PREPARATION AND EVALUATION OF DOMPERIDONE MICROPARTICLES USING NATURAL POLYMERS BY IONIC GELATION METHOD

B.V.Ramana, G.MEGHANA , , C S Parameswari S K Rubina, P Ershad Khan

ABSTRACT

Ocular bioavailability is always poor from conventional ophthalmic drops due to spillage and nasolachrymal drainage. Ocular in situ gels can increase the drug residence time thus increasing bioavailability. Purpose of current study is to prepare sustained release In situ ocular gels of Levofloxacin hemihydrate using gelrite as gel forming polymer, which is used in treatment of various bacterial infections. Formulations were evaluated for physical parameters like Clarity, pH, drug content, gelation, sterility test, rheological studies, *in vitro* drug release study and ocular irritancy studies, anti microbial efficiency study and stability studies. The formulated gels were transparent, uniform in consistency and had spreadability with a pH range of 7.1 to 7.4. Six different formulations with increasing polymer concentrations were prepared which was found to have drug content of 92-98%. From the preliminary studies it was observed that as the concentration of polymer was increased, the rate of drug release decreased to produce sustained drug delivery for prolonged period of more than 8 hours with a maximum of 90.2% drug release observed. Further *in vivo* results and stability studies conclude that it is be possible to formulate in situ ocular gel containing Levofloxacin hemihydrate.

Keywords: Gelrite, Rheological, ocular, gels, Bioavailability, Anti microbial, *In vitro*

1. INTRODUCTION

Eye is unique and vital organ. It is considered as window of the soul. We can enjoy and view the whole world only with this organ. There are many eye ailments which affect this organ and one can loss the eye sight. Therefore many ophthalmic drug delivery systems are available. These are classified as conventional and newer drug delivery systems. 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.

1.2 ABSORPTION AND BIOAVAILABILITY OF THE DRUGS FROM THE EYE

The drug solution instilled as eye drops into the ocular cavity may disappear from the precorneal area of the eye by any or a composite of the following routes.[5] shown in Fig.1.2.

1. Nasolacrimal drainage,
2. Tear turnover,
3. Productive corneal absorption,
4. Nonproductive Conjuctival uptake.

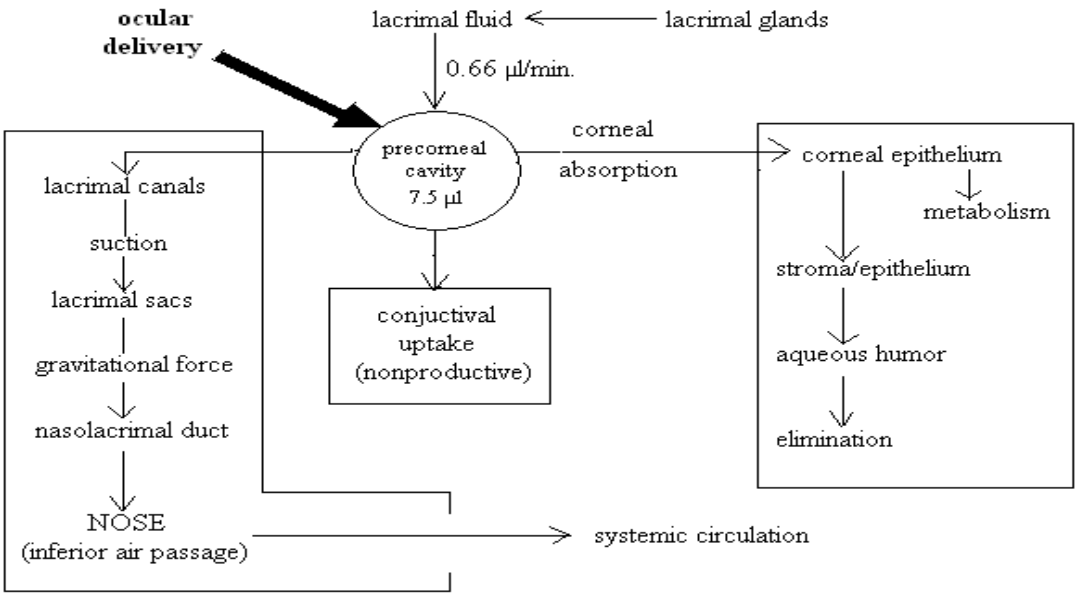

Fig.1.2 Routes of ocular absorption of drugs.

