An insight to ocular in situ gelling systems

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Abstract

Eye is delicate organ of body whose defence mechanism restricts entry of exogenous substances. Conventional drug delivery systems get washed off within a short period of time and results in poor ocular bioavailability. Development of in situ gel having protracted ocular residence time is one of the mile stone triumphs by pharmaceutical researcher for treatment of eye ailments. This polymeric system showed sol-to-gel phase transition by change in physiological parameters in pre-corneal area which includes pH, temperature or ionic interactions etc. Three types of in situ gels are well known based on mechanism involved in phase transition viz. temperature dependent, pH sensitive, ion activated systems. Gel formed after phase transitions have high viscosity along with bio-adhesive property, which increases resistance for instantaneous rinse off due to defence contrivance. Plus it prevents nasal-lachrymal drainage and avoids the systemic side effects. Recently, in situ gels have been vexed in amalgamation with another drug delivery system. It may include use of two or more stimuli systems together or added advantages of nano-technology. Combination of in situ gel with nanoparticles is utmost remedy for ocular treatment with water insoluble drugs.

Keywords: In situ gel; Smart polymer; Nanotechnology; ocular bioavailability, Prolong Release.

1. Introduction

Eye is one of the most important sensory organ which converts light to an electric signals which is interpreted by brain [1]. It gets suffer from various diseases like glaucoma, dry eye syndrome, trachoma, keratitis, conjunctivitis etc [2]. Eye is unique organ in terms of its anatomical-physiological structure and defence mechanisms [3]. It is highly protected organ which restrict the entry of any exogenous substances [4]. Therefore, to target the drug at required ocular site in therapeutic dose is one of the most challenging tasks to scientist [5]. Various factors like nasolacrimal drainage of drug, binding of drug to lachrymal protein, induced lachrimation, availability of limited corneal area creates as barrier for absorption of drug through ocular route [2,6].

An ocular treatment mainly focuses on topical application than systemic administration of therapeutic moiety. Drug can be delivered topically by solution, suspension or ointment of which eye drops account for 90% of marketed formulations [2,7]. The reason may be attributed to ease of administration and patient compliance. Nonetheless, the ocular bioavailability is very low with topical drop administration [8].

When drug is administered topically it reaches to targeted site by corneal followed by the non-corneal routes. Corneal route is foremost important for absorption of ophthalmic drugs [9]. However; cornea acts as barrier for topical absorption of these agents [10]. Anatomical structure of cornea exert unique differential solubility requirement for drug candidate. Various factors viz. partition coefficient, concentration gradient, diffusivity plays important role in transcorneal absorption [11].

Grass and co-workers studied the mechanism of corneal drug penetration from kinetic modeling point of view. The model was developed by relating permeability coefficient with partition coefficient and molecular weight. This model represents the cornea as a laminated membrane with a lipid layer (epithelium) and an aqueous layer (stroma).
The permeability coefficient of this laminated membrane is expressed as:

\[ K_{\text{per}} = 1 / \left( L_s / D_s \right) + Le(D_p + PDe) \]

Where;

- \( K_{\text{per}} \) = permeability coefficient,
- \( P \) = partition coefficient
- \( L_s \) and \( Le \) = thickness of stroma and epithelium
- \( D_s \), \( De \) and \( Dp \) are apparent diffusion coefficient for stroma, epithelium, epithelial pores respectively

Pores present on epithelial layer are of very small size and hence diffusion through pores can be neglected. Therefore when \( Dp = 0 \) Eq. 2 can be rearranged to give Eq. 2

\[ K_{\text{per}} = P / P \left( L_s / D_s \right) + Le / De \]

From Eq. 2 one can conclude that the epithelium is the barrier for small values of partition coefficient and the stroma for large values of partition coefficient [12-14]. The optimal log partition coefficient appears to be in the range of 1–3 [15].

Additionally, normal capacity of cul-de-sac is 7-10µl which can be extended up to 30µl without blinking [16]. Due to this, conventional eye drops are eliminated from the precorneal area immediately and only 1-10% of topically applied drug get absorbed [17]. Moreover, due to tear drainage more than 75% of the administered dose passes via nasolacrimal duct into the GI tract, leading to systemic side effects [18,19].

Short pre-corneal contact time combined with poor corneal permeability results in low ocular bioavailability (10% or less) and as a result, frequent instillation of concentrated solutions is needed in order to achieve the desired therapeutic effects [20,21]. A high frequency of eye drop instillation is associated with patient non-compliance [22]. It has been reported that nearly 50% of glaucoma patient discontinued topical ocular therapy within six month because of high frequency of instillation [19]. Inclusion of excess drug in the formulation is an attempt to surmount problems of bioavailability is potentially dangerous if drained drug solution get absorbed systemically through nasolacrimal duct [22,23].

