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FORMULATION AND EVALUATION OF THERMO SENSITIVE *IN-SITU* GEL FOR LOCAL ACTION: A REVIEW

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Abstract: Eye is the most sensitive part of body. Now a days in market there are not much types of formulations. The major trouble encountered is quick precornel drug loss. To improve ophthalmic drug BABE study, there are considerable efforts directed towards newer drug delivery systems for ophthalmic administration. Newer research in ophthalmic drug delivery systems is directed towards amalgamation of several drug delivery technologies, that includes to build up systems which is not only extend the contact time of the vehicle at the ocular surface, but which at the same time slow down the removal of the drug. There are various new dosage forms like *in-situ* gel, collagen shield, minidisc, ocular film, ocusert, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis etc. Conventional delivery systems often result in poor bioavailability and therapeutic response because high tear fluids turn over and dynamics cause rapid elimination of the drug from the eyes. For that, minimization of bioavailability problems, ophthalmic in situ gels were developed. In this review, ophthalmic *in-situ*gel having thermosensitive approach discussed.

Keywords: Sol-to-gel, Photopolymerization, Thermal analysis, Ocular irritancy test, Evaluation

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INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist. The anatomy, physiology, and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [1]. The extent of absorption of an ophthalmic drug is severely limited by physiological constraints. Among the factors that limit ocular absorption is the relatively impermeable corneal barrier. The cornea consists of three membranes, the epithelium, the endothelium and inner stroma which are the main absorptive barriers. The epithelium facing the tears with lipophilic cellular layers, acts as a barrier to ion transport. The tight junctions of the corneal epithelium serve as a selective barrier for small molecules and they prevent the diffusion of macromolecules via the paracellular route. The stroma beneath the epithelium is a highly hydrophilic layer making up 90% of the cornea. The corneal endothelium is responsiblefor maintaining normal corneal hydration. Clearly then, the more lipophilic the drugs are, the more resistance they will find crossing the stroma. The more hydrophilic a drug, the more resistant the epithelium, whereas the stroma and endothelium are limited in their resistance.

Great efforts are being directed towards the refabrication of existing drug molecules in a fashion, capable of solving problem related to poor water solubility, poor bioavailability, dosing problem, stability, toxicity etc. This trend of working has led to the development of new drug delivery system. Eye, as a portal for drug delivery is generally used for the local therapy as against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of drug, which are not intended for eye.1) Most of the ocular treatments call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. Several types of dosage forms can be applied as the delivery systems for the ocular delivery of drugs. The most prescribed conventional ocular dosage forms for the de-livery of drugs are eye drops, eye ointments and suspensions have major disadvantages like poor bioavailability due to rapid precorneal elimination, normal tears turnover and conjunctiva absorption, frequent instillation of concentrated medication, side effects due to systemic absorption of drugs, blurred vision due to presence of viscous vehicles.2) The present study aims at formulating ocular inserts using biodegradable polymers to overcome the drawbacks of conventional eye preparations.

APPROACHES OF *In Situ* GEL DRUG DELIVERY

There are four broadly defined mechanisms used fortriggering the in situ gelformation of biomaterials: Physiological stimuli (e.g., temperature and pH), physical changes in biomaterials

(e.g., solvent exchange and swelling), and chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).In situ formation based on physiological stimuli.

Thermally trigged system:

Temperature-sensitive hydrogels are probably the mostcommonly studied class of environment-sensitive polymer systems in drug delivery research5. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The ideal critical temperature range for such system is ambient and physiologic temperature, such that clinical manipulation is fascinated and no external source of heat other than that of body is required for triggergelation. A useful system should be tailorableto account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity.

Three main strategies are exists in engineering of thermoresponsive sol-gel polymeric system. For convenience, temperature-sensitive hydrogels are classified into negatively thermosensitive, positively thermosensitive, and thermally reversible gels [2]. Negative temperature-sensitive hydrogels have alower critical solution temperature (LCST) and contract upon heating above the LCST. Polymers with low critical temperature (LCST) transitionbetween ambient and physiologic temperature is used for this purpose. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (N- isopropylacrylamide) (PNIPAAm). PNIPAAm is a water soluble polymer at its low LCST, but hydrophobic above LCST, which result onprecipitation of PNIPAAm from the solution at the LCST. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO- PEO) triblock co-polymer that are fluid at low temperature, but forms thermo responsible gel when heated as a consequences of a disorder-order transition in micelle packing which makes these polymerssuitable for in situ gelation6. A positive temperature-sensitive hydrogel has an upper critical solution temperature (UCST), such hydrogel contracts uponcooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAM) or poly (acrylamide-co-butyl methacrylate) havepositive temperature dependence of swelling7. The most commonly used thermoreversible gels are these prepared from poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (Pluronics®, Tetronics®, poloxamer). Polymer solution is a free- flowing liquid at ambient temperature and gels at body temperature8. Cappello ET al.developed novel “protein polymers” ProLastins, which undergo an irreversible sol gel transition. When injected as a solution into the body, the material forms a firm, stable gel within minutes. It remains at the site of injection providing absorption times from less than one week to manymonths. Such a system would be easy to administer into desired body cavity. [3]

pH triggered systems:

Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH.The polymers with a large number of ionisable groups are known as polyelectrolytes. Swelling of hydrogelincreases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. [4] Likewise polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition [5]. Drug formulated in liquid solutions have several limitations , including limited bioavailability and propensity to be easily removed by tear fluid.Kumar and Himmelstein sought to minimize this factors and maximize this drug delivery by making a poly (acrylic acid) (PAA) solution that would be gel at pH 7.4.The author found that at concentrations high enough to cause gelation, however, the low pHof PAA solution would cause damage to surface of eye before being neutralized by the lacrimal fluid. This problem was solved by partially by combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH responsive polymer mixtures that was solution at pH 4 and gel at pH 7.4[6]. Mixtures ofpoly (methacrylic acid) (PMA) and poly (ethylene glycol) (PEG) also has been used as a pH sensitive system to achieve gelation.[7]

***in situ* FORMATION BASED ON PHYSICAL MECHANISM:**

Swelling: *in situ*formation may also occur when material absorbs water from surrounding environment and expand to occur desired space [8]. One such substance is mineral. (Glycerol mono-oleate), which is polarlipid that swells in water to form lyotropic liquid crystalline phase structures. It has some Bioadhesive properties and can be degraded in vivo by enzymatic action.[9]

Diffusion: This methodinvolves the diffusion ofsolvent frompolymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N- Methyl pyrrolidone (NMP) has been shown to beuseful solvent for such system.[10]

In situ formation based on chemical reactions Chemical reactions that results insitu gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

Ionic crosslinking: Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones. [11] While k-carrageenan forms rigid, brittle gels in reply of small amount of K^+, i-carrageenan forms elastic gels mainly in the

presence of Ca^{2+}. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono- and divalent cations, including Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}.

Gelation of the low-methoxy pectins can be caused by divalent cations, especially $Ca2^{+}$. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca^{2+} due to the interaction with guluronic acid block in alginate chains. [12]

Enzymatic cross-linking: In situ formation catalysed by natural enzymes has notbeen investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injectedbefore gel formation.[13]

Photo-polymerisation: Photo-polymerisation is commonly used for in situformation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similarpolymerizable functional groups are typically used as the polymerizable groups on the individual monomeers amd macromers because they rapidly undergo photo-polymerisation in the presence of suitable photoinitiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2, 2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo- polymerization, whereascamphor Quinoneand ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo [14]. Photopolymerizable systems when introduced to the desired site via injection get photocured in situ with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation. A photopolymerizable, biodegradable hydrogel as a tissue contacting material and controlled release carrier is reported by Sawhney et al. [15]

MATERIALS AND METHODS

MATERIALS

Antibiotic drugs which are used in eye application, Natural gums, synthetic polymers, Gelrite, ingredient for tonicity adjustment, Benzalkoniam Chloride, buffers, NaCl (Sodium Chloride)

METHODS

Preparation of Sol-To-Gel System

Sol-to-gel systems are prepared by two methods depending on the sterilization method employed. Gamma radiation sterilization is the usual method recommended for sterilization of polymeric de- vices. To evaluate the effect of gamma radiation on the physical properties, viscous systems were compared with the formulations sterilized by filtration.

METHOD:

The calculated amount of drug are placed in a volumetric flask and dissolved in acetate buffer of pH 4.4 under aseptic conditions. The required amount of sodium chloride and Benzalkoniam chloride are added and mixed thoroughly. The resulting solution is sterilized by filtration through 0.22-mm Millipore membrane filter paper. Solutions containing both HPMC and carbopol 934 are prepared by adding appropriately weighed amounts of the HPMC and carbopol. The solutionis thoroughlymixed and equilibrated in a biologicalshaker for 24 hr. at258 °C. The mixingis continued until a uniform and clear solution was formed. The resulting solutions were sterilized by autoclaving. The solutions were again shaken for 3 h.