Drug administered by instillation must penetrate the eyes and do so primarily through the cornea. Corneal absorption is much more effective than scleral or conjuctival absorption, in which removal by blood vessels into the general circulation occurs.[3]

1.3 GENERAL BACTERIAL INFECTIONS OF EYE AND ITS MEDICATIONS

Common bacterial infections of eye, which are listed as below: [7]

- **Conjuctivitis**

It is a common superficial eye disorder and may be caused by infection with a wide range of bacteria, viruses, and less frequently fungi. Staphylococci or Streptococci commonly cause acute bacterial conjunctivitis in adults, and Haemophilusinfluenzae and Morexellacatarhallis particularly in children. Other causes of bacterial conjuctivitis include Gonococci and Chlamydia trachomatis.

Uncomplicated bacterial conjuctivitis may be self limiting but empirical treatment with topical antibacterial is often given.

- **Blepharitis**

It is an infection of the lid margins. It is usually present in a chronic condition and may require prolonged treatment, typically involving local hygiene to remove encrustations and topical application of a broad-spectrum antibacterial ointment.

- **Endophthalmitis**

A considerable amount of effort has been made in ophthalmic drug delivery since the 1970s.A number of approaches to the delivery of drugs for ocular treatment has been investigated and proposed. These range from simple systems such as aqueous suspensions where the viscosity, and hence the residence time, has been increased by cellulosic polymer to complex system such as penetration enhancers, external devices (collagen shields, Preformed gels iontophoresis, and pumps), ion-exchange resins, Liposomes, microspheres and micro particles, polymeric films, inserts, prodrugs, mucoadhesives and metabolism based drug design.[8]The commercially available antibacterial agents for ophthalmic use are as follows:

- **Ocular Inserts**

Ocular inserts, one of the new classes of drug delivery systems, which are gaining worldwide praise release drugs at a pre-programmed rate for a longer period by increasing the precorneal residence time.[10]The goal of this delivery system is to provide a therapeutic amount of drug to the ocular tissues to achieve promptly and then maintain the desired drug concentration by increasing the contact time between the preparation and the Conjuctival tissue.[11]To achieve this goal particularly for chronic diseases such as glaucoma, it would be advantageous and more convenient to

maintain a dosing frequency to once, or at most, twice a week regimen.[12]An appropriately designed extended release ocular insert can be a major advance in this direction compared to conventional immediate release dosage forms.

- **Collagen Shield**

Collagen is regarded as one of the most useful biomaterials. The excellent biocompatibility and safety is due to its biological characteristics, such as biodegradability and weak antigenicity, these properties made collagen the primary resource in medical applications. Collasomes show promise among drug delivery systems to the human eye. They are first fabricated from porcine scleral tissue, which bears a collagen composition similar to that of the human cornea. The shields are hydrated before they are placed on the eye. Typically the drug is located into the drug solution for a period of time prior to application. Shields are not individually fit for each patient, as are soft contact lenses and therefore, comfort may be problematic and expulsion of the shield may occur.

forming gels are formulations, applied as solutions, sols, orSuspensions, that undergo gelation after instillation due to physicochemical changes inherent to the eye .Currently two groups of hydrogels are distinguished (Fig.1.3), namely preformed and in situ forming gels. Preformed hydrogels can be defined as simple viscous solutions, which do not undergo any modifications after administration, while in situ forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the eye.

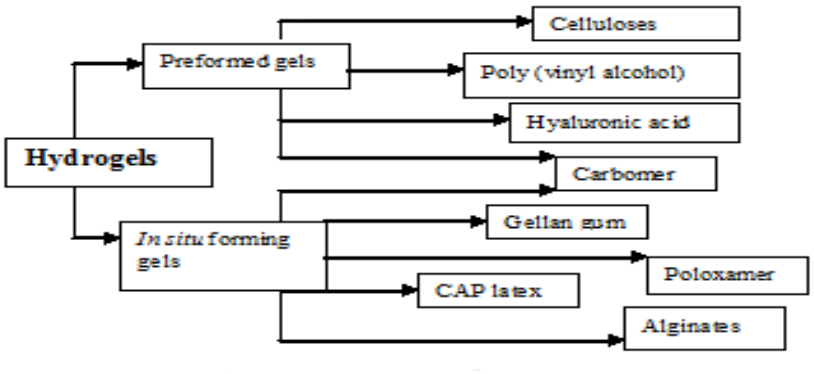

Fig.1.3 Classification of hydrogels.

