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An Overview - Stimuli Sensitive Hydrogels in Ocular Drug Delivery System.

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Abstract:
The aim of this review is to explore the advanced progress of ophthalmic products; drug delivery is one of the mainly tricky and complicated fields for researchers. The conventional formulations such as solutions, suspensions, ointments, etc. shows some constraint in increase of pre-corneal elimination, elevated changeability in efficiency, blurred vision and also diminish their bioavailability.
Hydrogels is a gifted novel biomaterial with the intention of effectual tool of delivering medication. Hydrogels own numerous properties and characteristics to facilitate capable drug carriers. The structure and composition of the hydrogels permit the medicine to particular site inside the body and discharge the drug according to exterior physical changes. There are various types of hydrogels, which are able to use according to their anticipated purpose. Hence, hydrogels are called stimuli-sensitive hydrogels.

Keywords: Drug Delivery, Hydrogels, Polymer, Stimuli Sensitive, Solutions.

INTRODUCTION:

Eye is a vital organ and unique. Eye is considered as window of the soul. By using this organ we can view and enjoy the whole world. Drug delivery to eye is a tricky job to the scientists because its typical anatomy restricts drug absorption into deep tissues. There are various ophthalmic drug delivery systems available as conventional dosage forms such as eye drops, eye ointments and eye lotions. In several cases, the eye ointment damages the organ eye and even leads to loss of eye sight. The major problems with ocular route include non- productive absorption, drainage of tear fluid, induced lacrimation, tear turn over and impermeability of drugs to cornea (1). Mostly the eye drops are conventional ophthalmic delivery systems often results in poor bioavailability and therapeutic response, because of high fluid turnover and dynamics cause rapid precorneal removal of the drug. An elevated occurrence of eye drop instillation into the eye is leads to patient non-compliance. Administration of drugs in the form of eye drops for treating topical eye diseases like dryness of the eye, conjunctivitis in the eye, keratitis, and eye flu is the most common approach and more than 90% of marketed ophthalmic formulations are still available as eye drops for water soluble drugs. (2)

Numerous *in-situ* gels had been developed to extend the pre-corneal residence time of the drug and to get better bioavailability. These ocular drug delivery systems consist of polymers that reveal sol to gel phase transition appropriate to change in specific physiochemical parameters such as (pH, temperature) in their environment, in the cul-de-sac in this case. Depending on the technique engaged to cause sol to gel phase transitions on the eye surface the subsequent three types of systems are accepted, pH triggered system, temperature dependent system and ion activated system. By these three techniques the above *in-situ* gelling ophthalmic delivery system is developed. (3)

Diverse Approach of *In-Situ* Gelation:

Preferably, an in-situ gelling system is supposed to be low viscous free flowing liquid to allow for reproducible administration to the human eye as eye drops, and the gel created following phase transition must be strong enough to hold out the shear forces in the cul-de-sac also demonstrated extended residence period in the eye. Sequentially to raise the efficacy of the drug, a dosage form must be chosen which increases the contact time of the drug in the eye. This might then prolong the residence time of the gel formed in-situ along with its ability to release drugs in sustained manner will assist in enhancing the bioavailability, diminish systemic absorption and reduce the need for repeated administration leading to improved patient compliance. Depending upon the method employed to cause sol to gel phase transition on the ocular surface, the subsequent types of systems are predictable. (4) (5)

pH – triggered systems: Cellulose acetate phthalate (CAP) latex, Carbopol, Polymethacrilic acid (PMMA), Polyethylene glycol (PEG) and Pseudo latexes.

