JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON OPHTHALMIC IN SITU GEL – NOVEL TRENDS

[1]Ms. Mali S.N. [2] Dr. Chaus W.N., [3] Ms.Savalsure S.M. , [4] Ms. Ambad S.S.,
[1,3,4]Lecturer, Dayanand Institute of Pharmacy, Latur
[2] Principal, Dayanand Institute of Pharmacy, Latur

Abstract

The field of ocular drug delivery is one of the most challenging and interesting field for pharmaceutical scientist. In ophthalmic in situ gels various polymers are used. Generally hydrogels are used. These polymers will increase solution viscosity the field of ocular drug delivery is one of the most challenging and interesting field for pharmaceutical scientist. From last 10 to 20 years the field has been significantly improved. The ocular drug delivery system is considered as crucial and challenging as the delivery of drug is quite difficult.Moreover, the conventional ophthalmic formulations exhibit a short pre-corneal residence time and poor bioavailability. To overcome these problems newer drug delivery system like In situ gel has been developed. The 'in situ gel' system has emerged as one of the best novel drug delivery systems; it helps for the sustained and controlled release of the drugs by its special characteristic feature of 'Sol to Gel' transition. Various biodegradable polymers like carbopol,pluronics, alginate, gelrite etc. are used. In situ gelling system is a convenient, easy to administer and has better patient compliance. Formulation were evaluated for physical parameters like clarity, pH, drug content, gelation, sterility test, ocular irritancy study, in vitro drug release and rheological studies.

Key Words: In situ gel, Polymer, Ophthalmic Drug Delivery , Drug release

Introduction

Drug delivery into the ocular region by using conventional delivery systems like drops and ointment requires frequent dosing the reason being that when they are introduced in the precorneal region they are quickly washed away due to the lachrymal nasal drainage and tear flow. Further, the efficacy of the administered drug is hindered by the barrier system of the eye making it nearly impossible for drugs to easily penetrate the eye .A very small fraction of drug gets access to the active site i.e., approximately less than 1%of total drug instilled, this is why ocular delivery of the drug is a major challenging endeavour faced by the pharmaceutical scientist today .Ophthalmic drug delivery is a most challenging and interesting area for

upcoming pharmacists and formulation chemists due to its unique anatomy and physiology. which consists of three layers namely: Epithelium, Stroma, and Endothelium.Epithelium is outer layer of eye which acts as a barrier for hydrophilic drug, while lipophilic drugs facing difficulties to cross the stroma. Endothelium layer is lipoidal in nature. There are about 70% conventional dosage forms are available in the market.Ophthalmic solutions have poor bioavailability and therapeutic response, because of high tear fluid turnover and dynamics that cause rapid precorneal elimination of the drug. A high frequency of ophthalmic solutions instillation is main cause of patient non-compliance. Various ophthalmic vehicles such as inserts, ointments, Suspensions, and aqueous gels, have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts.

