Review Article

A review on: Smart polymer use in novel ophthalmic drug delivery systems.

Abstract

Loss of sight or vision loss are utmost concerned worldwide health problems resulting in significant financial and public loss. Eyesight helps one to capture signals from the surrounding environment and depict clear visual images. Ophthalmic drug delivery has been seen as a significant challenge because of different defensive boundaries and drug clearance system resulting from an exceptional structural and functional nature of eyes. Formulation administered into the eye in the form of regular ophthalmic formulations leads to less absorption of drug. Therefore, approaches have been carried out to advance a novel, nontoxic and effective ophthalmic drug delivery system to control the difficulties occurring by the regular formulations. Latest innovation in pharmaceutical drug design has produced stimuli responsive *in-situ* gel system providing continuous release action and increased ocular drug bioavailability. They act by undergoing phase conversion from solution to gel due to change in ocular pH, temperature and ions in the ocular environment. Stimuli transformed gel is formed in the region of cul de sac thus combating the limitations of regular ophthalmic formulations such as less retention time and fast nasolacrimal drainage within the eyes**.**

Keywords- *in-situ* gel, controlled release, pH responsive polymers, temperature responsive polymers, ion responsive polymers.

1) Introduction

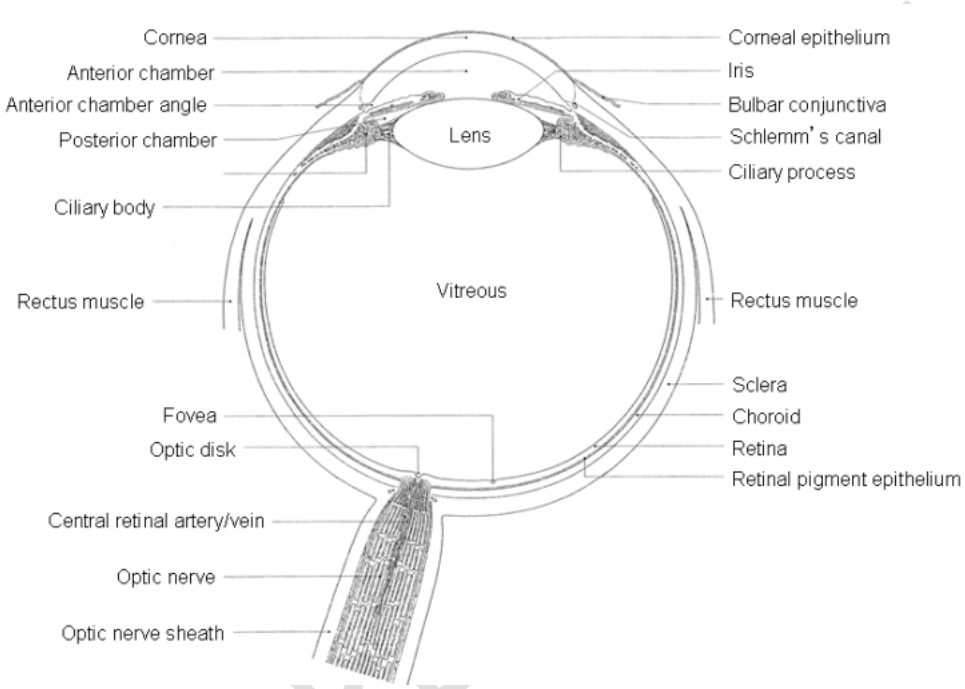
An eye is an important sensory organ that serves a doorway and receives outer images by transferring these images towards the brain in the form of signals through the optic nerve. In this way the link between the body and our surrounding is established.[1] The eye is proved to be the fundamental organ of vision. Eyeballs are located in the orbit where almost one-fifth of the orbital region is occupied by each of the two eyeballs. The unoccupied area is covered up by the blood vessels, extraocular muscles, lacrimal gland, nerves of fascia and the fats.[2] Several disease conditions can influence the eye despite the fact that it is highly secured and self-contained. Infections can be shallow in the external layer of the eye like conjunctivitis, or inside the eye like glaucoma, or the issue might be in the vitriol portion and identified with the retina, for example, age-related macula edema.[3] The eye is an expansion of the focal sensory structure. It has numerous usual physiological and anatomical features similar to cerebrum. Each of them has firm fibrous coverings, and are protected by bony walls and a double supply of blood to the vital layer of the nervous tissue. Internal compartments within brain and eye are filled fluids of similar composition and pressure. Since retina and the optic nerves arise from the brain, the diseases influencing the central nervous system and eye are similar.[2]