Temperature dependent systems: Chitosan, Pluronics, Tetronics, Xyloglucans, and Hydroxypropylmethyl Cellulose or Hypromellose (HPMC)

Ion activated Systems (Osmotically induced gelation): Gelrite, Gellan, Hyaluronic acid and alginate. (6)

pH –triggered in-situ gelation:

The combination of Polyacrylic acid (i.e. used as a gelling agent) and Hydroxypropyl methylcellulose (i.e. used as a viscosity enhancing agent) to form the formulation by adjusting the pH from acidic to basic pH, in order to form the transition of sol to gel. Here the formulation with pH triggered in-situ gel is therapeutically efficacious, stable, non irritant and provide sustained release of the drug tear fluid. (7)

Lokhande Umesh Ramachandra et al. (8) have designed and developed pH-triggered in-situ gelling system of Ciprofloxacin, in which the poor bioavailability and

therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of in-situ gel forming systems that are instilled as drops into eye and undergo sol-gel transition in the cul-de-sac. They had prepared the gel by using the polymers such as Carbopol 934, HPMC-K4M, HPMC-E15V and HPMC-E50LV. Under gone the studies, such as clarity, drug content, gelling capacity, *in-vitro* release studies, sterility studies, ocular irritancy studies and FT-IR studies. The *in-vitro* release studies showed the F_3 formulation around 97.33% as maximum among all formulations for the period of 8 hours. Moreover, they had gone for ocular irritant studies in albino rabbits and proved the formulation does not cause any ocular damage to the cornea, iris and conjunctiva.

Vinod Singh, et al. (9) had developed hydrogels that are sensitive to stimuli i.e. pH, in the cul-de-sac of the eye for providing a prolonged effect and increased bioavailability with reduction in frequency of administration. The drug chosen was timolol maleate, polyacrylic acid as the gelling agent, HPMC as viscolizer and NaCl as isotonic agent. Dug release studies were carried out by using dynamic dialysis technique. By using albino rabbits the reduction for intraocular pressure was carried out. Student t-test was performed for *in-vivo* study.

Rathod K B et al. (10) had prepared the controlled release in-situ gel of Norfloxacin for ocular drug delivery. The polymers Carbopol 934 and HPMC KM re used to increase the contact time, to achieve controlled release, reduction in frequency of administration and greater therapeutic efficacy of drug. The prepared in-situ gels were evaluated for clarity test, pH, drug content analysis, *in-vitro* gelation, rheological studies, sterility testing and *in-vitro* drug release studies.

Nisha Shetty G et al. (11) had formulated Naphazoline and antazoline in-situ gelling systems for ocular delivery and stability studies. The polymers used for preparation of in-situ gel are Carbopol 940 and HPMC K4M. The formulations are evaluated by using their pH, Isotonicity, gelling capacity, rheological characteristics, *in-vitro* release, sterility and *in-vivo* studies. Their formulations proved to be very stable at room temperature and at higher temperature (40ºC).

Prachi Saxena et al. (12) had developed the pH sensitive hydrogels of Levofloxacin hemihydrates for ophthalmic drug delivery. Levofloxacin is a widely used for the treatment of acute conjunctivitis. The drug suffers the drawbacks of poor bioavailability due to its pH dependent solubility. In order to increase, the solubility of Levofloxacin in addition to the polymers Carbopol 940 and HPMC. The β- cyclodextrin were also added. The author had designed the formulation (2^3) as 2 level 3 factors of factorial designs. Under gone the characterization studies as clarity, drug content, gelling capacity, viscosity studies, in-vitro release studies, release kinetics, sterility studies, Pyrogen testing, ocular irritancy studies and accelerated stability studies. In-vitro release studies reveals that PF7 showed the best release of 84.31% of among all other formulations. Further, they had gone for ocular irritant studies in albino rabbits and showed the formulation does not cause any ocular damage to the cornea, iris and conjunctiva.

Shashank Nayak N et al. (13) had formulated and evaluated the pH triggered in-situ ophthalmic gel of Moxifloxacin Hydrochloride. The Moxifloxacin Hydrochloride ophthalmic in-situ gel is prepared by the combination of polymers HPMC K15 and Carbopol 934. They had studied the characterization studies such as FT-IR studies, DSC studies, clarity, drug content, gelling capacity, viscosity studies, *in-vitro* release studies, sterility studies, ocular irritancy studies and accelerated stability studies. In the FT-IR studies, no specific interaction was observed between the drug and the polymers used in these formulations. Here, the F5 shows the good sustained release for the period of 8 hours. The stability data's at the end of the 45 days that the formulations was found to be stable and efficacious. No change in pH, drug content, gelling capacity were observed.