❖ **In situ gel**

Distinguishing from preformed hydrogels, in situ forming gels are formulations, applied as a solution, which undergoes gelation after instillation due to physicochemical changes inherent to the biological fluids. In this way, the polymerswhich show sol-gel phase transition and thus triggerdrug release in response to external stimuli are the most investigated. In situ hydrogels are providing such 'sensor' properties and can undergo reversible sol-gel phase transitions upon changes in the environmental condition. These "intelligent" or "smart" polymers play important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released.[15]

A polymer used to prepare in situ gels should have following charteristics: [16]

- It should be biocompatible.
- It should be capable of adherence to mucus.
- It should have pseudo plastic behaviour.
- It should have good tolerance and optical clarity.
- It should influence the tear behaviour.

In vitro drug release from the formulations was studied by the diffusion process. Here the pH of the Lacrimal fluid and the blinking rate of the eye were taken into consideration and were simulated.

- **Procedure**

The *in vitro* release of Levofloxacin hemihydrate from the prepared formulations was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked over night in the receptor medium (Simulated Tear Fluid, pH 7.4). *In vitro* release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell). *In vitro* release of Levofloxacin hemihydrate was carried out in formulations with different concentrations of Gelrite using cellophane membrane. The diffusion medium 100ml of simulated tear fluid stirred at 50rpm at 37^0 C $\pm 0.5^0$ C. One end of the diffusion tube was covered by a cellophane membrane. The1 ml formulation were spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a UV spectrophotometer at 287.5nm using simulated tear fluid as blank.

❖ **Comparative Evaluation of Marketed Products with prepared in Situ Gels**[75]

Prepared in situ gelling systems and marketed products of eye drops were taken for the preliminary studies and *in vitro* release studies. The *in vitro* release of marketed products was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked over night in the receptor medium (Simulated Tear Fluid, pH 7.4). *In vitro* release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell).*In vitro* release of Levofloxacin hemihydrate was carried out in formulations with different concentrations of Gelrite

using cellophane membrane. The diffusion medium 100ml of simulated tear fluid stirred at 50rpm at 37^0 C $\pm 0.5^0$ C. One end of the diffusion tube was covered by a cellophane membrane. The 1ml formulation were spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a UV spectrophotometer at 287.5nm using simulated tear fluid as blank (Table 5.8-5.9 and Figures 5.8-5.9).

❖ **Pharmacokinetic Release Studies[6]**

All the optimized formulations were subjected to study the release kinetics and the best fit kinetic model was determined for the optimized formulations using analysis software PCP Disso V2. (Table5.9-5.14, Figure 5.9-5.13)

❖ **Antimicrobial Efficacy Studies[76]**

The Antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations. Staphylococcus Aureus, Pseudomonas Aeruginosa and E.coli were used as the test organisms. These were determined by Agar diffusion test employing Cup-Plate method. Sterile solutions of Levofloxacin hemihydrate (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured in to cups bored into sterile nutrient agar previously seeded with test organisms (Pseudomonas Aeruginosa, E.coli and Staphylococcus aureus),After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37^oC for 24hrs. The zone of inhibition (ZOI) measured around each cup and was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained the study (Table 5.15).

Ocular therapy could be significantly improved if the pre-corneal residence time of drugs could be increased; several new preparations have been developed for ophthalmic use not only to prolong the contact time of the vehicle at ocular surface, but also to slow down the elimination of the drugs.

Conventional ophthalmic solution dosage forms have advantage such as, ease of instillation and proper dosage administration. Beside ophthalmic ointments have the advantages of increased contact time. By utilizing these advantages of different dosage forms, the newer approach, in situ gelling system was developed. These gels exhibit a unique property of sol-to-gel transition when a change in their physicochemical property takes places. This type of novel ocular drug delivery can provide increased bioavailability by increasing residence time of gel formed and better patient compliance due to ease of administration.

The aim of the present work envisaged “Preparation and evaluation of in situ opthalmic gel of an anti infective drug for sustained ocular delivery” for the treatment of various bacterial diseases of eye, by providing comfortness, compliance to the patients and improved therapeutic performance of the drug over conventional ocular dosage forms.