The solutions just described are mixed under aseptic conditions in a sterilized flask and are thoroughly shaken until a uniform and clear solution was formed. The solution is transferred into previously sterilized ambercolor Ed bottles, each with a cap that is fitted and that carried a dropper fitted with a teat.[16]

Measurement of gelation temperature

Gelation temperature is the temperatureat which the liquid phase makes a transition to gel. A gelation temperature range suitable for ophthalmic in situ gel formulation would be 33-36ºC. If the gelation temperature of is lower than 33ºC, gelation occurs at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than 36ºC, the formulation will stays as a liquid at body temperature, resulting in lacrimal drainageand washout from the corneal surface of the eye. Therefore, the formulation must have a suitable gelation temperature, 33-36ºC21, so that, it can remain in sol phase at room temperature (25ºC) and converted to a gel phaseinstantly upon administration to the cull-de-

sac. PF 127 and PF 68 are selected due to their thermosensitive gelling properties. In addition, PF 127 and PF 68 are known to have low toxicity, less skin irritation, excellent water-solubility, high solubilizing capacity for drug, good drug release characteristics and compatibility with wide range of chemicals and polymers.The prepared gels were evaluated for gelation temperature as described by Gilbert ET al22. The gelation temperature was measured by heating the formulation (1-2°C/min) in a 15 ml borosilicate glass test tube. In each test tube 2 ml of formulation solution was placed heated with gentle stirring until formulation solution gets gelled. Gel formation was considered as the point where there was no flow when the test tubes were gently immersed.[17]

EVALUATION OF IN-SITU GEL SYSTEM:

The prepared in-situ gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, in vitro diffusion study, is tonicity, antibacterial activity, in vivo ocular testing in rabbits and accelerated stability studies. The pH of in-situ gel solution was found to be 7.4 for all the formulations. The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange).

Physical parameters

The formulated in-situ gel solution is tested for clarity, pH, gelling capacity, and drug content estimation.

Gelling capacity

The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted.[18]

Rheological studies

The viscosity measurements can be calculated using Brookfield viscometer, Cone and Plate viscometer. The *in-situ* gel formulations were placed in the sampler tube. From the literature it was evident that, the formulation before gelling should have a viscosity of 5 to 1000 map's. And after ion gel- activation by the eye, will have a viscosity of from about 50-50,000 mPas10, 13. The samples are analyzed both at room temperature at 25°C and thermo stated at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement. The angular velocity of the spindle was increased 20, 30, 50, 60, 100, 200 and the viscosity of the formulation is measured. All the formulations exhibited

Newtonian and pseudo plastic flow characteristics before and after gelling in the simulated tear fluid respectively.[19]

In vitro drug release studies

In vitro release study of in-situ gel solution was carried out by usingFranz diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for 6 hrs. and same volume of fresh medium is replaced. The withdrawn samples are diluted to 10ml in a volumetric flask with respective solvent and analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using the equation generated from standard calibration curve. The % cumulative drug release (%CDR) calculated. The data obtained is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers Peppas and Fickinian diffusion mechanism for their kinetics. [20]

Texture analysis

The consistency, firmness and cohesiveness of in-situ gel are assessed by using texture profile analyzer which mainly indicated gel strength and easiness in administration in vivo. Higher values of adhesiveness of gels are needed to maintain an intimate contact with mucus surface. [21]

Isotonicity evaluation

Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations are subjected to Isotonicity testing, since they exhibited good release characteristics and gelling capacity and the requisite viscosity. Formulations are mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation. [22]

Drug polymer interaction study and thermal analysis

Interaction study can be performed with Fourier Transform Infra-Red (FTIR) spectroscopy. During gelation process the nature of the interacting forces can be evaluated using the technique by employing KBr pellet method. Thermo gravimetric Analysis (TGA) can be conducted for in situ forming polymeric system to quantitate the percentage of water in

hydrogel. Differential Scanning calorimetry (DSC) conducted to observe if there are any changes in thermo grams as compared with pure active ingredients used for gelation.[23]

Antibacterial activity

The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic. To carryout microbiological assay serial dilution method is employed.[24]

Ocular irritancy test

The Raise irritancy test was designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Raise test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of1hr, 24hrs, and 48hrs. 72hrs, and 1week after administration. Three rabbits (male) weighing 1.5 to 2kg are used for the study. Thesterile formulation is instilled twice a day for a period of 7 days, and a cross-over study is carried out (a 3 day washing period with saline was carried out before the cross-over study). Rabbits are observed periodically for redness, swelling, watering of the eye. [25. 26]

Accelerated stability studies

Formulations are placed in ambient colour vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution.[27]

Statistical analysis

The results obtained from the experiments of mucoadhesiveness strength and release studies were analysed statistically using multivariate tests. A statistically significant difference was conducted by using various SPSS software and difference was considered to be significant at $P<0.05$.[28]

CONCLUSION:

Ophthalmic drug delivery system is burgeoning field in which most of the researchers are taking challenges to combat various problems related to this delivery. Steady advancement in the understanding of principles and processes governing ocular drug absorption and disposition and continuing technological advances have surely brought some improvements in the efficacy of ophthalmic delivery systems. The primary requirement of a successful controlled release product focuses on increasing patient compliance which the in-situ gels offer. Exploitation of

polymeric in-situ gels for controlled release of various drugs provides a number of advantages over conventional dosage forms. Sustained and prolonged release ofthe drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the in-situ gel formulations can make them more acceptable and excellent drug delivery systems34-35.

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