The poor bioavailability and therapeutic response shown by conventional eye drops can be overcome by the use of novel ophthalmic drug delivery system (NODDS) [24]. In last two decades several NODDS have been developed in order to improve ocular bioavailability of drug. It includes aqueous gels, liposomes, nanoparticles, dendrimer, nanomicells, implant, contact lenses, nanosuspension etc [8, 20]. Additionally rapid progress in bioscience opens new possibilities to meet need of posterior treatment [25]. However these ocular drug delivery systems have not been used extensively because of some drawbacks such as blurred vision from ointment, non-reproducibility and poor stability of liposomes, high cost of nano-preparation and poor patient compliance from contact lenses [3, 26].

The development of \textit{in situ} gel systems has received significant interest of ophthalmologist over the past few years. \textit{In situ} gel forming system has showed their potential in increasing the residential time because of bio-adhesiveness of formed gel. Additionally polymers used to achieve \textit{in situ} gelling may results in sustained release of drug molecules [27, 28, 29]. This system described as low viscosity solution that undergoes phase transition in cul-de-sac to form viscoelastic gel. This sol-to-gel phase transition is due to conformational changes of polymer in response to physiological environment. \textit{In situ} formulations are more acceptable for patient because they are administered as solution or suspension which immediately undergoes to gelation as come in contact with eye [30].

Depending upon method employed to cause sol-to-gel phase transition on ocular surface three types of \textit{in situ} gels are widely accepted as pH triggered systems, ion-activated systems and temperature sensitive system [5]. Use of \textit{in situ} gel-forming polymeric formulations may increase patient compliance by decrease in frequency of administration and overall cost of treatment [31, 32]. The technology can be commercially acceptable, less complex and low manufacturing costs [3].

In recent years, extensive investigations have been dedicated to develop newer \textit{in situ} gelling system which involved combination of one or more NODDS with \textit{in situ} gelling systems [33]. It involved use of two different stimuli sensitive system together, nanoparticles loaded \textit{in situ} systems etc. These combinations at least theoretically prove its benefit in modulating drug release at the ocular surface. Further studies are required to examine the benefits of these combined approaches [33, 34].

Article will summarised concept of all approaches used in stimuli sensitive systems along with information of different polymers that can be used in respected approach, advance in stimuli sensitive system, FDA approved formulation based on concept of \textit{in situ} gel and different evaluation parameters of \textit{in situ} gel.

1.1 Ideal properties for \textit{in situ} formulations

\textbf{Physical state:} Formulation should be free flowing liquid which allows ease of administration with reproducible dose delivery

\textbf{Phase transition:} Upon instillation it should undergoes sol-to-gel formation by phase transition [35].

\textbf{Strength of gel:} Formed gel should be strong enough to withstand the shear force in cul-de-sac which prolongs residence time of drug [6].

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1.2 Mechanism behind increase in ocular bioavailability

Conventional ophthalmic formulations get eliminated within short period of time because of poor ocular retention. It is widely accepted that increase in viscosity of formulation results in more resistance to wash off because of which drug remain in contact at site for a longer period of time. Increase in contact time shows improved bioavailability [37].

As in situ system undergoes phase transition in normal physiological condition to form viscoelastic gel, it can be results in increased bioavailability along with sustained release of drug and less or no systemic side effect [2].

2. Various approaches of in situ gelation

Various approaches used for in situ gelling systems are as follows:
- **Stimuli-responsive in situ gel system**
  - Temperature induced in situ gel system
  - pH induced in situ gel system
- **Chemically induced in situ gel system**
  - Ionic cross linking (Ion activated systems)
  - Enzymatic cross linking

2.1 Stimuli responsive in situ gel system

Polymers used in stimuli responsive system are also known as stimuli-sensitive, intelligent, smart or environmentally sensitive polymer. These polymers adapt small external changes in environment and undergo relatively large and abrupt, physical or chemical changes [37]. These polymer systems may recognise a stimulus as a signal and then change their chain conformation in direct response [29].

2.1.1 Temperature induced in situ gel system

Temperature-sensitive systems are the most commonly studied class of stimuli responsive polymer systems for ocular targeting [28]. The use of a biomaterial whose transitions from sol to gel is triggered by change in temperature is an attractive way to achieve in-situ formation [38]. The ideal phase transition temperature for this type of systems is physiologic temperature where there is no need of external source of heat other than that of body for gelation. For convenience, temperature-sensitive in situ gels are classified into negatively thermo-sensitive, positively thermo-sensitive, and thermally reversible gels [39]. Negative temperature-sensitive in situ gels have a lower critical solution temperature (LCST) and contract upon heating above the LCST. A positive temperature sensitive in situ gel has an upper critical solution temperature (UCST) and contracts upon cooling below the UCST [40,41].