Preparation of in situ gelling Systems

In the present work the in situ gelling systems were prepared by ion exchange and temperature dependent methods with the help of gelling agent Gelrite and Humectant propylene glycol.

Evaluation of in situ gelling systems

For the conformation of the intactness of the drug in formulations all formulations were subjected to IR study and compared to IR absorption spectra of pure drug. Studies reveal that there was no definite changes in bands were observed with respect to pure drug. So it was conformed that formulations did not have any drug polymer interactions.

Optimized in situ gels were subjected for preliminary evaluation such as, visual appearance, clarity, pH and drug content. All formulations were found transparent and clear, pH of the formulations was within 7.1 to 7.4 and drug content was found within 92-98% in all optimized in situ gelling systems.

Using Simulated Tear Fluid a simple Spectrophotometric method for estimation of Levofloxacin hemihydrate was developed. The absorption maxima by UV spectrophotometer were obtained at 287.5 nm in Beer's range of 2-12 μg/ml.

During blinking the shear rate on the preparation is large. If the viscosity is too high, this will result in irritation. On the other hand, if the viscosity is too low, it will give rise to increased drainage. So the formulation should have optimum viscosity for easy instillation into the eye as liquid, which will undergo a rapid sol-to-gel transition, hence the good gelling capacity. But administration of the formulation should influence the pseudoplastic character of precorneal film. In order to evaluate the rheological behavior, viscosity of the formulations before and after addition of STF was evaluated using Brook Field viscometer (RVT MODEL). It showed that viscosity of all formulations decreased as the shear rate increased, which showed the character of pseudoplastic fluid.

Further, all formulations were subjected for sterility testing using nutrient agar media and incubated for 7 days under daily observation. This study showed that formulations did not having any microbial contamination, and was sterile.

The *in vitro* release studies were carried out for all Formulations using cellophane membrane and STF as the medium. Release kinetic studies of prepared in situ gels showed that the in situ gels followed first order drug release mechanism. Higuchi matrix equation confirmed the release by diffusion controlled mechanism. Korsemeyer-Peppas 'n' value of prepared in situ gels was found to be above 0.5 this indicated that the formulation followed non-fickian diffusion controlled mechanism. Obtained results indicated that F6 showed better sustaining effect amongst all formulations. This may be due to the higher concentration of gelrite.

Antimicrobial efficacy study carried out by using Staphylococcus Aureus, Pseudomonas Aeruginosa and E.coli as test microorganisms. After incubation up to 24 hours, it was found that all formulations were having effective anti microbial action.

Lastly, formulations were evaluated for the stability studies (at RT and 34ºC, 75±5 % RH) for 42 days. Results reveal that no changes were found in visual appearance, clarity and pH. These formulations were also analyzed for % drug remaining. This study showed that there was no definite change observed in the intactness of the drug after accelerated study of 42 days.

Hence from the above the above results we can conclude that aim of the study was achived ie., formulation of in situ gels of Levofloxacin hemihydrate using Gelrite for treatment of various bacterial infections.

In this study in situ opthalmic gel of Levofloxacin hemihydrate, which is broad spectrum anti bacterial agent used in the treatment of ocular infections was prepared by using gelrite as a release retardant, it was found that increase concentration of gelrite decreased the decreased the drug release.

Optimized formulations F6 (0.6 % Gelrite and Propylene glycol 8%), F5 (0.5 % Gelrite and Propylene glycol 8%), F4 (0.4 % Gelrite and Propylene glycol 8%) and F3 (0.3 % Gelrite and Propylene glycol 8%) were liquid before instillation in to eye and underwent rapid gellation upon instillation in to eye, the formulations were found to be clear, having good in situ gelling capacity , having drug content 92-98%, optimized formulations were sterile and showed sustained drug release over 8 hours period as compared to marketed eye drop, release kinetic study showed that the formulation followed first order diffusion controlled and non fickian release mechanism, the optimized formulations was having good antibacterial efficacy, as per the Draize test protocol the ocular irritancy studies were carried out, results showed that formulations were non irritant. As per ICH guidelines the stability study of formulations were carried out results showed that formulations were stable (transparent and clear) at room temperature as well as at 40°C.