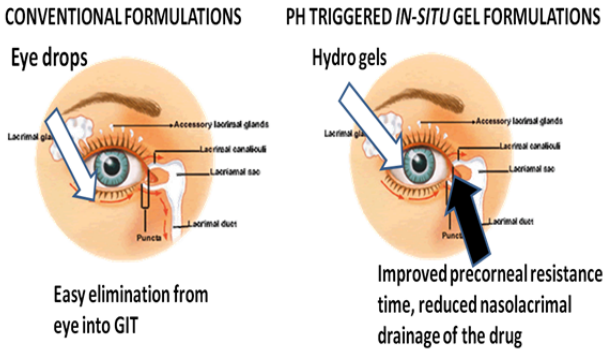
Padma Preetha J et al. (14) had formulated and evaluated in-situ gels of Diclofenac sodium. These formulations were prepared by combination of HPMC, HPC, HEC and Carbopol derivatives. They had performed the characterization studies such as drug content, pH of the ophthalmic gels, in-vitro diffusion study, sterility testing, anti-microbial studies, anti-bacterial & anti fungal studies. The percentage cumulative drug release of the formulation F1 showed the maximum release of 87.23% compared to others formulation F2, F3 and F4 for the period of 8 hours. The selected formulations showed the antimicrobial, anti bacterial and anti fungal efficacy.

Patel PB et al. (15) had developed and evaluated pH triggered in-situ ophthalmic gel formulation of Ofloxacin. Ofloxacin is a 2nd generation Fluoroquinolone used in external infections of the eye such as acute and sub acute conjunctivitis, bacterial keratitis and Keratoconjunctivitis. The formulation optimization was carried out using (3^2) 3 level 2 factors full factorial design. The ophthalmic gel formulation was prepared by using the polymers Carbopol 974P and Noveon® AA- 1 USP and Polycarbophil. They had studied the characterization studies such as drug content, in-vitro gelling capacity, rheological studies, in-vitro drug release studies, Anti-microbial efficacy studies, Adhesiveness study, effect of sterilization study, and accelerated stability studies. From the kinetics study all the formulations (A1 –A9) obeys Koresmeyer – Peppas matrix model. In this paper they proved factorial design; multiple regression analysis, contour plots and desirability function were found to be useful for the optimization of formulations.

Nagalakshmi S et al. (16) had formulated and evaluated stimuli sensitive pH triggered in-situ gelling system of fluconazole in ocular drug delivery. The polymer used to formulate the in-situ gel is HPMC and Carbopol 940. The Evaluation parameters had been performed such as gelling capacity, drug content, in-vitro release studies, in-vitro release studies using goats cornea, sterility testing, anti-fungal assay, FTIR studies and accelerated stability testing. F2 formulation shows the good sustained release when compared to other formulations for the period of 8 hours.

Jovita kanoujia et al. (17) had formulated and evaluated a novel pH-triggered in-situ gelling ocular system containing Gatifloxacin. The gatifloxacin is a 4th generation fluoroquinolone derivative used to treat external infections of the eye, using biodegradable polymers (HPMC, HPMC K15M and Carbopol 940). The characterization parameters of these formulations are pH, determination of viscosity, gelling capacity, drug content, bio-adhesive strength, in-vitro release of gatifloxacin, and kinetics drug release. The results of the drug release of this formulation shows anomalous and non- fickian diffusion controlled. The statistical analysis and ANOVA test was performed.