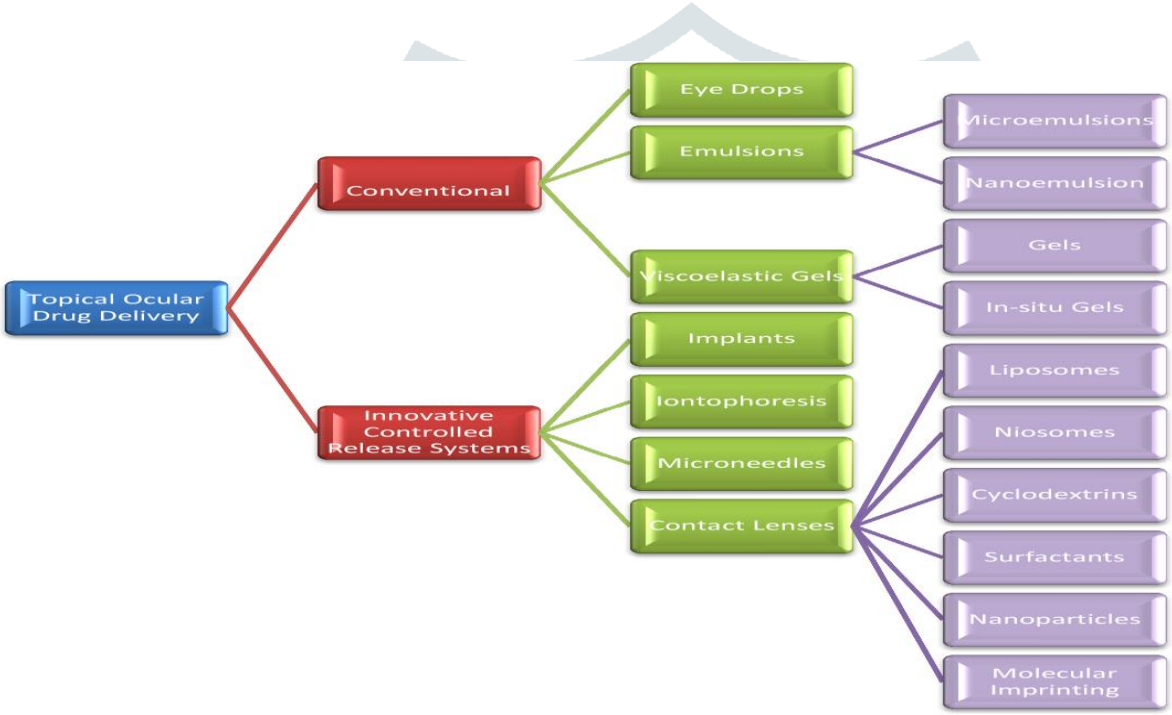

Fig: 1 – Classification of Ophthalmic Dosage Form

The following characteristics are required to optimize ocular drug delivery systems -

- A good corneal penetration.
- A prolonged contact time with corneal tissue.
- Simplicity of installation for the patient.
- A non-irritative and comfortable form (the viscous solution should not provoke lachrymation and reflex blinking).
- Appropriate rheological properties and concentration of viscolyzer.

Disadvantages of Conventional Ophthalmic Delivery Systems -

- Poor ocular bioavailability
- Poor therapeutic response
- Rapid precorneal elimination of the drug
- High frequency of administration
- Patient non-compliance
- Blurred vision
- Nasolacrimal drainage of the drug
- Irritation to the eye
- Cellular damage eat the ocular surface
- Toxic side effects.

Limitation of Conventional ophthalmic drug delivery system

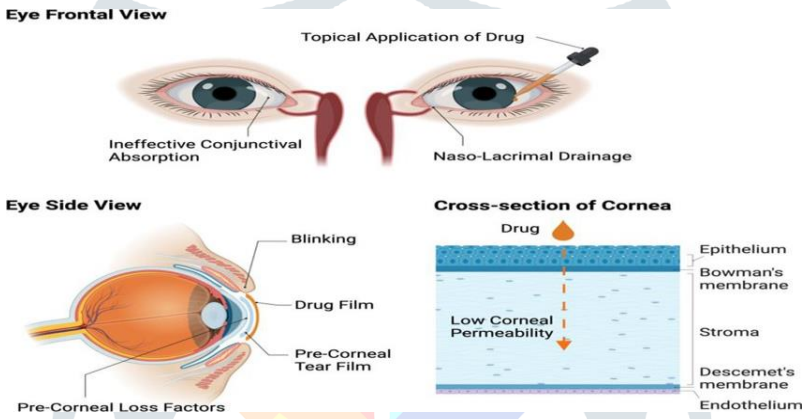

Fig: 2 – Limitation Conventional Ophthalmic Drug Delivery System

Ophthalmic In-Situ Gelling System

A more desirable dosage form would be one that can deliver drug in a solution form, create little to no problem of vision and need be dosed no more frequently than once or twice daily. In situ activated gel forming systems are those which are when exposed to physiological conditions will shift to a gel phase. This new concept of producing a gel in situ was suggested for the first time in the early 1980s. Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking). In situ gel-forming systems can be described as low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment. The rate of in situ gel formation is important because between instillation in the eye and before a strong gel is formed, the solution or weak gel is produced by the fluid mechanism of the eye. Both natural and synthetic polymers can be used for the production of in situ gels.