2) Anatomy and Physiology of eyes

Eye is vital organ in living beings where ophthalmic drug delivery system plays a very crucial role by acting directly at the site without entering into the systemic circulation[4]. Due to the distinctive structure it becomes very difficult for the entry of drug molecule into the eye to show its action at the targeted site[5]. The structure of the eye is segregated into the two sections, the frontal and the subsequent portion in which frontal part includes ciliary body, cornea, lens, aqueous humor, conjunctiva along with cornea while the subsequent part which is also known as the back of the eye deals with vitreous humor, choroid, retinal pigment

epithelium, sclera, neutral retina, optic nerve, and neutral retina. These segments are commonly affected by severe type of optic illnesses resulting in visual impairment. Diseases like cataract, glaucoma, allergic conjunctivitis affects anterior segment whereas macular degeneration and diabetic retinopathy mainly affects posterior segment.[6].

Fig no 1: Structure of an eye[7]



- The frontal (anterior) region covers around one third portion of an eye while the rest of the remaining portion of an eye comes under the posterior segment [5]. An eye is made up of 3 sections: the external part, middle part and internal part. External part mainly includes of the sclera and cornea where cornea helps to transfer the light towards retina and lens while sclera represents the connective tissue that helps to protect eye and assist in retaining its structure. Limbus is the connective point of the sclera and cornea. The middle part consists choroid and ciliary body. Choroid is in the form of vascular layer which supplies nutrients and oxygen to the coronial layer. The power of the lens and its shape is regulated by ciliary body, while the dimensions of the pupil and the capacity of incoming light to the retina is regulated by Iris. The internal part of an eye consist retina which helps in capturing and the processing of the light[8]. The cornea consists of the 90% of the epithelium cells which results in high membrane lipophilicity because of which it restricts the absorption of the drug as it forms very tight junction with protein. The lipophilic drug passes very easily through this membrane. The

absorption of the drug takes place through various mechanisms like paracellular, carrier mediated, transcellular, receptor mediated transport and active mechanisms in which paracellular is prevented by the corneal tight junction[9]. The structure of the cornea is completed with the help of 5 layers, which include bowman's layer and epithelium, Descemet's membrane, stroma, endothelium and the basement membrane[10].

3) Factors affecting ocular bioavailability.

Human optic structure is equipped with ocular barriers to avoid hazardous chemical substances as well as therapeutic drug substance from entering distinctive tissues of an eye. Retinal-blood barriers and tear dilution conjunctival completes the dynamic whereas the blood aqueous barrier, corneal stroma, corneal epithelium corneal endothelium deals with the static barrier hamper drug uptake which affect therapeutic efficiency of the topical formulations (<5%).[11] Following are few obstacles to ophthalmic drug distribution and bioactivity.[3]

a. Tears

The tear film is the primary hindrance which quickly eliminates introduced formulation from the eye, leading to limited drug absorption.[12] Because of the dilution of formulation by the tear turnover (about 1 μL/min), faster elimination and interaction of drug with tear proteins, the absorption rate of the administered medication decreases. Furthermore, the amount of the administration of the dose is often 20μL (micro litre) –50 μL(micro litre) while the capacity of cul-de-sac is just 7 –10 μL(micro litre). As a result, the surplus volume exits from the duct of nasolacrimal drainage, thus decreasing the therapeutic efficiency of the drug.[13]

b. Reflex blinking

With a typical capacity of 39 μL(micro litre), a standard eye dropper dispenses 25–56 μL(micro litre) of the preparation. On the other hand, an eye can temporarily contain approx. 30 μL(micro litre) of the drug with the remainder being eliminated by reflex blinking (5–7 blinks/min) or nasolacrimal drainage, thus reducing the quantity of the medication accessible for pharmacological activity.[14]

c. Cornea

The cornea comprises of numerous layers: Descemet's membrane, Bowman's layer, basement membrane, endothelium, stroma and corneal epithelium. It is highly specialized, transparent and avascular tissue.[15] The cornea is expected to be the primary route for drug absorption into the front chamber. It is formed of multiple levels and is roughly 0.5 mm wide in the middle, widening to nearly 0.7 mm (milli metre) at the outer edge.