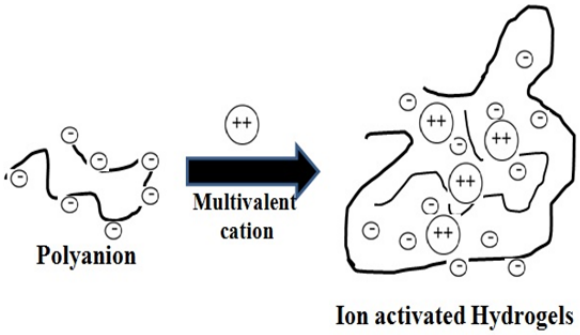
Hemalata Dol et al. (18) had formulated and evaluated in-situ ophthalmic gel of moxifloxacin hydrochloride. The polymers used to formulate were HPMC K4M and Carbopol 934P. The standard curve calibration of moxifloxacin hydrochloride was diluted by simulated tear fluid (STF). The (3^2) 3 level 2 factors factorial design was constructed for the polymers HPMC K4M and Carbopol 934P. The characterization studies of the formulations were carried out such as clarity, pH, drug content, *in-vitro* gelation studies, measurement of gel strength, rheological studies, sterility studies, *in-vitro* release studies and statistical analyses. *In-vitro* gelation studies of formulation F4 and F5 revealed more suitable gelling capacity when compared to other formulations. The statistical analysis and ANOVA test was also performed for the study.



Diagrammatic representation of pH triggered in-situ gel formulations increase the bioavailability–increased precorneal resistance time and decreased nasolacrimal drainage of the drug.

Ion activated Systems:

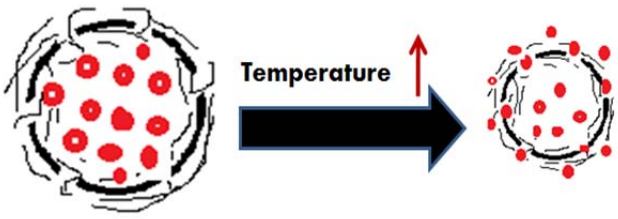
Dasankoppa F.S et al (19) had design, developed and evaluated cationic guar and hydroxypropyl guar based in-situ gels for ophthalmic drug delivery. In this investigation ion-induced gelation effect was used by combining cationic guar and hydroxypropyl guar. The evident of the literature says that a cationic polymer in conjunction with a non ionic polymer leads to enhanced gelling ability and sustained release drugs. The interactions were done by drug - polymer compatibility studies. Evaluation of gel formulations done by gelling capacity, pH measurements, Rheological studies, drug content, Effect of sterilization, in-vitro release study, kinetic studies, Short term stability studies and in-vivo toxicity studies. The compatibility studies were clearly explained by using DSC analysis. The in-vitro release profiles of the formulation were shown for the period of 12 hours. The formulation (CG-HP2) optimized showed maximum release after the stability study testing done for 6 months. Finally the ocular toxicity studies were carried out on rabbits. No ocular lesions were observed from the both eyes of the rabbit. The Draize test is also found to be zero which revealed non-irritant property of the formulation.



Diagrammatic representation of ion activated in-situ gel formulations where anion and cation both gets activated in the form hydrogels.

Temperature Dependent Systems:

Binu chaudhary et al. (20) had prepared and evaluated novel in-situ gels containing acyclovir for the treatment of oral herpes simplex virus infections. The preparation of in-situ gel done by cold method. Poloxamer 407 and poloxamer 188 were polymers used in the preparation at 4ºC and maintained the temperature. The other ingredients were added to these formulations are HPMC K100, Carbopol 934, Methyl paraben and propyl parabens. They had undergone the studies such as gelling time, determination of pH, viscosity of the formulation, spread ability test, gelling strength, drug content, Ex-vivo permeation study and mucoadhesive studies. They had concluded that the formulation were prepared within the range of 28-38ºC. The release kinetics of the formulations followed higuchis and Korsmeyer peppas model mechanisms. Further the in-situ gel formulation is used to treat oral herpes simplex infection locally by improving the patient compliance.



Diagrammatic representation of temperature dependent in-situ gel formulations. The drug molecules encapsulated by the polymer large molecules were triggered by the raise of temperature (25-35ºC) and the drug gets released out or the molecules get shrunken into small molecules.

