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CURRENT STATUS OF OPHTHALMIC *IN-SITU* FORMING HYDROGEL

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ABSTRACT

Ophthalmic drug delivery is one of the most interesting and challenging endeavors faced by the pharmaceutical scientist. The conventional ocular drug delivery systems like solutions, suspensions and ointments show drawbacks such as increased precorneal elimination, high variability and blurred vision. Low absorption results in short duration of action and high frequency of eye drop instillation are associated with discomfort to the patient. Delivery of a therapeutic agent can be improved by developing novel systems for example through penetration enhancers or by altering its physicochemical properties. In the novel delivery system various approaches are used like *In-situ* gelling, use of mucoadhesive polymers, polymer coated Nanoparticle and Liposomal formulation. The primary focus of the review is to give an insight in to the novel drug delivery to delay elimination from eye and to improve corneal penetration of drug molecule.

KEY WORDS: Novel ophthalmic formulation penetration enhancers mucoadhesive polymers i*n-situ* gel.

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1. INTRODUCTION

The eye is very unique organ because of its trilaminate structure consisting of Epithelium, Stroma and Endothelium. Outermost epithelium is rate limiting barrier for transcorneal diffusion of most hydrophilic drugs. Stroma acts as diffusion barrier to highly lipophilic drugs owing to the hydrophilic nature of the former. Endothelium is Lipoidal in nature which does not offer significant barrier to the transcorneal diffusion of most drugs. In case of formulations, conventionals like solutions, suspensions have many disadvantages exemplified by like rapid precorneal elimination, drainage by gravity, normal tear turnover, frequent instillation, enzymatic metabolism, nasolacrimal drainage, conjunctival absorption and absence of controlled release. The residence time of most conventional ocular solutions ranges between 5 and 25 minutes. Only 1-10% of topically applied drug is absorbed in to the eye and from the rest major part of drug absorbed systemically results in systemic side effects. By increasing formulation viscosity, drainage rate will be decreased, and it leads to increase in precorneal residence time and prolongation in the time of Ocular absorption. There are certain characteristics required for optimizing ocular drug delivery system like Good corneal penetration, prolonged contact time with corneal tissue, simplicity of instillation, non irritative and comfortable form, appropriate rheological properties, sterility and isotonicity.

There are two major barriers in local ophthalmic drug delivery viz., short residence time in precorneal area and poor permeability of the cornea. The cornea has an isoelectric point (pI) of 3.2. At pH above the pI, it carries a negative charge and is selective to positively charged molecules. On the other hand, at pH below the pI, it carries a net positive charge. As a result a positively charged molecule can pass across the cornea more effectively at physiological pH [1, 2].

Various problems encountered leading to poor bioavailability of the eye installed drugs are viz.,[3, 4] binding by the mucus proteins, drainage through eye, lacrimation and tear turnover, limited corneal area and trilaminate structure of eye, metabolism and high ocular shear rate ranging from 0.03 s^{-1} during inter-blinking periods to 4250 - 28500 s^{-1} during blinking [5, 6]. This review provides a glance on Mucoadhesive polymers, penetration enhancers, *In-situ* gel and future demand.

2. MUCOADHESIVE POLYMERS

2.1. Introduction:

Literature gives two applications for mucoadhesion one it increase in intimate contact of delivery system with corneal surface and second it may result in high drug concentration in local area.

To study the mucoadhesive polymers it is important to add a glance on the composition mucin which composed of glycoprotein, proteins, lipids, electrolytes, inorganic salts, water, enzymes, mucopolysaccharide, cysteine rich subdomain, galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine, N-acetylneuraminic acid (sialic acid). Each carbohydrate chain terminates in either a sialic acid or an L-fucose group. Hence, the mucin molecules behave as anionic polyelectrolyte.

From the composition of mucin, the researchers found out the two ways to achieve mucoadhesion one as mucin contains negatively charged Sialic acid group for the same researchers have used positively charged polymers leading to interactions with mucin for mucoadhesion and second as mucin contains disulfide bond forming groups. Researchers have employed thiol group containing polymers (thiomers). After ophthalmic instillation there is formation of disulfide bond between polymer and mucus which gives mucoadhesion [7-14].

2.2. POLYMERS

2.2.1. Anionic Polymers:-

a. Poly (acrylic acid) (Carbopol)
b. Polycarbophils

2.2.2. Cationic polymer:-

a. Chitosan

2.2.3. Non-ionic polymer:-

a. Hydroxypropylmethylcellulose (H.P.M.C)
b. Methylcellulose

2.2.4. Thiolated Polymer:-

a. Thiomers: Thiolated Chitosan.