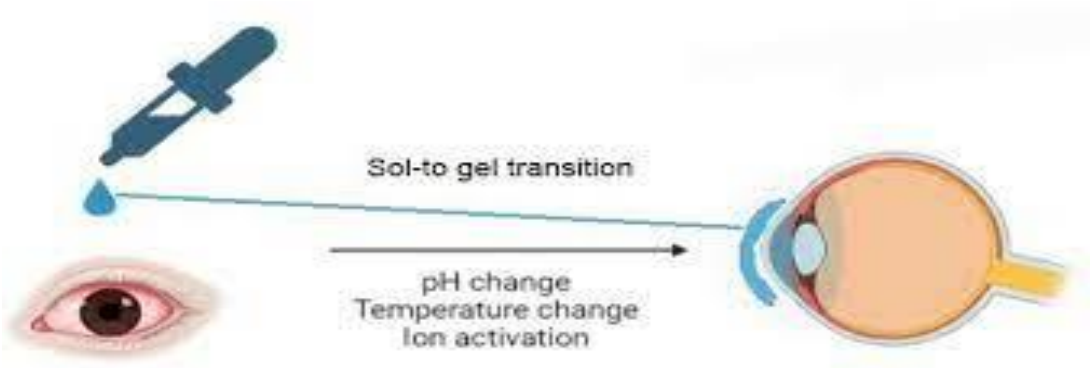

Fig: 3 – Mechanism of Sol-To-Gel Transition

Advantages of ophthalmic In Situ forming gel.

1. By increasing the precorneal residence time increases the ocular bioavailability
2. Provide better fit and housing of the delivery system
3. Due to the decreased loss of the active drug from the ocular region increases accurate dosing.
4. Provides prolong, sustained and ultimately controlled drug delivery
5. Due to ease to administer and increased comfort, provides better patient compliance.
6. Due to increased precorneal residence time, dosing frequency is significantly decreased.
7. Absorption of the drug or trans-barrier (protective barriers of the eye) permeation is enhanced. Provides targeted drug delivery in the ocular region and prevents the loss of drug to other ocular tissues
8. Less blurred vision as compared to ointment.
9. Decreased nasolacrimal drainage of the drug which may causes undesirable side effects due to systemic absorption (i.e. reduced systemic side effects).
10. The possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention.
11. Generally more comfortable than insoluble or soluble insertion.
12. Increased bioavailability due to increased precorneal residence time and absorption.
13. Avoidance of hepatic first pass.

Various approaches of In-situ gelation:

Depending upon the method employed to cause sol to gel phase transition on the ocular surface, the following types of systems are recognized.

1.pH-sensitive systems: Polyacrlic acid (Carbopol 940) is used as the gelling agent in combination with hydroxy propyl-methylcellulose (Methocel E50LV) which acted as a viscosity enhancing agent. The formulation with pH-triggered in-situ gel is therapeutically efficacious, stable, non-irritant and provided

sustained release of the drug for longer period of time than conventional eye drops. Another example cellulose acetate phthalate (CAP) is a polymer undergoing coagulation when the original pH of the solution (4.5) is raised to 7.4 by the tear fluid.

2. Temperature sensitive system: The system is designed to use Poloxamer as a vehicle for ophthalmic drug delivery using in-situ gel formation property. Thegelation temperature of graft copolymers can be determined by measuring the temperature at which immobility of the meniscus in each solution was first noted. The bioadhesive and thermally gelling of these graft copolymers expected to be an excellent drug carrier for the prolonged delivery to surface of the eye. Other example of Poloxamer-407 (a polyoxyethylene polyoxypropylene block copolymer) is a polymer with a solution viscosity that increases when its temperature is raised to the eye temperature.

3. Ion-sensitive systems (osmotically induced gelation): In this polymer may undergo phase transition in presence of various ions. Gellan gum commercially available as gelrite is an anionic polysaccharide Ca2+, Mg2+, k+ and Na+. Formulation undergo liquid- gel transition under influence of an increase in ionic strength and gel formation take place because of complexation with polyvalent cations in lacrimal fluid. Example: gelrite, gellan, hyaluronic acid, alginates.

