohen S, Lobel E, Trevgoda A, Peled Y. A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. Journal of controlled release. 1997 Feb 17;44(2-3):201-8.

10. Crescenzi V, Dentini M, Coviello T. Solution and gelling properties of microbial polysaccharides of industrial interest: the case of gellan. InNovel biodegradable microbial polymers 1990 (pp. 277-284). Springer, Dordrecht.

11. Eaga Chandra Mohan, Jagan Mohan Kandukuri, Venkatesham Allenki; Preparation and Evaluation of In-Situ-Gels for Ocular Drug Delivery; Journal of Pharmacy Research 2009, 2(6),1089-1094.

12. Edsman K, Carlfors J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. European journal of pharmaceutical sciences. 1998 Apr 1;6(2):105-12.

13. Eguchi H, Shiota H, Oguro S, Kasama T. The inhibitory effect of vancomycin ointment on the manifestation of MRSA keratitis in rabbits. J Infect Chemother. 2009;15:279–283. doi: 10.1007/s10156-009-0708-6.

14. Fukuda M, Hanazome I, Sasaki K. The intraocular dynamics of vancomycin hydrochloride ophthalmic ointment (TN-011) in rabbits. J Infect Chemother. 2003;9:93–96. doi: 10.1007/s10156-002-0219-1.

15. Gallarate M, Chirio D, Bussano R, Peira E, Battaglia L, Baratta F, Trotta M. Development of O/W nanoemulsions for ophthalmic administration of timolol. Int J Pharm. 2013;440:126–134. doi: 10.1016/j.ijpharm.2012.10.015.

16. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. AAPS J. 2010;12:348–360. doi: 10.1208/s12248-010-9183-3.

17. Gaudana R, Jwala J, Boddu SH, Mitra AK. Recent perspectives in ocular drug delivery. Pharm Res. 2009;26:1197–1216. doi: 10.1007/s11095-008-9694-0.

18. Gebhardt BM, Varnell ED, Kaufman HE. Cyclosporine in collagen particles: corneal penetration and suppression of allograft rejection. J Ocul Pharmacol Ther. 1995;11:509–517. doi: 10.1089/jop.1995.11.509.

19. Grasdalen H, Smidsrød O. Gelation of gellan gum. Carbohydrate Polymers. 1987 Jan 1;7(5):371-93.

20. Gulsen D, Chauhan A. Ophthalmic drug delivery through contact lenses. Invest Ophthalmol Vis Sci. 2004;45:2342–2347. doi: 10.1167/iovs.03-0959.

21. Gunda S, Hariharan S, Mitra AK. Corneal absorption and anterior chamber pharmacokinetics of dipeptide monoester prodrugs of ganciclovir (GCV): in vivo comparative evaluation of these prodrugs with Val-GCV and GCV in rabbits. J Ocul Pharmacol Ther. 2006;22:465–476. doi: 10.1089/jop.2006.22.465.

22. Gupta H., Aqil M., KharR.K., Ali A., Bhatnagar A., Mittal G., An alternative in situ gel-formulation of levofloxacin eye drops for prolong ocular retention., J Pharm Bioallied Sci 2015:Vol 7 (1) 9-14.

23. Gupta H., Velpandian T., and Jain S., Ion- and pH-activated novel in-situ gel system for sustained ocular drug delivery, Journal of Drug Targeting, 2010: Vol 18(7): 499–505.

24. Gupta H.,Jain S., Mathur R., Mishra P., Mishra A.K.&VelpandianT., Sustained Ocular Drug Delivery from a Temperature and pH Triggered Novel in Situ Gel System, Drug Delivery, 2007: Vol 14(8), 507-515.

25. Gurny R, Ibrahim H, Buri P. The development and use of in situ formed gels triggered by pH-in biopharmaceutics of ocular drug delivery. Edman, P., Crs Press199381-90. 1993.

26. Gurny R, Ibrahim H, Buri P. The development and use of in situ formed gels, triggered by pH. CRC press; 2019 Aug 15

27. Hornof MD, Bernkop-Schnürch A. In vitro evaluation of the permeation enhancing effect of polycarbophil-cysteine conjugates on the cornea of rabbits. J Pharm Sci. 2002;91:2588–2592. doi: 10.1002/jps.10258.

28. Jain S, Jain P, Mishra A, Pathak A. A Review on Triggered Gel for Ocular Drug Delivery.

29. Kawasaki N, Ohkura R, Miyazaki S, Uno Y, Sugimoto S, Attwood D. Thermally reversible xyloglucan gels as vehicles for oral drug delivery. International journal of pharmaceutics. 1999 Apr 30;181(2):227-34.

30. Kim SH, Lutz RJ, Wang NS, Robinson MR. Transport barriers in transscleral drug delivery for retinal diseases. Ophthalmic Res. 2007;39:244–254. doi: 10.1159/000108117.