Carbopol

Carbopol is the lightly crosslinked commercial form of Poly(acrylic acid). The mucoadhesive properties of poly(acrylic acid) are due mainly to hydrogen bonding, while hydrophobic interaction with mucin is not significant [15]. When anionic polymers interact with mucin, the maximum interactive adhesive force occurs at an acidic pH, suggesting that the mucoadhesive polymer in its protonated form is responsible for the mucoadhesion[16]. The swollen polymer entangles with mucin on the eye surface, stabilizing a thick hydrogel structure[17, 18]. In contrast, in the precorneal area, the neutral pH value of the tears and the shielding of the carboxyl groups by cations present in the tear fluid diminish the interaction of carbomer with the functional groups on mucin. A decrease in mucoadhesion is measured[19].

Polycarbophils

Polycarbophil is also the lightly crosslinked commercial form of Poly(acrylic acid) exhibits stronger mucoadhesion same as Carbopol. The polycarbophil is used to prolong the precorneal residence time due to *in-situ* gel formation and mucoadhesion. Polycarbophil–cysteine showed a 2.2-fold and 2.4- fold increase in the transcorneal permeation of sodium fluorescein and dexamethasone phosphate, respectively, when compared to the unmodified polycarbophil [20].

Chitosan

Chitosan is a cationic superior mucoadhesive due to an ability to develop the electrostatic interactions between its positively charged amino group and negatively charged sialic acid residues of mucin. Chitosan is a polysaccharide consisting of copolymers of glucosamine and N-acetylglucosamine. The primary amino group accounts for the possibility of relatively easy chemical modification of chitosan and salt formation with acids. At acidic pH, the amino groups are protonated, which promotes solubility, whereas chitosan is insoluble at alkaline and neutral pH. Because of its favorable properties, such as enzymatic biodegradability, non-toxicity and biocompatibility chitosan has received considerable attention as a novel excipient in drug delivery systems[21-23]. Chitosan is reported to act as a penetration enhancer that increases transcorneal permeation of the drug by opening the tight junctions between epithelial cells[24-26] or by intracellular routes[27] or some researchers also proposed the mechanism underlying this permeation enhancing effect seems to be based on the positive charges of the polymer which interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins[28]. Besides this, chitosan has ability to convert into hydrogel at ocular pH (pH 7.4) which makes it out of the best suited candidate for the development of this type of delivery systems[29-31].

Cellulose derivatives: Methyl cellulose and H.P.M.C

Methyl cellulose solutions transform into opaque gel between 40–50^{0}C, whereas H.P.M.C shows phase transition between 75-90^{0}C. These phase transition temperatures can be lowered by chemical or physical modifications.

Mechanism: Gelation of cellulose solutions is primarily caused by the hydrophobic interactions between molecules containing methoxy substitution. At low

temperature, the macromolecules are hydrated and there is little polymer-polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity[32].

Thiomers:[33]

can be classified as Cationic thiomers (like Chitosan-cysteine, Chitosan- thiobutylamidine, Chitosan –thioglycolic acid) and Anionic thiomers (like Poly (acrylic acid)–cysteine, Poly(acrylic acid)–homocysteine, Poly(acrylic acid)–cysteamine, Alginate–cysteine). It was found that Thiolated polymer could increase mucoadhesion by 2 to 140 fold. Bernkop et al, studied retention time of Fluorescine in cornea using Polyacrylate polymer insert and Thiolated Polyacrylate polymer insert where they found out Controlled delivery with Thiolated polymer insert which could not be achieved by Polyacrylate polymer insert.

Mechanism of mucoadhesion:

Thiolated polymer found to show its action by two ways.

a. Formation of disulfide bonds with the mucus gel: formation of covalent bond (see in Fig 1).

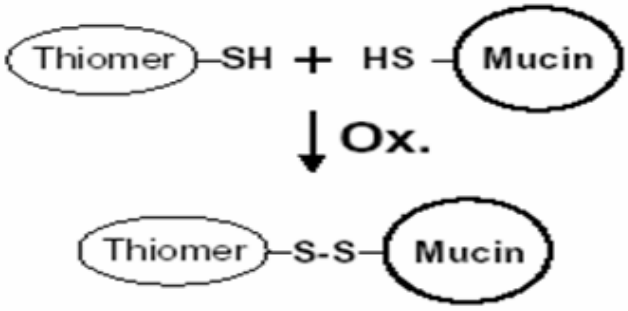

Figure 1
Formation of disulphide bond between the polymer and mucus gel [21].

b. *In situ* cross-linking process: (See Fig 2)

- Oxidation of the thiol groups at physiological pH-values, which results in the formation of inter and intramolecular disulfide bonds.
- This Crosslinking process can be observed within the pH range of **5–6.8**.