Ideal Characteristics of Polymers

- The polymer should be capable of adhering to the mucous membrane.
- It should be well compatible and should not
- provide any toxic effects.
- It should have good tolerance.
- It should be biocompatible.
- It should have pseudo plastic behaviour. .
- Polymer should be capable of decreasing viscosity with increasing shear rate there by lowering viscosity during blinking eye.

Some of the most important polymers used as in-situ gelling agents are described in table.

Table No. 1 Polymers Used As In-Situ Gelling Agents

Polymer	Mechanism	Properties
In temperature sensitive in situ gelling system		
1.POLOXAMER/ PLURONICS	At room temperature (25 ^{O}C), it behaves as viscous liquid and is transformed to	Poloxamers or pluronic are the series of commercially available difunctional triblock

	transparent gel when temperature increases (37^0C). At low temperature, it forms small micellar subunit in solution and increase in temperature results increase in viscosity leads to swelling to form large micellar cross linked network.	copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide. The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms.
2.CELLULOSE DERIVATIVES (Methyl Cellulose, Hydroxy Propyl Methyl Cellulose, Ethyl Hydroxy Ethyl Cellulose)	Gelation of cellulose solution is caused by hydrophobic interactions between molecules containing methoxy substitution. At low temperature, molecules are hydrated and little polymerpolymer interaction occurs, whereas at high temperature, polymers lose their water of hydration .	Cellulose is a linear homopolymer polysaccharide consisting of D anhydroglucopyranose units joined together by β-1,4-glycosidic bonds. Extensive intramolecular and intermolecular hydrogen bonding present in cellulose renders it insoluble in water. Various cellulose ethers (CEs) have been prepared by etherification of the three hydroxyl groups on anhydroglucose units of cellulose producing water-soluble derivatives.
PH sensitive in situ gelling system		
1. CARBOPOL	At specific pH there is Electrostatic, hydrophobic interaction and Hydrogen	Carbopol is the lightly crosslinked commercial form of Poly(acrylic acid), which

	bonding takes place, hence leads to interdiffusion. The observed phase transition for carbopol solution was mediated by the variation of pH from 4.0 to 7.4 and can be attributed to ionization of Carbopol polymer.	stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. As the concentration of carbopol increases, due to its acidic nature it causes irritation to the eye. Addition of viscosity enhancer like HPMC, MC will reduce the concentration without affecting its gelling property.
2. POLYCARBOPHILS	Polycarbophil is insoluble in water, but its high swelling capacity in a neutral medium permits the entanglement of the polymer chains with the mucus layer. The nonionized carboxylic acid groups of polycarbophil bind to the mucin by means of hydrogen bonds	Polycarbophil is also the lightly crosslinked commercial form of Poly(acrylic acid) exhibits stronger mucoadhesion same as Carbopol. As concentration increases, acidic nature may cause lacrimation, hence combination of polymers are used.
In ion sensitive in situ gelling system		
1.GELLAN GUM/GELRITE	Gellan gum produce a cation induced in situ gelation (Ca2+, Mg 2+, K+, Na+) due to the cross linking between negatively charged helices and mono or divalent cations (Na+, Ca+, Mg+) present in tear fluid.	Gellan gum is anionic heteropolysaccharide that is, tetrasaccharide repeat unit of 2 β-D-glucoses, 1 β-D-glucuronate, and 1 α-Lrhamnose. GelriteR is a low-acetyl Gellan gum, which forms a clear gel in the presence of mono- or divalent cations. It has the tendency of gelation which is temperature dependent or cations induced.