- Epithelium has 56 layers of squamous stratified cells (rising to 810 layers at the edge), has a cumulative thickness of 50100 mm, and a cell layer turnover rate of roughly one per day. The strong interconnections and hydrophobic regions make it the most important restriction for the absorption of the drug.
- Bowman's membrane is a 814 mm-thick acellular uniform layer. It is arranged between the membrane of basement epithelium and the stroma.
- Almost 90% of the corneal thickness is made up of stroma, also known as substantia propria. It is mostly made up of water, with roughly 200–250 collagenous lamellae layered on the top of one another and extending equivalent to the surface. The physical strength and optical transparency are provided by lamellae. Structurally the stroma seems to be open which allows hydrophilic solutes to circulate freely.
- Descemet's membrane is sandwiched between the endothelium and stroma. It is generated by endothelium.
- Endothelium is a thin film of compressed hexagonal cells 5 mm thick and 20 mm broad which is crucial to preserve appropriate corneal moisture. The endothelium is in close interaction with the front compartment and hence it experiences continuous inflow of watery fluid through aqueous humor which further enters stroma. To efficiently penetrate the cornea, formulation must have both polar and lipid soluble characteristics, as well as have small atomic size to pass across complex connections.[16]

d. Conjunctiva

Owing to the combination of lymphatics and conjunctival blood capillaries that may lead to considerable medication escape into the blood stream thus decreasing therapeutic efficiency, conjunctival drug uptake is believed to be inefficient as compared to corneal drug penetration. Inactive transport of polar drug molecule can be slowed even more by conjunctival epithelium complex cell inter connections.[17]

e. Sclera

Considering polar substances migrating through the collagen content, the sclera seems to have greater trans scleral penetration as compared to cornea. The molecular radius, molecular weight and charge all play a significant role in sclera penetration. All particles penetrate scleral holes more easily unlike macromolecules. Hence macromolecules such as anti-VEGF agents possess small distribution rate through the sclera and must be given intravitreally.[11]

f. Choroid/Bruch's Membrane

The supply of blood to the retina is done with the help of choroid which is the most extremely vascularized tissue of the body. An OCT (Optical Coherence Tomography) can detect the size of choroid and retina. The study of OCT shows that the thickness of the choroid decreases with ageing while the broadness of the membrane increases with age. Increase in the turnover of glycosaminoglycans, cross-linking of collagen fibres, and calcification of elastic fibres all are the effects of these variations. Absorptivity of the drug from the episcleral or subconjunctiva area into the vitreous and retina may be affected by choroid and BM thickness alterations.[13]

g. Blood-Ocular Barriers

Blood ophthalmic obstacles are the combinations of blood-aqueous and blood-retinal barriers. These are real limitations between the eye and blood that plays a significant role in drug absorption, ocular route drug excretion, and preserving homeostatic regulation.[18] The BRB (blood-retina barrier) consist of the two constituents, viz, (i) the RPE (retinal pigment epithelium) cells and (ii) the endothelium (complex walls) of retinal capillaries, which represent the external and internal BRB, respectively. This barrier creates an obstacle for the formulation to enter into the retina. BRB turns as a significant obstacle which avoid the entrance of the unwanted substances into the retina through choroid. The entrance of the medication into the highly vascularized choroid is very quick after systemic delivery, but they often fail to cross the BRB. Due to its specific structure, the BRB is highly active at providing its obstructive role.[20]

h. Drug metabolism

Several enzymes are present in both anterior and the posterior segments (cytochrome P450, ketone reductase, cyclooxygenase) which carry out metabolism of the active drug administered into the eye. This further leads to reduction of ophthalmic drug bioavailability.[21]

4) Novel Ophthalmic Drug Delivery

1. Thermosensitive polymers

One of the most frequently examine group of stimuli responsive polymeric material in drug delivery studies is thermally responsive drug gel formulations[22]. The liquid to gel phase transformation is induced by a change in temperature caused due to an increase in hydrophobicity in the temperature sensitive gelling systems[23]. Temperature responsive polymers that transform from sol-gel phase at the temperature of the eye (37^{o}C) can be used to attain continuous drug delivery. While developing a temperature sensitive gelling system there are three primary options available. They are positively thermo responsive, negatively thermo responsive and thermally reversible gel.[24] Polymers with a positive temperature profile of swelling, contract when cooled below their UCST, separating them into two phases.[25] Negative thermo-responsive in-situ gels consists a sufficient LCST (low critical solution temperature) than positive thermo-responsive gels and shrink when heated beyond the LCST. This will be accomplished by using polymers having a LCST (low critical solution temperature) that change within the physiologic and ambient temperatures. At room temperature (20-25°C), the preparation is liquid, however when it interacts with ocular environment (35-37°C), it gels. Gelling occurs due to degeneration of long chain of polymers which in turn causes the emergence of lipophilic regions and the conversion of watery fluid to in-situ gel. Poloxamer's (Synthetic polymers) and PNIPAAM (Poly N-isopropylacrylamide) and Natural polymers (cellulose derivatives) are the most often utilized polymers.
Eg: Poloxamer/Pluronic's, Cellulose derivative.[26]