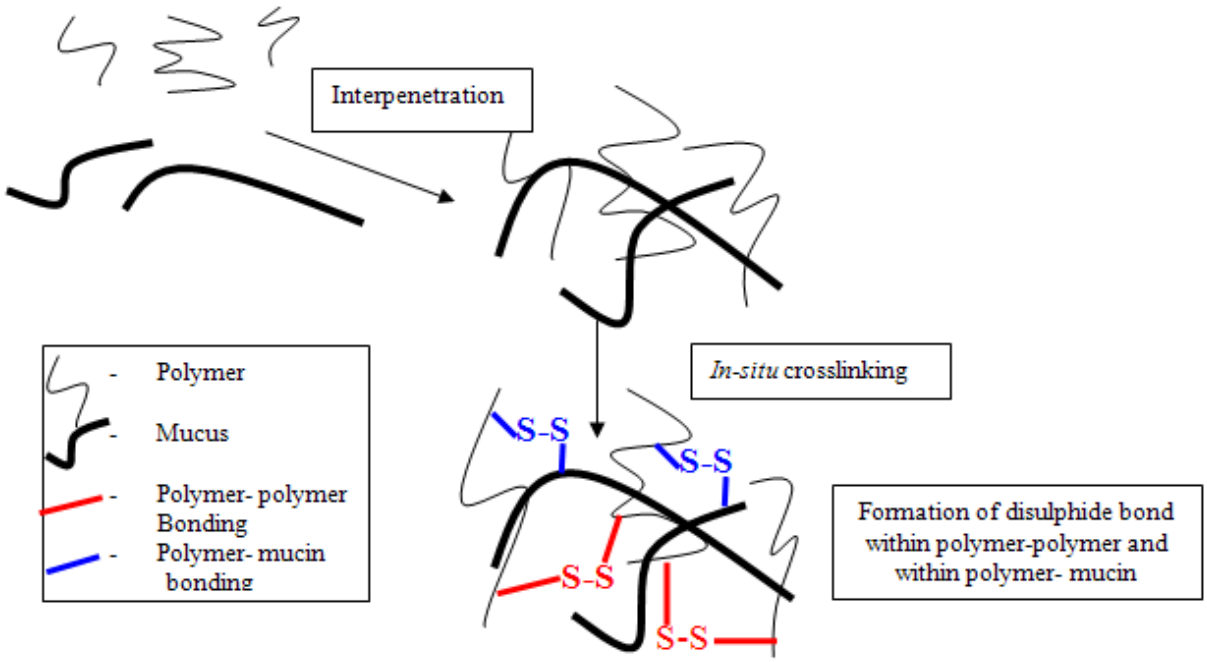

Figure 2
In-situ crosslinking after instillation.

Thiolated Chitosan

Thiolated chitosans make them highly suitable excipient for controlled drug release dosage forms. Moreover, solutions of thiolated chitosans display *in-situ* gelling properties at physiological pH values. Literature shows that chitosan-thioglycolic acid conjugates a 5–10-fold increase in mucoadhesion in comparison to unmodified chitosan; also the mucoadhesive properties of chitosan-TBA (chitosan-4-thio-butyl-amidine) conjugates found to be further improved. Medium molecular mass chitosan-TBA conjugate displaying 264 mM thiol groups per gram polymer shows more than 100-fold improvement in mucoadhesion compared to unmodified chitosan. The permeation enhancing effect of chitosan can be greatly improved by the immobilization of thiol groups[28, 34-36].

3. PENETRATION ENHANCERS[12]:

Penetration enhancer is the other approach to improve ocular bioavailability.

3.1. ***Ideal characteristics:*** Ideal characteristics for the penetration enhancers are as follows:

a. Action should be immediate.
b. There should be immediate recovery of the tissue after removing the absorption enhancers.
c. There should not be any systemic and local effect associated with the penetration enhancers.
d. The enhancers should be physically and chemically compatible with a wide range of drugs and excipient.

3.2. ***Mechanisms of Penetration enhancers***[12, 37]*:* Mechanism of penetration enhancers are as follows:

i. Altering the membrane structure by extracting membrane components.
ii. Chelating Calcium ions leads to opening of tight junction.
iii. Inducing high osmotic pressure that opens the tight junction.
iv. Altering the mucus structure & rheology so that diffusion barrier weakens.

v. Modifying the physical properties of the drug-enhancer entity.
vi. Inhibiting enzyme activity.

3.3. Examples of Penetration enhancers[12]:

3.3.1. ***Cyclodextrin****:* Cyclodextrin acts as a true carrier by keeping hydrophobic molecules in solution by their hydrophobic core. α-Cyclodextrin has a significant penetration enhancing effect 10 times on the corneal permeability of Pilocarpine [38].

3.3.2. ***Cytochalasins****:* are the groups of small molecules that bind specifically to act in microfilaments, the major component of the cytoskeleton. It has been shown that the cytoskeleton participates in regulation of epithelial permeability in a variety of conditions. Therefore, it is reasonable strategy to design penetration enhancers to act specifically on cytoskeleton in order to improve Paracellular transport[39]. Rojanaskul et al, showed that Cytochalasin B decreases transepithelial electrical resistance (TEER) of the cornea in dose dependent manner, as barrier property of the tight junction can be reflected by the TEER [40].