2. SODIUM ALGINATE	The monomers of alginate (βD-mannuronic acid (M) and α-L- glucuronic acid (G) are arranged as M-M block or G-G block with alternating sequence (M-G) block. Upon interaction of G block of polymer with calcium moieties in tear fluid, resulting in the formation of homogenous gel.	It consist of (1→ 4) linked β-D-mannuronic acid and α-L-guluronic acid. A prolonged precorneal residence of formulations containing alginic acid looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties.
3. XANTHAN GUM	The anionic character of this polymer is due to the presence of both glucuronicacid and pyruvic acid groups in the side chain which results in gel formation when comes in contact with (ions present in) tear fluid.	The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β-D glucoseresidues) and a trisaccharide side chain of β-D-mannose-β-D glucuronicacid -α-D mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain.

EVALUATION AND CHARACTERIZATION OF IN-SITU OPHTHALMIC GEL

1] Physical parameter.

The formulated In-situ solution is tested for clarity, pH, gelling capacity, appearance.

2] Viscosity.

Viscosity can be calculated by using Brookfield viscometer, cone and plate viscometer. The Insitu gel formulation was placed in sampler tube. The samples are analyzed both at room temperature at 25 °c and thermo stated at 37 °c ± 0.5 °c by a circulating bath connected to viscometer adaptor prior to each measurement.

3] Gelling Capacity:

Gelling capacity of prepared formulation is determined by placing the drop of formulation in vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for gelling was noted.

Composition of Simulated Tear Fluid.

Sodium bicarbonate: 0.20gram Sodium chloride: 0.67gram Calcium chloride dehydrate: 0.08gram De ionized water: 100ml

4] Isotonicity Evaluation.

Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation.

5] Drug content.

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 1 ml was withdrawn and further diluted to 10 ml with distilled water. Concentration was determined at 200-400nm by using UV visible spectroscopy.

6] In Vitro Drug Release Studies.

In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated tear fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 µm pore size). The whole assembly is placed on thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37 0c± 0.5 0c.

1ml sample is withdrawn at predetermined time interval of 1hr for 6hrs the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The

obtained data is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism for their kinetics.

By using dialysis tube.

This study is performed in the Dialysis tube containing 1 ml of the formulation, which is then suspended in beaker at 37 ± 0.50C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring at 20 RPM to stimulate the blinking effect. Dialysis membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid is mounted by tied and sandwiched between the donor and receiver compartment.

Aliquots of 1 ml withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples analyze by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.

7] Drug Polymer Interaction Study and Thermal Analysis.

Interaction study was performed with Fourier Transform Infra Red (FTIR) spectroscopy. During gelation process the nature of interacting forces can be evaluated using the technique by employing kBr pellet method. Thermo Gravimetric Analysis (TGA) can be conducted for in- situ forming polymeric system to quantitate the percentage of water in hydrogel. Differential Scanning Calorimetry (DSC) conducted to observe if there are any changes in thermograms as compared with pure active ingredients used for gelation .

8] Antibacterial Activity.

The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotics. To carry out microbiological assay serial dilution method is employed.

9] Accelerated stability studies.

Formulations are placed in ambient coloured vials and sealed with aluminium foil for a short terms accelerated stability study at 40 ±2°c and 75±5% RH as per International Conference on Harmonization (ICH) states guidelines. Samples are analyzed every month for clarity, pH, gelling ability, drug content etc.

10] Ocular Irritancy Studies.

For these studies generally male albino rabbits, having weight around 1-2 kg are selected. The modified Draize technique is used to determine ocular irritation potential of ophthalmic preparation. The in-situ gel preparation is incorporated in lower cul-de-sac & at time interval of 1hr, 2hrs, 48hrs, 72hrs, & 1 week after drug

administration irritancy will be tested. The albino rabbits are observed for swelling, redness & watering of eyes periodically.

CONCLUSION

In ophthalmology, many efforts have been made to develop a delivery system with prolonged residence time in the ocular region, which could ultimately result in the increased ocular bioavailability by making many changes and modifications in the product formulation and product content. In situ gelling system is novel and technically superior to existing technologies. Development of ophthalmic drug delivery system has proved to be beneficial as compared to the conventional drug delivery. Various natural, synthetic, semi synthetic polymers are being used by the pharmaceutical researchers for controlled release of drug. These polymers are very useful in the formulation of in-situ gel systems. The evaluation of in-situ gels can be carried out based on the parameters like gelling capacity, rheological studies, in-vitro drug release studies, drug-polymer interaction study, thermal analysis, antibacterial activity and ocular irritancy test.There is high scope for research work on in situ gel system in order to provide advanced techniques in drug delivery systems.