Hence from the above the above results we can conclude that aim of the study was achived ie., formulation of in situ gels of Levofloxacin hemihydrate using Gelrite for treatment of various bacterial infections.

The aim of the present work envisaged "Preparation and evaluation of in situ opthalmic gel of an anti infective drug for sustained ocular delivery" for the treatment of various bacterial diseases of eye, by providing comfortness, compliance to the patients and improved therapeutic performance of the drug over conventional ocular dosage forms.

Formulation containing Gelrite (0.2-0.7 %) was prepared along with propylene glycol a water miscible co solvent and humectant, as adjuvant in order to improve the solubility of Levofloxacin hemihydrate.

Optimized formulations F6 (0.6 % Gelrite and Propylene glycol 8%), F5 (0.5 % Gelrite and Propylene glycol 8%), F4 (0.4 % Gelrite and Propylene glycol 8%) and F3 (0.3 % Gelrite and Propylene glycol 8%) were liquid before instillation to the eye and underwent rapid gelation upon installation to the eye.

FTIR study of physical mixture of drug and polymer, prepared in situ gels was carried out and were compared with IR absorption spectra of pure drug. Studies reveal that there were no definite changes in bands observed with respect to pure drug. So it was confirmed that formulations do not have any drug polymer interactions.

Optimized in situ gels were subjected for preliminary evaluation such as, visual appearance, clarity, pH and drug content. All formulations were found transparent and clear, pH of the formulations was within7.1 to7.4, drug content was found within 92-98% in all optimized in situ gelling systems.

In order to evaluate the rheological behavior, viscosity of the formulations before and after addition of STF was evaluated using Brook Field viscometer. It showed that viscosity of all formulations decreased as the shear rate increased, which showed the character of pseudoplastic fluid.

Sterility testing was done by using nutrient agar media and incubated for 7 days under daily observation. This study showed that formulations do not having any microbial contamination, and was sterile.

In vitro release of Levofloxacin hemihydrate from the selected formulations was studied through diffusion cell using cellophane membrane for 8 hours. It was compared with the marketed eye drop. Results reveal that all formulations exhibited sustained release of the drug from gelrite polymeric network over 8 hours.

Release kinetic studies showed that the in situ gels followed first order drug release mechanism. Higuchi matrix equation confirmed the release was diffusion controlled. Korsemeyer-Peppas 'n' value of 0.55-0.85 indicated that the formulation followed non-fickian diffusion controlled release mechanism.

Antimicrobial efficacy study carried out by using Staphylococcus Aureus, Pseudomonas Aeruginosa and E.coli as test microorganisms. After incubation up to 24 hours, it was found that all formulations had effective anti microbial action.

The stability study was carried out for all optimized formulations up to 42 days. Results reveal that no changes were found in visual appearance, clarity and pH. These formulations were also analyzed for % drug remaining; this study showed that there were no definite changes observed in the intactness of the drug after accelerated stability study of 42 days.

Hence from the above the above results we can conclude that aim of the study was achived ie., formulation of in situ gels of Levofloxacin hemihydrate using Gelrite for treatment of various bacterial infections.

1. Mohan EC, Jagan Mohan K, Venkatesham A. Preparation and evaluation of in situ gels for ocular drug delivery. J pharm Res 2009; 2(6):1089- 94.
2. www.google.com Primary care ophthometry news, a guide to Topical anti infectives.
3. Martindale. The complete drug reference, 34th ed. Pharmaceutical press. 1996; 127-227.
4. Hill JM, Callaghan O, Hobden RJ, Kaufman E, Mitra AK. Ophthalmic drug delivery systems. New York, Marcel Dekker Inc; 1993. vol 58. p. 1-204.
5. Chein YW, Novel drug delivery systems, 2nd ed. New York, Marcel Dekker Inc; 1993. vol 29. p. 269-300.
6. Bourlais CL, Acar L, Zia H, Sado PA, Needhan S, Leverge R. Ophthalmic drug delivery systems – Recent Advances. *Progress in Retinal and Eye Research* 1998; 17(1):33-58.
7. Lieberman HA, Rieger MM, Banker GS. Pharmaceutical dosage forms: disperse system, 2nd ed. New York, Marcel dekker Inc; vol 2. p. 357-97.
8. Geeta MP, Madhubhai MP, Recent advances and challenges in ocular drug delivery systems, Pharma Times 2007; 39(1):21-25.
9. Peppas N. A., Bures P., Leabandung W., and Ichikawa H. Hydrogels in pharmaceutical formulations, Eur J Pharm and Biophar 2000; 50:27-46.
10. Sreenivas SA, Hiremath SP, Godbole AM. Ofloxacin ocular inserts: Design, formulation and evaluation. Iranian J pharmcol 2006; 5(2):159-62.
11. Sasaki H, Tei C, Nishida K, Nakamura J. Drug release from an ophthalmic insert of a beta-blocker as an ocular drug delivery system. J Contr Rel 1993; 27:127-37.