3.3.3. ***Azone ®***

3.3.4. ***(1-Dodecylazocylazacycloheptane-2-one):*** changes the structure and fluidity of the cell membrane. Increase penetration of Hydrophilic compounds and decrease the penetration of lipophilic compounds. It is interesting to note that 0.1% can enhance corneal penetration of hydrophilic compounds by at least 20 fold but inhibit corneal penetration of lipophilic compound.

3.3.5. ***EDTA*** [12, 41]: Calcium-chelating agent has been shown to act on:
a) Cell junctions by chelating with calcium ions as proper functioning of tight junction depends on calcium ion, because of chelate formation there is alteration in intercellular integrity.
b) EDTA also disrupts the plasma membrane and, consequently, increases intercellular permeability.

3.3.6. ***Benzalkonium Chloride:***
Cationic surfactant has been reported to increase the *in vitro* permeation of Ketorolac (an anionic drug) through rabbit cornea [42].
Mechanisms:
a) Formation of a more lipid soluble ion pair and
b) Disruption of the corneal epithelium

3.3.7. ***α-Amino Acid:*** It enhances transcellular pathway with higher extent. These carriers increase the permeability coefficient of many drugs.

3.3.8. ***Pz – peptide*** (4-phenylazobenzoxy carbonyl-pro-leu-gly-pro-D-arg): It is Pentapeptide, capable of opening tight junctions in reversible manner.

3.3.9. ***Cationic polymers:*** such as chitosan, aminated gelatin, and poly-L-arginine are reported to increase the transepithelial absorption of peptide drugs by dissociation of tight junction assemblies which restrict the Paracellular permeation in intestinal and nasal epithelia without producing significant epithelial damage. Thus poly-cationic polymers may be useful penetration enhancers for ocular drug delivery [43].

4. IN-SITU OPHTHALMIC GEL

4.1. Introduction:

In-situ gel forming formulations undergo phase transition from liquid to semisolid gel upon exposure to physiological environments. These formulations are free flowing liquid at room temperature to allow easily reproducible administration into the eye as a drop and after instillation formulation undergoes *in- situ* phase transition to form a strong a firm gel that is capable of withstanding shear forces in the cul-de-sac and of sustaining drug release at physiological conditions. Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye [32, 44].

4.2. Advantages of in- situ forming gel [45]:

a) Generally more comfortable than insoluble or soluble insertion for example less blurred vision as compared to ointment.
b) Increased bioavailability due to precorneal retention and in turn increase retention leads to decreased nasolacrimal drainage. As nasolacrimal drainage of the drug causes undesirable side effects due to systemic absorption of the drug.
c) Drug effect is prolonged hence frequent instillation of drug is not required.
d) The principle advantage of this formulation is the possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention.

4.3. ***Principles of gelling:*** Following are the triggering mechanisms by which we can achieve *in-situ* gelling with examples of polymer [6, 32, 46, 47]

4.3.1. Temperature triggered:

i) Pluronic (Poloxamer)
ii) Cellulose derivatives
iii) Polymethacrylates

4.3.2. pH triggered:

i) Cellulose acetate phthalate
ii) Polyacrylic acid (Carbopol)
iii) Polycarbophils

4.3.3. Ion activated:

i) Gellan gum (Gelrite®)
ii) Sodium Alginates

4.4. In-situ gel forming polymers

4.4.1. Poloxamer (Pluronic): (Temperature triggered)

Poloxamer is the tri-block polymer of Polyethylene oxide–Polypropylene oxide–Polyethylene oxide. Poloxamer is composed of a central hydrophobic part (polyoxypropylene) surrounded by hydrophilic part (polyethylene oxide). Pluronic F-127, a very commonly used Poloxamer for ophthalmic use converts into a colorless and transparent gel at temperature above 35^0C. Mechanism: The mechanism involving the sol-to-gel transformation, after increase in temperature there is gradual desolvation of the polymer and increased micellar aggregation (entanglement of the polymeric network). The micelles formation takes place due to the polyoxypropylene block dehydration, at definite point micelles come in contact and no longer move. Micelles composed of central Hydrophobic part (polyoxypropylene) surrounded by Hydrophilic part (Ethylene oxide). Drawback: Poloxamer has weak mechanical strength which leads to rapid erosion. Therefore blend of Poloxamer with the other polymer is used [48-53].

4.4.2. Cellulose acetate hydrogen phthalate (CAP)

CAP is the only polymer known to have a buffer capacity which is low enough to gel effectively in the cul-de-sac of the eye. The pH change of 2.8 units after instillation of the native formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel [54-56].