References

1. Al-Bazzaz FY, Al-Kotaji MY. Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. Int J App Pharm. 2018; 10(4):153-61. https://doi.org/10.22159/ijap.2018v10i4.26885 2. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An overview. World journal of pharmacology. 2013; 2(2):47. https://doi.org/10.5497/wjp.v2.i2.47

3. Savita Gambhire, Karuna Bhalerao, Sushma Singh, In-situ hydrogel: different approaches to ocular drug delivery, International Journal Of pharmacy and pharmaceutical sciences, 2013; 5(2).

4. Desi H.A, Bhalla,H.L.Preparation and Evaluation of new eye drops containing a combination of ciprofloxacin and dexamethasone,Indian drugs37(4),2000.

5. Keister JC, Cooper ER, Missel PJ, Lang JC, Hager DF. Limits on optimizing ocular drug delivery Journal of Pharmaceutical Sciences 80; 1991: 50-53.

6. Shyale S. Preparation and evaluation of ocular inserts containing norfloxacin. Turk J Med Sci 2004; 34;230 – 246.

7. Mali Mahesh N,Hajare Ashok A.In-situ gel forming systems for sustained ocular drug delivery system.European Industrial Pharmacy., 2010; 5: 17-20.

8. Patil Rajeshwari N,Kumar Rachana S.In-situ gelling system:Novel approach for ophthalmic drug delivery.World Journal of Pharmacy and Pharmaceutical Sciences., 2014; 3(7): 423-440.

9. Kumar JRK, Muralidharan S, Dhanaraj SA. (A Review : Polymeric In-situ Gel System). Research and Reviews : Journal of Pharmacy and Pharmaceutical Sciences, 2013; 2(1): 1–7.

10. Almeida H, Amaral MH, Lobão P, Lobo JM. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations. Drug discovery today. 2014 Apr 1; 19(4):400-12. https://doi.org/10.1016/j.drudis.2013.10.001

11. Agarwal K. In-situ gel formation for ocular drug delivery system an overview. Asian Journal of Biomedical and Pharmaceutical Sciences. 2011 Oct 1; 1(4)

12. Nerkar TS, Gujarathi NA, Rane BR, Bakliwal SR, Pawar SP. (In-Situ Gel: Novel Approach In Sustained And Controlled Drug Delivery System). Pharma Science Monitor, 2013; 4(4): 1-18.

13. Mitan R, Gokulgandhi Jolly R , Parikh ,Megha B, Dharmesh MM. A pH triggered in-situ gel forming ophthalmic drug delivery system for Tropicamide. Drug Deliv Technol 2007; 5; 44-49.

14.Sultana Y, Aqil M, Ali A, Zafar S. Evaluation of carbopol-methyl cellulose based sustained release ocular delivery system for pefloxacin mesylate using rabbit eye model. Pharm Dev Technol 2006; 11(3):313-9.

15. Srividya B, Cardoza RM, Amin PD, Sustained ophthalmic delivery of ofloxacin from a pH triggered in- situ gelling system. J Control Release. 2001; 73(2-3):205-11.

16. Katarina E, Johan C, Roger, P. Rheological evaluation of poloxamer as an in- situ gel for ophthalmic use. Eur J Pharm Sci 1998; 6: 105–112.

17. ElKamel AH, in vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. Int J Pharm 2002; 241(1):47-55.

18. Mitan,R, Gokulgandhi jolly R,Parikh,Megha B,Dharmesh MM;2007,5,44-49.

19. Sumedha Meshram, Surendra Kumar Jain, Nishiprakash Jain, formulation and evaluation of insitu ophthalmic gel of ketorolac tromethamine, World journal of pharmacy and pharmaceutical sciences, 2(3): 1370-1384.