EVALUATION PARAMETERS:

Physical appearance:

The appearances of the formulation were done by visual observation, which includes the color, homogeneity, phase separation and consistency (Mohan et al 2009, Mohamed, 2004)

Drug content:

Determinations of the drug content were done by dissolving the weight quantity of formulation (1g in 100ml) in pH (6-7) phosphate buffer. The diluted solutions were subjected into the 0.45µm membrane filter and the filtered solutions were analyzed by using UV- visible spectrophotometer at appropriate wavelength.

Determination of pH:

The formulation in-situ gels were dissolved in 50-100ml of distilled water and the pH was measured in triplicate by using pH meter (Model 111E).

Determination of Gelling Capacity:

By using visual method the *in-vitro* gelling capacities were determined. The colored solutions such as (Amaranth dye, Congo red dye and indigo blue dye) can be used by dissolving 1g of dye in distilled water and mixed with the formulation of in-situ gel. The *in-vitro* gelling capacity of the formulations were measured by placing 5ml of gelation solution (STF fluid 7.4) in the glass test tube and maintained the temperature at 37º±2ºC. 1ml of dye solution is taken in the pipette and added into the gelation solution (STF fluid 7.4); it immediately converted into stiff gel like appearance. The *in-vitro* gelling capacities were evaluated by the appearance of stiffness of the gel. And the time period for which the gel converted into stiff gel remains as such. Further, the color was added to give the visual appearance to the gel. Based on the 3 categories the *in-vitro* gelling capacity time period was calculated. (17)

1. (+) Gel forms after few minutes, disperses rapidly.
2. (++) Immediately gelation occurs, remains for few hours.
3. (+++) immediately gelation occurs, remains for extended period of time.

Viscosity measurement:

Determinations of viscosity of the prepared formulations were done using the Brookfield Viscometer (Brookfield Model DV2T). The formulations pHs were adjusted to 6-7 by neutralizing with 0.5N NaOH. Similarly, the temperature of the formulation is gradually increased to 25ºC- 37ºC. Further, the viscosities of the formulations were recorded before and after gelling. The experiment is performed for 3 times / triplicate. (21)

In-vitro release studies:

The *in-vitro* release studies for the prepared formulations were done by cellophane membrane using Franz diffusion cell apparatus (FDC Apparatus) / modified dissolution method. The phosphate buffer 7.4 pH was used as the dissolution medium. The membrane cellophane is soaked overnight in the Glycerin solution (Smoothening agent) and washed with distilled water. Further, the membrane was soaked in dissolution medium for few minutes. The open ended cylinder was taken and tied to one end and other was free to add the formulation. The cylinder tube was kept in such a position that the cellophane membrane dipped into the dissolution medium with constant stirring and the temperature should be maintained at 37ºC±2ºC. The sample was withdrawn (2ml/5ml) to maintain the sink condition. Similarly the cellophane membrane was kept in the Franz diffusion cell apparatus in between the donor compartment and the receptor compartment. Moreover, the formulations were taken in the donor compartment and the receptor contains diffusion medium (Phosphate buffer pH 7.4/ Simulated Tear Fluid (STF fluid 7.4 – the STF fluid contains 0.67g of NaCl, 0.20g of $NaHCO_3$, 0.008g of $CaCl_2$ and makes up to 100g with deionized water) (22) (23). The samples were withdrawn and replaced by using sampling port to maintain sink conditions. Finally, then the collected samples for certain time intervals are subjected to do with UV - visible spectrophotmetric analysis.

Ex-vivo release studies:

The prepared formulations / optimized formulations were used for these studies by using modified Franz diffusion cell (FDC) apparatus. The fresh goat cornea is removed with help of slaughter and the cornea act as a diffusion membrane (24). The cornea membrane was soaked in the diffusion medium and placed in between the donor and receptor compartment. The diffusion medium used is Simulated Tear Fluid (STF fluid 7.4 – the STF fluid contains 0.67g of NaCl, 0.20g of $NaHCO_3$, 0.008g of $CaCl_2$ and makes up to 100g with deionized water). The diffusion medium was maintained at 37ºC±2ºC temperature and the samples were collected at regular intervals by using sampling port. Further the samples were analyzed by using UV-visible spectrophotometer.