4.4.3. Carbopol

Carbopol shows sol-to-gel transition in aqueous solution when pH rises above 5.5. Mechanism: At specific pH there is Electrostatic, hydrophobic interaction and Hydrogen 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. At pH 7.4, the mutual repulsion of ionized carboxyl groups may produce more stretched carbopol backbone and those carboxyl groups may also form stable hydrogen bonds with water molecules through hydrophilic interactions [49, 57, 58]. On the other hand, the hydrophobic nature of carbopol backbone may form hydrophobic interchain aggregation; this cross-linking phenomenon may result in

formation of more viscous gel at pH 7.4 environment [59].

Drawback: As concentration increases, acidic nature may cause lacrimation, hence combination of polymers are used.

4.4.4. Polycarbophils

Polycarbophil is pH-triggered *in-situ* gelling systems. 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 non-ionized carboxylic acid groups of polycarbophil bind to the mucin by means of hydrogen bonds [60, 61]. Noveon® AA-1 polycarbophil, USP is a high molecular weight polyacrylic acid polymer crosslinked with divinyl glycol and exhibits a definite sol to gel transition in aqueous solutions as the pH is raised above its pKa of about 6.0±0.5. The use of Noveon® AA-1 polycarbophil *in-situ* gelling system is substantiated by transformation into stiff gels when the pH is raised. However, the concentration (0.3–0.5%, w/w) of Noveon® AA-1 polycarbophil required to form stiff gels results in highly acidic solution which are not easily neutralized by the buffering action of the tear fluid. The lower the concentration of Noveon® AA-1 polycarbophil in the gel forming solutions, the lower is its buffering capacity and the faster is the gel is formed in eye. Although phase transition does occur when the Noveon® AA-1 polycarbophil concentration is 0.2% (w/w), the gel formed has a much lower viscosity than those containing the higher concentration of 0.3% or 0.4% (w/w) which indicate that the gels formed at higher concentration are stronger [62].

4.4.5. Deacetylated Gellan Gum (Gelrite®): (Ion activated)

Gellan gum is anionic heteropolysaccharide. Gelrite® is a low-acetyl Gellan gum, which forms a clear gel in the presence of mono- or divalent cations. The electrolytes of the tear fluid and especially Na^+, Ca^{2+} and Mg^{2+} cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution into the cul-de-sac. There is cross linking of negatively charged polysaccharide and cations. Once gelled, the formulation resists the natural drainage process from the precorneal area. Residence at the site of drug absorption is prolonged and, subsequently, the bioavailability of the drug is increased. 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 prone to elimination by the fluid mechanics of the eye. It is assumed that the rate at which electrolytes from the tear fluid are adsorbed by the polymer depends on the osmotic gradient across the surface of the gel. As a result, the osmolality of the solutions might have an influence on the sol–gel transition rate. The hypotonic samples were non-irritating, whereas isotonic and hypertonic solutions caused an increase in lachrymation and blurred vision. The high tolerance of the hypotonic samples is due to the rapid formation of a gel residing in the conjunctival sac, thus avoiding any solution spreading over the sensitive cornea. When instilling hypotonic Gelrite® solutions, the gels remain in the human eye for 20 h [63, 64].

4.4.6. Alginates: (Ion activated)

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 [65, 66].

Mechanism: Alginate is a block –copolymer two types of monomers, β- D-mannuronic acid (M) and α-L-guluronic acid (G), arranged as homopolymeric blocks of M-M blocks or G-G blocks together with blocks of alternating sequence (M-G). The polymer forms 3-dimensional Ionotropic hydrogel matrices, generally by the preferential interaction of calcium ions with G moieties resulting in the formation of inhomogeneous gels. The characteristic properties of these hydrogels, such as mechanical strength and porosity, are

dependent upon the G:M ratios, type of ionic cross-linker (bi- or poly- valent cations) and concentration and viscosity of the initial alginate solution etc [67, 68].

4.5. *Evaluation parameter:* following evaluation parameters generally followed for *In-situ* gel.

4.5.1. *Clarity:* The clarity of the formulations before and after gelling is to be determined by visual examination of the formulations under light, alternatively against white and black backgrounds [69, 70].

4.5.2. *Morphological Analysis:* SEM characterization: The gelling ability of the prepared formulations determined either visually or by studying the surface morphology of the formulations at solution state and at gel state by using scanning electron microscopy (S.E.M). By SEM image we can study compact and loose surface morphology of *In-situ* gel [71].

4.5.3. *Rheological studies:* Rheology of formulation need to be determined before and after gelation by using either the Brookfield's viscometer (RVT model) in the small volume adaptor or Cone and plate geometry viscometer (Brookfield RVCP DV-III). Also rheological study needs to be performed for formulations with and without drug to analyze the effect of addition of drug on rheological behavior of polymer blend [69].