20. Kakad V , Kumar R, Rupvate S, Nagare R, Madagul J. (Review On Polymers Used For In Situ Gel For Ophthalmic Drug Delivery System). International Journal of Pharmaceutical Research And BioScience, 2015; 4(3): 52-68.

21. Sonawane SD, Patil RY, Lad M. (A Review on polymers used in novel in situ gel formulation for ocular drug delivery and their evaluation). Journal of Biological and Scientific Opinion, 2013; 1(2): 13237.

22. Ludwig A. (The use of mucoadhesive polymers in ocular drug delivery). Advanced Drug Delivery Reviews, 2005; 57: 1595– 1639.

23. Sechoy O, Tissie G, Sebastian C, Maurin F, Driot JY, Trinquand C. A new long acting ophthalmic formulation of carteolol containing Alginic acid. Int J Pharm, 2000; 207: 109-16.

24. Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD et al. Novel injectable solution of chitosan form biodegradable gels in situ. Biomaterials, 2000; 21: 2155-61.

25. Nanjawade Basavraj K,Manvi FV,Manjappa AS.In situ-forming hydrogels for sustained ophthalmic drug delivery.Journal of Controlled Release., 2007; 122: 119-134

26. Rowe,Sheskey,Owen.Handbook of Pharmaceutical Excipients.Fifth edition.Published by the Pharmaceutical Press., 2006.

27. Nirmal H.B.*, Bakliwal S.R., Pawar S.P, In-Situ gel: New trends in Controlled and Sustained Drug Delivery System, International Journal of Pharm Tech Research, April-June 2010; 2(2): 1398-1408.

28. Gourav Rajoria, Arushi Gupta, In-situ gelling system: A novel approach for ocular drug delivery, American journal of pharmaceutical research, 2012; 2(4).

29. Rathore KS .In Situ Gelling Ophthalmic Drug Delivery System: An Overview International Journal of Pharmacy and Pharmaceutical Sciences, 2010.Vol 2, Suppl 4,30-34

30. Gupta A, Manocha N. Formulation and Evaluation of In-Situ Ophthalmic Drug Delivery System. International Journal of Pharmaceutical & Biological Archives 2012; 3(4):715- 718.

31. Mitan R, Gokulgandhi Jolly R , Parikh ,Megha B, Dharmesh MM. A pH triggered in-situ gel forming ophthalmic drug delivery system for Tropicamide. Drug Deliv Technol 2007; 5; 44-49.

32. Pandit D, Bharathi, A, Srinatha,Ridhirkar,Singh S. Long acting ophthalmic formulation of indomethacin : Evaluation of alginate gel system . Indian J Pharm Sci 2007; 69:37-40.

33. Sudam Nagargoje, Atul Phatak, Chandrashekhar Bhingare, Shilpa Chaudhari, Formulation and evaluation of ophthalmic delivery of fluconazole from ion activated in situ gelling system, Der Pharmacia Lettre, 2012, 4(4): 1228-1235.

34. Katrina E, Johan C, Roger P. Rheological evaluation of Poloxamer as an in situ gel for ophthalmic use. Euro J pharm Sci; 1998. 6:105-112.

35. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH triggered in situ forming opthalmic drug delivery system for tropicamide. Duug Deliv. Technol; 2007. 5:44-49

36. Sautou –Miranada V, Labret F, GrandBoyer A , Gellis C, Chopineau J. Impact of deep-freezing on the stability of 25mg/ml vancomycin ophthalmic solutions. Int J pharm 2002; 234:205-207.

37. Doijad RC, Manvi FV, Malleswara Rao VSN , Prajakta , Alsae. Sustained ophthalmic delivery of gatifloxacin from In-situ gelling system. Indian J pharma sci 2006; 8:814-818.

38. Draize J, Woodward G, Calvery O. Method for the study of irritation and toxicity of substance applied topically to the skin and Mucous Membrane. J Pharmacol exp ther, 1994; 82:377-390.

39. Gambhire Savita, Bhalerao Karuna, Singh Sushma. In-situ hydrogel: different approaches to ocular drug delivery. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(2): 27-36