Mechanism of gel formation:

The temperature triggered in-situ gel process employs temperature responsive polymers which are liquid below their low critical solution temperature (LCST) but gels once the ambient temperature exceeds the LCST. [27] They occur as clear, homogeneous, free-flowing polymeric solutions at temperatures just under the low critical solution temperature (LCST) but turn turbid once they approach the low critical solution temperature (LCST). Beyond the LCST, phase transition begins, splitting the solution into a solvent and a gel phase, which is usually liquid. It is mostly because of the entropy effect, that favors phase separation as temperature rises. [28]

2. pH-responsive polymers

A further essential bioenvironmental component at the ocular location involves pH, which develops a gel almost instantly when exposed to bio-stimulus. [22] Researchers have witnessed significant pH variations in the human body that could be employed to target curative medicines to a particular bodily location, tissue, as well as cellular division. As a result of such characteristics, pH responsive polymers are appropriate medicinal methods for precise therapeutic drug delivery. When the pH of a solution changes, pH responsive polymers alter their structure and physical features like chain arrangement, surface activity solubility, and so forth. [29] The capacity of receiving or donating protons in accordance to pH variations is a significant characteristic of ionic pH responsive polymers. These polymers have carboxylic or sulphonic (acidic) or ammonium salts (basic) groups in their structure. [30] The pH-responsive systems are ionizable with a pKa value between 3 and 10, and therefore is one of the most investigated stimuli-response systems. Polyacids, polybases, or a blend of both make up pH-responsive polymers. There are four different types of pH-responsive polymers: Artificial polypeptides, biopolymers weak polyacids, polyacids and pH-responsive degradable polymers. The extent of ionization of the polyelectrolyte is caused by changes in the surrounding pH, that affects the solubility or structure of the polyelectrolyte. The swelling and deswelling of a polymer is caused by changing the pH of a solution. As a result, medication devices composed of such polymers will have pH-dependent release profile. [31]

Eg: Carbomers (polyacrylic acids, PAA) and Cellulose acetate phthalate latex

Mechanism of gel formation

Alteration in the ionization potential of the polyelectrolyte's which have carboxylic or phosphoric (weakly acidic) or ammonium (weakly basic) groups cause the sol-gel phase transformation. The pH at which these groups ionize is determined by their pKa values (3–10) and also the polymer's molecular weight. Modifications in any of these group's ionization states induce alterations in structure and solubility, and also system swelling. The ionic strength, temperature and salt concentration all influence the process of forming gel and features of certain pH sensitive polymers. [28]

3. Ion-responsive polymers

In the context of an ionic environment given by the ophthalmic surface (Ca++ and several other ions found in tear fluid), some polymers undergo phase transitions. Therefore, the anionic character of these polymers leads to the connection among the oppositely charged ions and polymers, which has been intensively studied in the production of the in situ gel system for ophthalmic drug delivery. [22] Due to an ionic property of these polymers, they form a strong association with different charge particles. As these electrostatic forces approach unity, because of which shift occurs in the arrangement of polymer, which results in the transformation of sol-gel phase. [32]

Ex: Gellan gum and sodium alginate.

Method of formation of gel:

Anionic polysaccharides reinforced with Na^{+} (monovalent) and Mg^{2+} and Ca^{2+} (divalent) cations present in the lacrimal fluid can cause the solution to gel phase transformation, thus increasing the viscosity of polymer. The viscosity and concentration of cations are correlated to each other. Hence increase in viscosity results in increase of concentration of cation. As a result, the dilution of viscous solution takes place due to increase in the formation of tear which leads in greater concentration of cation and hence viscosity of the polymer also increases. This further leads to improved drug ophthalmic retention time, increasing therapeutic efficiency and decreased nasolacrimal drainage.