4.5.4. *Drug Polymer interaction study:* Interaction studies can be performed in three ways one is by using UV, second is by taking IR spectra and third is by using DSC instrument. In first method by UV the solutions of Polymer and drug prepared separately and in combinations and are autoclaved. The ultraviolet spectra taken before and after autoclaving using double beam ultraviolet-visible spectrophotometer. Compare both the spectra for any possible change in solution content due to interactions between different ingredients [72]. In the second method the IR spectra the FTIR graph of pure drug and combination of drug with excipient are recorded, then compared [1] and in the third method DSC scan is runned for individual component and the mixture for the interaction study [71]. The interaction studies were carried out to check any possible physiochemical interaction among the formulation ingredients. If UV spectra, IR spectra and DSC graph of the ingredients before and after mixing found to be identical and no additional peak emerged or existent peak shifted that confirms the formulation ingredients were compatible to each other and no physicochemical reactions taking place.

4.5.5. *Gelling capacity:* The gelling capacity is determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid which should be freshly prepared and equilibrated at 37^0C. After this visual assessment for the gel formation is performed, the time of gelation and the time taken for the gel formed to dissolve is to be noted. The composition of the artificial tear fluid: Sodium chloride 0.670g, Sodium bicarbonate 0.200g, Calcium chloride dihydrate 0.008g, and purified water q.s. 100 g [6].

4.5.6. *Texture Analysis* [73]*:* Texture analyses provide information on mechanical properties of samples, namely hardness, compressibility and adhesiveness. These properties can be directly correlated with Sensory parameters in vivo and therefore, are valuable in the development of product with desirable attributes that contribute to patient acceptability and compliance [74].

- Hardness: perform to measure the force required to produce deformation of the gels [75].
- Compressibility: measures the work required to achieve compression of the product along a definite distance.
- Adhesiveness: work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe [76].

Texture analysis performed using TA-XT2 Texture analyzer. Formulation (35 gm) is to be taken in 50 ml bottles, taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (35 mm diameter) forced down into each sample at defined rate (1 mm/s) and to a defined depth (10 mm).

4.5.7. In Vitro Drug Release Profile: can be studied by either of the following method:

4.5.7.1. This study performed in the Dialysis tube containing 1 ml of the formulation, which is then suspended in beaker at 37 ± 0.5^0C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring. Aliquots of medium withdrawn at different time intervals and equal volumes of fresh media added to replace the withdrawn samples. Withdrawn samples studied for the drug content by using suitable analytical method, then calculate the cumulative percent drug release [72].

4.5.7.2. *In vitro* release studies can also be carried out by using bi-chambered donor receiver compartment model (Franz diffusion cell). In this method 1ml of gel spread uniformly on a dialysis membrane, which is then contacted with receptor medium which is stirred continuously at 20 r.p.m to simulate blinking action of eyelids. Samples withdrawn at periodic intervals and analyzed for concentration of drug by using suitable analytical method [77].

4.5.8. In-Vitro Transcorneal Permeation Study: Goat corneas often used to study the permeation of drug across the corneal membrane. Whole eyeballs of goat procured from a slaughter house and transported to laboratory in cold condition in normal saline maintained at 4^0C. Then corneas carefully removed along with a 5–6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas kept in cold freshly prepared solution of tear buffer of pH 7.4. This study performed by using modified Franz diffusion chamber. The upper chamber serves as a donor compartment in which 100 *µ*l of drug solution / *In-situ* gel formulation under study placed. The upper and lower chamber separated by goat cornea. The lower chamber served as a receiver compartment which is infused continuously with simulated tear fluid at the rate of 20 *µ*l/min. The whole system maintained at 37 ± 0.5^0C. Collect the perfusate at periodic time intervals and analyzed for concentration of drug by using suitable analytical method [72].

4.5.9. *In-vivo precorneal residence measurement in Rabbit:* The rabbits weighing 1.5-2 kg generally used for this study, during study 25 µl of formulation instilled in to the centre of lower cul-de-sac of rabbit eye. The rabbits lacrimal fluid 2 µl will be withdrawn from the conjunctival sac after predetermine intervals by means of capillary for the determination of residence time and concentration of drug [78].

4.5.10. *Gamma Scintigraphy study:* In this study the rabbits of either sex weighing 2–3 kg are used. The radiolabeled drug solution will then mixed with other formulation ingredients and required concentration of polymers. Gamma camera is autotuned to detect the radiation of radiolabeled material. Rabbits need to be anesthetized, then they are positioned under gamma camera and 25 *µ*l of the radiolabeled drug solution and *in-situ* gel formulation containing radiolabeled drug instilled onto the left corneal surface of the rabbits (two groups). Recording will be conducted 5 sec after instillation and continued for 20 min using 128 ×128 pixel matrix. Individual 68 frames (68×16 sec) will be captured by dynamic imaging process. Region of interest (ROI) will be selected on the one frame of the image and time-activity curve will be plotted to calculate the rate of drainage from eye. A single whole body static image also will be taken after 2 hr of instillation of drug

solution and formulation to see whether the drug entering in the blood or not [72].