12. Bawa R. Ophthalmic drug delivery systems. New York Marcel Dekker, Inc.; 1993. vol 58. p. 224-48.
13. Robinson JR. Ocular evaluation of polyvinyl alcohol vehicle in rabbits. J. Pharm. Sci 1975; 64: 1312-16.
14. Felt O, Baeyens V, Zignani M, Buri P,Gurny R. Mucosal drug delivery ocular Encyclopaedia of controlled drug delivery. Geneva, University of Geneva, 1999. vol 2.p. 605–22.
15. Soppinath KS, Aminabhavi, TM, Dave AM, Kumar SG, Rudzinski WE. Stimulus responsive“smart” hydrogels as novel drug delivery systems. Drug Dev. Ind. Pharm.2002; 28: 957-74.
16. Van M. Biopharmaceutics of ocular drug delivery, P. Edman ed, Boca Raton, CRC press, 1993, P.27-42.
17. Wichterle O, Lim D. Hydrophilic gels for biological use. Nature 1960; 185:117-18.
18. Miller SC, Donovan M.D. Effect of Poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. Int. J. Pharm 1982; 12: 147–52.
19. Gurny R, Boye T, Ibrahim H. Ocular therapy with nanoparticulate systems for controlled drug delivery. J. Contr. Rel 1985; 2: 353–61.
20. Moorhouse R, Colegrove G.T, Sandford P.A, Baird J.K, Kang K.S. A new gel forming polysaccharide, Solution Properties of Polysaccharides. ACS Symposium series, Washington-DC 1981; 111–24.
21. Hoffman A.S, Afrassiabi A, Dong A. Thermally reversible hydrogels: II. Delivery and selective removal of substances from aqueous solutions, J. Control. Release 1986; 4: 213–222.

22. Gariepy E.R, Leroux J.C. In situ forming hydrogels review of temperature-sensitive systems. Eur. J. Pharm. Biopharm. 2004; 58: 409–426.
23. Cabana A, Ait-Kadi A, Juhasz J. Study of the gelation process of polyethylene oxide-polypropylene oxide polyethylene oxide copolymer (Poloxamer 407) aqueous solutions. J. Colloid Interface Sci 1997; 190: 307–312.
24. Harrington W.F, Von Hippel W.F. The structure of collagen and gelatin. Adv. Protein Chem 1961; 1: 1–138.
25. Shirakawa M, Yamatoya K, Nishinari K. Tailoring xyloglucan properties using an enzyme. Food Hydrocoll 1998; 22: 25–28.
26. Miyazaki S, Suisha F, Kawasaki N., Shirakawa M, Yamatoya K, Attwood D. Thermally reversible xyloglucan gels as vehicles for rectal drug delivery. J. Contr. Rel 1998; 56: 75–83.
27. Gariepy ER, Leroux JC. In situ forming hydrogels reviews of temperature-sensitive systems. Eur. J. Pharm. Biopharm 2004; 58: 409–26.
28. Lin HR, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery. J Contr Rel 2000; 69:379.
29. Boye T, Gurny R, Ibrahim H. Ocular therapy with nanoparticulate systems for controlled drug delivery. J Contr Rel 1985; 2:353-61.
30. Le Bourlais CA, Treupel-Acar L, Rhodes Ct, Sado PA, Leverge R. New ophthalmic drug delivery system. Drug Dev Ind Pharm 1995; 21:19-59.
31. Hui HW, Robinson JR. Ocular drug delivery of progesterone using of bioadhesion polymer. Int J Pharmaceut Sci 1985; 26:203-213.
32. Davis NM, Farr SJ, Hadgraft J, Kellaway IW. Evaluation of mucoadhesive polymers in ocular drug delivery: part 1, viscous solution. Pharm Res 1991; 8(8):1039-1043.

33. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Contr Rel 2001; 73:205-211.
34. Kumar S, Haglund BO, Himmelstein KJ. In situ-forming gels for ophthalmic drug delivery. J Ocul Pharmacol 1994; 10:47- 56.
35. Thilak kumar M, Bharathi D, Balasubramaniam J, Kant S, Pandit J.K. pH induced *in situ* gelling of Indometacin for Sustained ocular delivery. Ind J Pharm Sci 2005; 67(3):327-333.
36. Doijad RC, Manvi FV, Malleswara VSN, Prajakta Alase. Sustained ophthalmic delivery of Gatifloxacin from in situ gelling system. Ind J Pharm Sci 2006; 68(6):809-814.
37. Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained release carriers. Drug Dev Ind Pharm 2000; 26:1025-1038.
38. Jansson P.E, Lindberg B. Structural studies of gellan gum, an extracellular polysaccharide elaborated by Pseuomonas elodea. Carbohydr. Res 1983; 124: 135–39.
39. Kuo M.S, Mort A.J, Dell A. Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. Carbohydr. Res 1986; 156: 173–87.
40. Morris VJ, Stephen A.M. Bacterial polysaccharides, in food polysaccharides and their application. Marcel Dekker, New York, 1995; 341–375.
41. Rozier A, Mazuel C, Grove J, Plazonnet B. Gelrite®: a novel ion activated in situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol. Int. J. Pharm 1989; 57: 163–168.

42. Kang KS, Veeder GT, Mirrasoul PJ, Kaneko T, Cottrell IW, Agarlike. Polysaccharide produced by a Pseudomonas species: production and basic properties. Appl. Environ. Microbiol 1982; 43: 1086–1091.
43. Maurice DM, Srinivas SP. use of fluorometry in assessing the efficacy of a cation-sensitive gel as an ophthalmic vehicle: comparison with scintigraphy. J. Pharm. Sci 1992; 81: 615–618.
44. Meseguer G, Gurny R, Buri P, Rozier A, Plazonnet B. Gamma scintigraphic study of precorneal drainage and assessment of miotic response in rabbits of various ophthalmic formulations containing Pilocarpine. Int. J. Pharm.1993; 95: 229–34.
45. Haug A, Larsen B, Smidsrod O.A study of the constitution of alginic acid by partial hydrolysis. Acta Chem Scand 1966; 20: 183–90.
46. Painter TJ, Smidsrod O, Haug A. A computer study of the changes in composition distribution occurring during random depolymerisation of a binary linear heteropolysaccharide. Acta Chem. Scand 1968; 22: 1637–48.
47. Larsen B, Smidsrod O, Painter TJ, Haug A. Calculation of the nearest neighbor frequencies in fragments of alginate from the yields of free monomers after partial hydrolysis. Acta Chem. Scand 1970; 24: 726–28.
48. Brittain HG. Analytical profiles of drug substances and excipients. Elsevier 23:321-361.
49. Lindell K. and Engstrom S. *In vitro* release of Timolol from an in situ gelling polymer system. Int J Pharm 1993; 95(1-3):219-28.
50. Kumar S, Himmelstein KJ. Modification of in situ gelling behavior of carbopol solutions by HPMC. J Phar Sci 1995; 84(3):344-8.

51. Cohen S, Lobel E, Trevgoda A, Peled Y. A Novel in situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J. contr.rel 1997; 44(2-3):201-8.
52. Ding S. Recent Developments in ophthalmic drug delivery. Pharmaceutical sciences and technology Today 1998; 1(8):328-35.
53. Dimitrova E, Bogdanova SV, Mitcheva M, Tanev I, Minkov E. Development of model aqueous ophthalmic solution of indomethacin. Drug Devel Ind Pharm 2000; 26(12):1297-01.
54. Sechoy O, Tissie G, Sebastian C, Maurin F, Driat J, Trinquand C. A new long acting ophthalmic formulation of carteolol containing alginic acid. Int J Pharm 2000; 207:109-16.
55. El-Kamel AH. *In vitro* and *in vivo* evaluation of pluronic F127-based ocular delivery system for timolol maleate. Int J Pharm 2000; 241:47-55.