4. Enzyme-Responsive polymers

Enzymes are bioabsorbable in nature, work in moderate surroundings, and have a high degree of selectivity, all of which make them suitable candidates for stimuli-sensitive release triggers. These enzymes can be degrading enzymes or the enzymes that are overexpressed in pathological situations (disease-specific). A study found that an enzyme-sensitive hydrogel using HNE (human neutrophil elastase), a triggering enzyme released by neutrophils at the area of inflammation, was effective in the administration of anti-inflammatory medicines. Due to the presence of neutrophils, release of the hydrogel takes place at the site of the inflammation and execute site-specific and local delivery, preventing systemic medication associated adverse-effects. For the sensitivity of the hydrogel, HNE-sensitive linkers and model peptides were added to PEGDA (poly (ethylene glycol) diacrylate). After diffusion of HNE into the hydrogel it breaks the substrate thus releasing the drug at targeted site. In vitro, the peptides was eliminated in an organized behavior in the presence of HNE.[33]

5. Multi stimuli responsive polymers

The use of the mixture of polymers with various gelling mechanisms, results in the enhanced therapeutic efficacy and the compliance of the patient, which is the unique strategy involved in the ophthalmic in-situ gelling systems. The various studies as per the literature includes the use of pH-sensitive, ion activated or thermo-responsive polymers in the similar ophthalmic preparation have been available in current years. Multi-responsive systems have potential to preserve the principal medication until the desired target is achieved.[34] Scientist used a mixture of pH and ion activated gelling systems i.e. methylcellulose and sodium alginate, which is used to design and analyze a sparfloxacin-loaded new in-situ gelling system for prolonged optic drug administration. The formulation was in the form of a solution at pH 4.7 and underwent rapid transformation from sol-gel which resulted in the rise of the pH to about 7.4. As compared with the eyedrops, the in-situ gel preparation exhibited continuous in vitro delivery of sparfloxacin absorption for more than a 24 hr period. [27]

Eg: Poly(ethylene glycol) methyl ether methacrylate

5) Estimation of *In-situ* gel formulations.

The parameters for the assesment of the in-situ gel preparations consist of pH determination, medication concentration, isotonicity, gelling capacity, rheological examination, in vitro diffusion study in vivo ocular testing in rabbits, antibacterial activity, clarity and accelerated stability studies. The medication should possess a required viscosity which permit it's compatible administration into the eye in the form of drops (liquid), which undergoes a quick transformation from solution to gel phase (activated by ion exchange, temperature or pH)[35]

1. **Determination of Clarity**

The clarity of the preparations earlier and later the process of gelling can be identified with the help of visual inspection below light or against white and black backgrounds.[26]

2. **pH Determination**

Determination of the pH is performed for all the preparations immediately after the sample is prepared and documented with the help of the digital pH meter.[36]

3. **Gelling capacity**

By introducing a small amount of the created preparation into an ampoule holding 2 mL of newly produced STF (simulated tear fluid) and immediately observing it, the gelling capability of the preparation can be evaluated. The amount of time it takes for the gel to set is recorded.[37]

4. **HET CAM TEST**

The Draize eye irritation test is one of the most important method to determine the injuries that are cause to the animals which are used in the experiments. Many of the invitro methods are used in the determination of the toxicity of the eye irritants by replacing it with in vivo eye irritation testing. HET-CAM (Hens Egg Test Chorioallantoic Membrane) examination is one of the alternative methods. HET-CAM test and Draize eye irritation test are carried out to avoid the potential risk cause on human, test animals due to the effect of chemical and helps to determine the harmful effect on the living things. Due to the irregular storage of these contaminated chemicals or during the manufacturing process, human or the animals directly

come in contact with these agents and it can cause injury. Draize eye irritation test is the very commonly used test for the detection of the ocular irritation of the eye, as this test has been used for more than 40 years. This test is proved to be very common and important but it may be painful for the test animals and because of this, from the ethical point of view this method is unaccepted. To overcome this problem Draize eye irritation test is replaced by Hens Egg Test Chorioallantoic Membrane (HET-CAM) test. The HET-CAM test, is carried out with the use of the CAM (chorioallantoic membrane) of the embryo of the chick. In the HET-CAM test, CAM is made up of the tissue which consist of arteries, capillaries and veins, and it is easy for the study. With the help of this method, chemicals are directly sited in the contact with the CAM of the hens egg.[38] The purpose of this method is to mark out the components and the procedures which are used to determine the ocular irritancy of the test substance.[39]

5. Rheological studies

Cone viscometers, Plate viscometers and Brookfield viscometers can all be used to determine consistency. In a test tube, the in-situ gel preparation is put. Before gelling, the preparation should have a consistency of 5m Pas-1000 m Pas, and after the development of the gel, it should possess a consistency of 50-50,000 m Pas.. [35]

6. Viscosity and rheology

This is a crucial point to consider when evaluating *in-situ* gels. The rheological and the viscosity characteristics of the polymeric preparations were evaluated using a Brookfield viscometer or Ostwald's viscometer. The preparation should be consistent enough to produce effect while also should show patient compliance particularly during intravenous and ophthalmic administration..[40]