4.6. General Safety Consideration [79]:

4.6.1. ***Sterility:*** Every ophthalmic product must be manufactured sterile and proved sterile on a lot basis before release of the product to the marketplace. USP XXI recognizes five methods of achieving sterilization, steam sterilization, dry heat sterilization, sterilization by filtration, gas sterilization and sterilization by ionizing radiation. It is the manufacturers responsibility to ensure the safety and efficacy of the process and the absence of any adverse effect on the product, such as the possible formation of toxic substances, which is an ever-present possibility with gas sterilization or when using ionizing radiation.

4.6.2. ***Ocular Toxicity and Irritation****:* Assessment of the ocular irritation potential of ophthalmic solutions represents an extremely important step in the development of both over-the-counter and prescription pharmaceuticals. Albino rabbits are currently used to test the ocular toxicity and irritation of ophthalmic formulations. Various governmental agencies have published guidelines for eye irritancy studies. These guidelines are directed towards ophthalmic formulations, chemicals, cosmetics, ophthalmic containers, and the materials that may accidentally or intentionally contact the eye during use.

4.6.3. ***Preservation****:* The FDA required that all ophthalmic solutions be manufactured sterile. Preservatives are included as major component of multiple dose eye solutions for the primary purpose of maintaining sterility of the product after opening and during use.

5. DIFFERENT COMBINATIONS OF POLYMERS FOR IN-SITU GEL

5.1. J. Padma Preetha *et al* prepared diclofenac sodium *in- situ* ophthalmic gels by using different combination of Alginate with different cellulose derivative. Author prepared the different combinations of Alginate, Hydroxy propyl methyl cellulose, hydroxy propyl cellulose, hydroxy ethyl cellulose and carbopol. Conclusion were drawn by varying the concentration of polymers with two different gums ratio there is increased residence time and sustained drug release was found [45].

5.2. Suresh V Kulkarni et al, prepared Latanoprost ophthalmic gel with the aim of promoting the prolong release of drug using Carbopol, HPMC, and HPC polymers. The prepared gels showed similar decrease in Intraocular pressure (IOP) as that of standard Latanoprost eye drops and was found that ophthalmic gels exhibited prolonged anti glaucoma activity over a period of 6 hrs, where as eye drop showed shorter duration of action. The results indicate that controlled drug delivery is a viable alternative in improving the therapeutic index [77].

5.3. Sathali A et al, formulated the levofloxain *in-situ* gelling system by using poly acrylic acid in combination with hydroxy propyl methyl cellulose (HPMC) which acted as viscosity enhancing agent. Carbopol (0.5%) and HPMC-E50LV (1.5%) showed better sustaining effect. The same formulation were compared with marketed Levofloxacin formulation for *in-vitro* release , it was found that at the end of two hours the release was 32.86% for that formulation and 99.05% for Marketed formulation [1].

5.4. Sirish Vodithala et al, formulated Ketorolac tromethamine gelling system involving Gellan gum (Gelrite®) as polymer. Gelrite® was selected as polymers for ion activated ocular gels due to its gelling property. Gelrite® in the concentrations of 0.75% w/v was found to be better carrier system because it shows optimum gelation. With the increase in the concentration of Gelrite the gelation capacity was found to be increased. Author concludes that the developed formulations containing

0.75% w/v of Gelrite® showed sustained release of drug for 6 hrs [69].

5.5. Basavaraj K. Nanjwade et al, formulated and evaluated an ophthalmic delivery system for a nonsteroidal anti-inflammatory drug, Ketorolac tromethamine, based on the concept of pH-triggered *in-situ* gelation where they used Polyacrylic acid (Carbopol® 934) (0.4% w/v) in combination with hydroxy propyl methyl cellulose (Methocel K4M) (0.5% w/v) which acted as a viscosity enhancing agent. The developed formulation provided sustained drug release over an 8-h period. Nanjwade et al, from experiment concluded that the developed formulation will be a viable alternative to conventional eye drops by virtue of its ability to enhance Bioavailability through its longer precorneal residence time and ability to produce sustained drug release [71].

5.6. Swati Gupta et al, formulated Carbopol/Chitosan Based pH Triggered *In-Situ* Gelling System for Ocular Delivery of Timolol Maleate with the aim of increasing poor bioavailability, therapeutic response and to reduce the systemic side effects. Polyacrylic acid (carbopol) was used as the gelling agent in combination with chitosan, which acts as a viscosity-enhancing agent. The 0.4% w/v carbopol/0.5% w/v chitosan based *in-situ* gelling system was in liquid state at room temperature and at the pH formulated (pH 4.6) and underwent rapid transition into the viscous gel phase at the pH of the tear fluid (lacrimal fluid) (pH 7.4). The *in vitro* drug release and *in vivo* effects of the developed *in situ* gelling system were compared with that of Glucomol® (a 0.25% Timolol ophthalmic solution). The results clearly demonstrated that developed carbopol-chitosan based formulation was therapeutically efficacious and showed a fickian (diffusion controlled) type of release behavior over 24 h periods. [80].