Accelerated stability studies:

The prepared in-situ gel formulations / optimized in-situ gel formulations were subjected to stability studies. Further, the formulations were kept for storage under temperature at 50ºC±1ºC for the period of 8 weeks / 2 months. Moreover, the formulations were evaluated by weekly for the clarity test, viscosity, pH, drug content and gelling capacity.

Sterility Studies:

The formulated / prepared formulations were filled in 50-100 ml capacity amber glass bottles. The bottles were closed by using aluminum foils / closed with grey butyl rubber closures, further sealed by using aluminum caps. The bottles were kept in autoclave for sterilization at 120ºC – 15 psi for 20 minutes. After that the samples were let to cool down to room temperature and the formulations were finally evaluated for the clarity test, pH, viscosity, drug content and gelling capacity.

In-Vivo Studies:

These studies were done by both Draize method and histological examination to evaluate the ocular irritation test of the rabbit eye.

Draize test method of Evaluation:

Ocular irritation test were done by most famous method Draize test. The albino rabbits were selected and treated with different in-situ gel formulations (control or gel group). This method ocular irritation scores were calculated by adding the irritation scores for the iris, cornea and conjunctiva. The rabbit eye irritation scores were evaluated by the total scores of all rabbits and divided by the total numbers of rabbits. They are classified into 4 groups as (25)

1. Non-irritating, score range from 0-3.
2. Slightly irritating, score range from 4-8.
3. Moderately irritating, score range from 9-12.
4. Severely irritating, score range from 13-16.

Histological Examination method:

The effects of particular drug on the structures of the cornea and integrity were evaluated by *in-vitro* method. After the ocular irritation test, the corneas of the freshly sacrificed rabbit was removed and incubated at 37ºC for about 2 hours in 0.5% control group solution or 0.5% gel group solutions. Sodium dodecylsulfate (SDS) solution added with phosphate buffer saline solution 0.1% as the positive control. After the incubation was over, corneas were washed with PBs solution and immediately fixed in formalin (8%). Finally the tissues were dehydrated in alcohol, place in melted paraffin and solidified in block form. Further, the cross section of the eye were cut and stained with haematoxylin and eosin (H&E). Moreover, the following changes were observed by using microscope, whether any pathological modifications were present or not.

In-vivo Elimination method:

To achieve the main goal of the study and the elimination time of the gel, the gel was dispersed with fluorescein disodium salt in the final preparations. The fluorescein disodium salt will be well retained in the gel and it can be easily observed by using slit lamp. 20-50µl of the each prepared formulations (fluorescein disodium salt and gel) were added to lower conjunctive sac of the rabbit eye (n-3), then the slit lamp blue light is used to observe the disappearance of the fluorescein disodium salt. At the preferred time intervals the rabbit eyes were inspected, when only 1 minute amount or none of the gel remained it was considered as lost of the fluorescein disodium salt from the eye. Then, the fore going time for inspection were defined as the elimination time.

CONCLUSION:

Since the studies have been discussed in the above review, that the formulations of ophthalmic pharmaceutical preparations with different in-situ gelling polymers that have exclusive physiochemical individuality of the ocular tissues is an outstanding plan that requires extra survey. In truth, the combination of two or more polymers (Carbopol & HPMC) in the similar formulations embrace assure for superior compliance and better therapeutic efficacy. For the reason, that it take benefits of the fundamental feature of the ocular sphere, greater than ever the retention time and consequently the bioavailability of the drug used. More than, modern years the make use of both biodegradable and biocompatible polymers in carrier systems have prove to be the most efficient approach (e.g. Hydrogels, polymeric micelles, Novel drug delivery systems and targeted drug delivery systems). Many advantages such as delivery systems for ophthalmic direction, for improving the bioavailability of poorly soluble drugs, supervision of a specific dosage forms, targeted and superior controlled drug release, reduced with the side effects and adverse effects.

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