7. Evaluation of the Texture analysis

The texture characteristic analyzer is used to examine the uniformity, stiffness, and compactness of the in-situ gel, which primarily signifies the strength of the gel and ease of injection in vivo. To sustain a close contact with the mucus surface, greater adhesiveness values of gels are required. [41]

8. Isotonicity evaluation

The isotonicity of ocular formulations is a significant feature. To avoid cellular injury or eye discomfort, isotonicity must be achieved. Isotonicity test is carried out on all ocular formulations that have acceptable drug release, gelling capability, and the required viscosity.[37]

9. Gamma scintigraphy studies in vivo:

Gamma scintigraphy is a well-known method for determining in vivo optic residence time. Even though rabbit is most frequently used animal for testing optic preparations, human subjects are favored for this research due to physiological variations between humans and rabbits, primarily the rate of blinking.[42]

10. Determination of the interaction of drug-polymer study and thermal analysis

Fourier Transform Infrared spectroscopy (FTIR) should be used to investigate Drug-polymer relationships. Using the KBr pellet approach, the type of the associated forces can be determined during the process of gelation. For in situ established polymer matrix, TGA (thermo gravimetric analysis) was taken into the action to examine the proportion of liquid in the hydrogel. Differential Scanning Calorimetry (DSC) was implemented to detect the variations in thermo grams as compared with pure active ingredients used for gelation. [35]

11. Drug content

Estimation of the medication concentration was done by correctly introducing 100 μL of preparations in the test tube and appropriately diluted with the help of STF (simulated tear fluid) to gain 10 μg/mL of the concentration and identified with the use of UV-Visible spectrophotometer.[36]

12. Sterility Testing

According to the Indian Pharmacopoeia, sterility testing for anaerobic and aerobic bacteria and fungi was carried out using SCDM (soybean casein digest medium) and fluid thioglycolate medium respectively. For sterility testing the direct inoculation method was used. In 100 mL of

culture medium, 10 mL of culture was introduced. Both media were incubated at 320°C for 7 days and any microbiological growth was noticed.[36]

13. Determination of the stability studies

Evaluation of the stability testing was carried out with the help of the ICH guidelines21. The designated preparations are exposed to 25, 30 and 40 ± 0.5˚C/75% R.H. respectively for 0, 30, 60, 90 and 180 days. The preparations are tested for pH, viscosity, drug content. While the shelf life of the preparations are examined by using Arrhenius Plot at 25˚C as per ICH guidelines.[43]

6) Conclusion

Ocular drug delivery system is one of the thought-provoking tasks for which many inventive methods are developed to control the difficulties experienced by the regular form of ocular preparations. The *In-situ* gelling approach is emerging as a favorable dosage form in ocular drug delivery resulting in prolonged precorneal drug retention time thereby offering sustained release mechanism. This further leads to increased drug bioavailability, therapeutic efficacy and decreased dosing frequency and side effects. *In-situ* gelling formulations having polymers which are sensitive to change in temperature, pH, ion concentration and enzymes are used. *In-situ* gels does not show any significant signs of toxicity or irritability to the eyes. Presently all the ocular *in-situ* formulations are formulated to contain only a single active ingredient. Hence future trends demands development of formulations containing multiple active ingredients for producing multi-target approach therapy. There are future expectations for producing many more dependable pH sensitive, ion sensitive (*in-situ* polymers) which may be sensitive to the biomarkers related with the disease condition of the eye.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

7) References

1. Cholkar K, Dasari S, Pal D, Mitra A. Eye: Anatomy, physiology and barriers to drug delivery. Ocul Transp Recept Their Role Drug Deliv. 2013 Oct 1;1–36.

2. Galloway NR, Amoaku WMK, Galloway PH, Browning AC. Basic Anatomy and Physiology of the Eye. In: Galloway NR, Amoaku WMK, Galloway PH, Browning AC, editors. Common Eye Diseases and their Management [Internet]. Cham: Springer International Publishing; 2016 [cited 2021 Jun 27]. p. 7–16. Available from: https://doi.org/10.1007/978-3-319-32869-0_2

3. Rawas-Qalaji M, Williams C-A. Advances in Ocular Drug Delivery. Curr Eye Res. 2012 May 1;37(5):345–56.

4. Pahuja P, Arora S, Pawar P. Ocular drug delivery system: a reference to natural polymers. Expert Opin Drug Deliv. 2012 Jul;9(7):837–61.

5. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular Drug Delivery. AAPS J. 2010 Sep 1;12(3):348–60.

6. Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery systems: An overview. World J Pharmacol. 2013 Jun 9;2(2):47–64.

7. Kuno N, Fujii S. Recent Advances in Ocular Drug Delivery Systems. Polymers. 2011 Mar;3(1):193–221.

8. Willoughby CE, Ponzin D, Ferrari S, Lobo A, Landau K, Omidi Y. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function – a review. Clin Experiment Ophthalmol. 2010;38(s1):2–11.

9. Ittoop sabita, Kahook malik. Cornea Permeability - an overview | ScienceDirect Topics [Internet]. [cited 2021 Jun 26]. Available from: https://www.sciencedirect.com/topics/neuroscience/cornea-permeability

10. Galloway NR, Amoaku WMK, Galloway PH, Browning AC. Basic Anatomy and Physiology of the Eye. In: Galloway NR, Amoaku WMK, Galloway PH, Browning AC, editors. Common Eye Diseases and their Management [Internet]. Cham: Springer International Publishing; 2016 [cited 2021 Jun 26]. p. 7–16. Available from: https://doi.org/10.1007/978-3-319-32869-0_2

11. Gote V, Sikder S, Sicotte J, Pal D. Ocular Drug Delivery: Present Innovations and Future Challenges. J Pharmacol Exp Ther. 2019 Sep;370(3):602–24.

12. Willoughby CE, Ponzin D, Ferrari S, Lobo A, Landau K, Omidi Y. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function – a review. Clin Experiment Ophthalmol. 2010;38(s1):2–11.

13. Kuno N, Fujii S. Recent Advances in Ocular Drug Delivery Systems. Polymers. 2011 Mar;3(1):193–221.

14. Bachu RD, Chowdhury P, Al-Saedi ZHF, Karla PK, Boddu SHS. Ocular Drug Delivery Barriers-Role of Nanocarriers in the Treatment of Anterior Segment Ocular Diseases. Pharmaceutics. 2018 Feb 27;10(1):E28.

15. Barar J, Javadzadeh AR, Omidi Y. Ocular novel drug delivery: impacts of membranes and barriers. Expert Opin Drug Deliv. 2008 May;5(5):567–81.

16. Mitra AK, Anand BS, Duvvuri S. Drug Delivery to the Eye. In: Fischbarg J, editor. Advances in Organ Biology [Internet]. Elsevier; 2005 [cited 2021 Jul 5]. p. 307–51. (The Biology of the Eye; vol. 10). Available from: https://www.sciencedirect.com/science/article/pii/S1569259005100123

17. Ocular Drug Delivery | SpringerLink [Internet]. [cited 2021 Jul 5]. Available from: https://link.springer.com/article/10.1208/s12248-010-9183-3

18. Dubald M, Bourgeois S, Andrieu V, Fessi H. Ophthalmic Drug Delivery Systems for Antibiotherapy—A Review. Pharmaceutics. 2018 Mar;10(1):10.

19. Sánchez-López E, Espina M, Doktorovova S, Souto EB, García ML. Lipid nanoparticles (SLN, NLC): Overcoming the anatomical and physiological barriers of the eye – Part I – Barriers and determining factors in ocular delivery. Eur J Pharm Biopharm. 2017 Jan 1;110:70–5.

20. Singh RRT, Tekko I, McAvoy K, McMillan H, Jones D, Donnelly RF. Minimally invasive microneedles for ocular drug delivery. Expert Opin Drug Deliv. 2017 Apr 3;14(4):525–37.

21. A comprehensive insight on ocular pharmacokinetics | SpringerLink [Internet]. [cited 2021 Jul 5]. Available from: https://link.springer.com/article/10.1007/s13346-016-0339-2

22. Agrawal AK, Das M, Jain S. In situ gel systems as “smart” carriers for sustained ocular drug delivery. Expert Opin Drug Deliv. 2012 Apr;9(4):383–402.

23. Pandey M, Choudhury H, binti Abd Aziz A, Bhattamisra SK, Gorain B, Su JST, et al. Potential of Stimuli-Responsive In Situ Gel System for Sustained Ocular Drug Delivery: Recent Progress and Contemporary Research. Polymers. 2021 Jan;13(8):1340.