5.7. Sindhu Abraham et al studied sustained Ophthalmic Delivery Of Ofloxacin From An Ion-Activated *In-Situ* Gelling System. Sodium alginate was used as the gelling agent in combination with HPC (Hydroxy Propyl Cellulose) that acted as a viscosity-enhancing agent. *In vitro* release studies indicated that the alginate/HPC solution retained the drug better than the alginate or HPC solutions alone. Formulation containing Sodium Alginate (1.5 % w/v) and HPC (0.5 % w/v) gave sustained drug release for 8 hours, which may be due to the higher concentration of sodium alginate along with HPC[66].

5.8. Himanshu Gupta et al formulated Sustained Ocular Drug Delivery for Timolol maleate from a Temperature and pH Triggered Novel polymer combinations of Chitosan / Pluronic F-127. A concentration of 0.25% chitosan and 9.0% Pluronic F-127 was selected as it had satisfactory attributes of viscosity and gelling capacity. Formulation was liquid at room temperature and at the pH formulated (pH 6.0–6.2) it underwent rapid transition into the gel phase at the pH of the tear fluid (pH 7.4) and physiological temperature (37^0C) [72].

5.9. Hong – Ru Lin, K. C. Sung formulated and characterized series of Carbopol / Pluronic phase change solutions for ophthalmic drug delivery for Pilocarpine hydrochloride. It was found that the optimum concentration of carbopol 934P solution for the *in-situ* gel system was 0.3% w/w, that of Pluronic F-127 solution was 14% w/w. *In-vitro* release studies Pilocarpine containing 0.3% carbopol / 14% Pluronic solution showed that only 1.3% Pilocarpine released in first minute, approximately 76% released after 6 hrs. *In-vivo* results indicated that 1.85 fold increases in total miotic response was obtained for the carbopol/Pluronic solution relative to the drug containing STF. Authors from *in-vitro* release and *in-vivo* pharmacological studies concluded that the carbopol/Pluronic solution had the better ability to retain drug than the carbopol or Pluronic solutions alone [49].

5.10. Tais Gratieri et al formulated Poloxamer/Chitosan gel with the aim of achieving prolonged retention time for ocular delivery by improving mechanical and mucoadhesive properties. Chitosan improves the mechanical strength and texture properties of Poloxamer formulations and also confers mucoadhesive properties in a concentration dependent manner. After a 10 min instillation of Poloxamer/chitosan 16:1 formulation in human eyes, 50-60% of the gel was still in contact with the cornea surface, which represents a fourfold increased retention in comparison with a conventional solution [73].

5.11. B. Srividya formulated and evaluated ophthalmic pH triggered *in-situ* delivery of Ofloxacin with the aim of getting sustained drug delivery. Aqueous solutions of varying concentrations of Carbopol 940 and HPMC of different grades were prepared and evaluated for gelling capacity and viscosity so as to understand the suitable composition of polymer. Authors finally selected 1.5% Methocel E50LV and 0.5% Carbopol 940 because of its satisfactory viscosity and gelling capacity. Results show that gel formed *in-situ* afforded sustained release over an 8 hr period [6].

5.12. Smadar Cohen et al studied novel *in-situ* forming ophthalmic drug delivery system of Pilocarpine from Alginates, where they used two grade viz. Manugel DMB (Alginate with guluronic acid more than 65%) and Kelton LV (low guluronic acid). *In-vitro* studies indicated that Pilocarpine is released slowly from alginate gel, over a 24 hrs. Intraocular pressure measurement of rabbit eyes treated with 2% w/v Pilocarpine nitrate in solution, or in the *in-situ* gel forming formulation composed of the high guluronic acid content DMB alginate, indicated that DMB significantly extended the duration of the pressure reducing effect of Pilocarpine, to 10 hrs, as compared to the 3hrs when Pilocarpine nitrate was delivered as a solution. In contrast, there was no apparent difference in the duration and extent of intraocular pressure decrease between rabbits treated with Pilocarpine in solution or in the Kelton LV alginate eye drop formulations [67].

6. CONCLUSION

- Conventional ophthalmic formulations are found to be insufficient for their activity because of less retention time of the formulation in ocular cavity.
- Less than 10% of the administered dose could cross the membrane.
- To satisfy the need, novel formulation with *In-situ* gelling technique came in the picture.
- This is able to solve the problem to a good extent.

7. FUTURE PROSPECT

- To find the other triggering factors for the *in-situ* gel formation.
- To increase the proportion of the drug that cross the corneal membrane by reducing the rapid elimination.
- To search for the penetration enhancers devoid of side effects.
- To target the drug in the posterior chamber through non-invasive route.

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