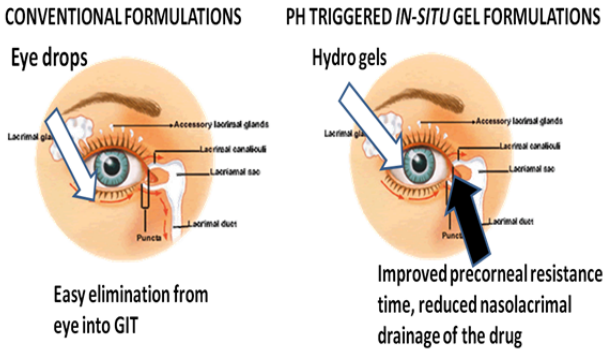
Jovita kanoujia et al. (17) had formulated and evaluated a novel pH-triggered in-situ gelling ocular system containing Gatifloxacin. The gatifloxacin is a 4th generation fluoroquinolone derivative used to treat external infections of the eye, using biodegradable polymers (HPMC, HPMC K15M and Carbopol 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.

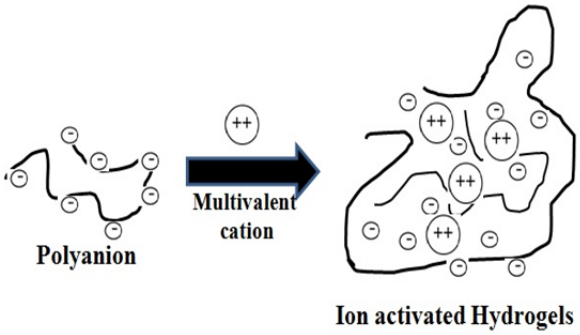
Hemalata Dol et al. (18) had formulated and evaluated in-situ ophthalmic gel of moxifloxacin hydrochloride. The polymers used to formulate were HPMC K4M and Carbopol 934P. The standard curve calibration of moxifloxacin hydrochloride was diluted by simulated tear fluid (STF). The (3^2) 3 level 2 factors 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:

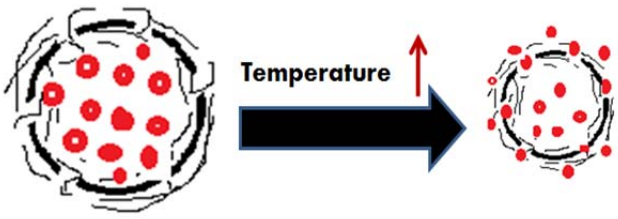
Dasankoppa F.S et al (19) had design, developed and evaluated cationic guar and hydroxypropyl guar based in-situ gels for ophthalmic drug delivery. In this investigation ion-induced gelation effect was used by combining cationic guar and hydroxypropyl guar. The evident of the literature says that a cationic 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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