24. In-Situ Gelling System: A Novel Approach for Ocular Drug Delivery [Internet]. [cited 2021 Jun 26]. Available from: https://www.researchgate.net/publication/268349482_In-Situ_Gelling_System_A_Novel_Approach_for_Ocular_Drug_Delivery

25. Singh B, Khurana RK, Garg B, Saini S, Kaur R. Stimuli-Responsive Systems with Diverse Drug Delivery and Biomedical Applications: Recent Updates and Mechanistic Pathways. Crit Rev Ther Drug Carrier Syst. 2017;34(3):209–55.

26. A NOVEL OPHTHALMIC DRUG DELIVERY SYSTEM: IN-SITU GEL | INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH [Internet]. [cited 2021 Jun 26]. Available from: https://ijpsr.com/bft-article/a-novel-ophthalmic-drug-delivery-system-in-situ-gel/?view=fulltext

27. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, et al. Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci. 2019 Jan 1;14(1):1–15.

28. Al-Kinani AA, Zidan G, Elsaid N, Seyfoddin A, Alani AWG, Alany RG. Ophthalmic gels: Past, present and future. Adv Drug Deliv Rev. 2018 Feb 15;126:113–26.

29. Mutalabisin MF, Chatterjee B, Jaffri JM. pH Responsive Polymers in Drug Delivery. Res J Pharm Technol. 2018 Nov 30;11(11):5115–22.

30. Smart Polymers and their Applications: A Review | A. | International Journal of Current Pharmaceutical Review and Research [Internet]. [cited 2021 Jun 26]. Available from: https://www.myresearchjournals.com/index.php/ijcprr/article/view/9220

31. R. Jadhav K, S. Pacharane S, V. Koshy P, J. Kadam V. Smart Polymers and Their Role in Drug Delivery: A Review. Curr Drug Ther. 2010 Nov 1;5(4):250–61.

32. Suhaime IHB, Majeed MTAABA. Environment Sensitive Polymers For Ophthalmic Drug Delivery. Int J Pharma Bio Sci [Internet]. 2012 Mar [cited 2021 Jun 26];Volume 3 Issue 1. Available from: http://ijpbs.net/abstract.php?article=1267

33. Mahlumba P, Choonara YE, Kumar P, Du Toit LC, Pillay V. Stimuli-Responsive Polymeric Systems for Controlled Protein and Peptide Delivery: Future Implications for Ocular Delivery. Molecules. 2016 Aug;21(8):1002.

34. Wells CM, Harris M, Choi L, Murali VP, Guerra FD, Jennings JA. Stimuli-Responsive Drug Release from Smart Polymers. J Funct Biomater. 2019 Sep;10(3):34.

35. Deka M, Ahmed AB, Chakraborty J. DEVELOPMENT, EVALUATION AND CHARACTERISTICS OF OPHTHALMIC IN SITU GEL SYSTEM: A REVIEW. Int J Curr Pharm Res. 2019 Jul 15;47–53.

36. Saini R, Saini S, Singh G, Banerjee DA. IN SITU GELS- A NEW TRENDS IN OPHTHALMIC DRUG DELIVERY SYSTEMS. 2015;6(05):5.

37. Meshram S, Thorat S. Ocular in Situ Gels: Development, Evaluation and Advancements. :7.

38. HET-CAM test for determining the possible eye irritancy of pesticides in: Acta Veterinaria Hungarica Volume 58 Issue 3 (2010) [Internet]. [cited 2021 Jul 11]. Available from: https://akjournals.com/view/journals/004/58/3/article-p369.xml

39. Wannaphatchaiyong S, Heng PWS, Suksaeree J, Boonme P, Pichayakorn W. Lidocaine loaded gelatin/gelatinized tapioca starch films for buccal delivery and the irritancy evaluation using chick chorioallantoic membrane. Saudi Pharm J. 2019 Dec 1;27(8):1085–95.

40. In-Situ gel: New trends in Controlled and Sustained Drug Delivery System. :11.

41. Kurniawansyah IS, Sopyan I, Wathoni N, Fillah DL, Praditya RU. APPLICATION AND CHARACTERIZATION OF IN SITU GEL. Int J Appl Pharm. 2018 Nov 22;10(6):34.

42. Patil AP, Tagalpallewar AA, Rasve GM, Bendre AV, Khapekar PG. A NOVEL OPHTHALMIC DRUG DELIVERY SYSTEM: IN-SITU GEL. 3:9.

43. Khan N, Aqil M, Ameeduzzafar, Imam SS, Ali A. Development and evaluation of a novel in situ gel of sparfloxacin for sustained ocular drug delivery: in vitro and ex vivo characterization. Pharm Dev Technol. 2015 Aug 18;20(6):662–9.