Formulation is liquid at room temperature (20-25°C) which undergoes gelation in contact with body fluid (35-37°C). Temperature increases degradation of polymer chains which leads to formation of hydrophobic domains and transition of an aqueous liquid to in situ gel [29]. Poloxamers, Xyloglucan, Chitosan and naturally occurring cellulose derivatives are most commonly used polymers in preparation of thermo-sensitive in situ gelling system.

**Poloxamers**

Poloxamers are a broad group of compounds that were introduced in the early 1950 as food additives and for pharmaceutical preparations. These water-soluble surfactants are triblock co-polymers prepared from poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) i.e. PEO-PPO-PEO. They are commercially available as Pluronic or Poloxamers which is marketed by BASF Corporation. These are the most commonly used thermosetting polymers and could be applicable for the development of effective ophthalmic drug delivery [2, 40].

The pluronic triblock copolymers are available in various grades differing in molecular weights and physical forms. Depending upon the physical designation for the grades are assigned, as F for flakes, P for paste, L for liquid [42]. Depending upon the ratio and the distribution along with the chain of hydrophilic and hydrophobic subunits, several grades are available having different gelling properties. Different grades of poloxamers are shown in Table 1.

**Table 1: Different grade of poloxamers**

<table>
<thead>
<tr>
<th>Poloxamer</th>
<th>Pluronic®</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>L 44 NF</td>
<td>2200</td>
</tr>
<tr>
<td>188</td>
<td>F 68 NF</td>
<td>8400</td>
</tr>
<tr>
<td>237</td>
<td>F 87 NF</td>
<td>7959</td>
</tr>
<tr>
<td>338</td>
<td>F 108 NF</td>
<td>14600</td>
</tr>
<tr>
<td>407</td>
<td>F 127 NF</td>
<td>12600</td>
</tr>
</tbody>
</table>

Pluronics F127, which gives colourless and transparent gel so commonly used in the pharmaceutical industries. Poloxamer formulation generally increased drug residence time at application sites, resulting in improved bioavailability and efficacy. Pluronic F127 was found to gel at a concentration of 20 wt. % at 25 °C, which is less than that of the other members of the Poloxamer series [29].

Three principal mechanisms have been proposed to explain the liquid-gel phase transition after an increase in temperature, including: i) Gradual desolvation of the polymer, ii) Increased micellar aggregation, iii) The increased entanglement of the polymeric network.

**Xyloglucan**

Xyloglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-D-glucan backbone chain, which has (1-6)-α-D xylose branches that are partially substituted by (1-2)-β-D-galactosylxylene. When xyloglucan is partially degraded by β- galactosidase, the
resultant product exhibits thermally reversible gelation by the lateral stacking of the rod like chains. The sol-gel transition temperature varies with the degree of galactose elimination. Gelation is possible only when the galactose removal ratio exceed to 35% [2, 43].

Chitosan

Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin [42]. Chitin is the second most abundant polysaccharides in nature after cellulose. The main commercial sources of chitin are the shell wastes of shrimp, crab, lobster, krill, and squid. It is a biologically safe, non-toxic, bio compatible, and biodegradable polysaccharide. Being a bioadhesive polymer and having antibacterial activity, chitosan is a good candidate for site-specific drug delivery. It is linear polysaccharide consisting of (1-4)-linked 2-amino-2-deoxy-b-D-glucopyranose which have the reactive amino groups and hydroxyl groups [2]. Chitosan is a biocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pH of 6.2. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. The pH gelling cationic polysaccharides solution are transformed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking by addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan aqueous solution [40].

Cellulose derivatives

Thermo reversible gels can be prepared with naturally occurring polymers. Most of natural polymer aqueous solutions form a gel phase when their temperature is lowered. Some examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation. Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10%), their aqueous solutions are liquid at low temperature but get gels upon heating. MC and HPMC are typical examples of such polymers. Cellulose derivatives have high phase transition temperature which can be lowered by chemical or physical modification [2].

2.1.2 pH induced in situ gel system

Sol to gel phase transition is achieved by change in pH. All 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 ionizable groups are known as polyelectrolytes. Swelling of in situ gel increases as external pH increases in case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. Most of anionic pH-sensitive polymers are based on PAA (Carbopol, carbermor) or its derivatives.

2.1.3 Polymer used in pH induced in situ gel system

Carbomer

It is cross-linked poly (acrylic acid), commercially available as Carbopol. It is widely used in ophthalmic formulation in order to enhance pre-corneal retention of drug [29]. Carbomer are white-coloured, ‘fluffy’, acidic, hygroscopic powders with a characteristic slight odour. It has glass transition temperature in range of 100-105°C [44, 45]. Carbopol offers the advantage of exhibiting excellent mucoadhesive properties as compared to other polymers. Interaction between mucin and poly (acrylic acid) occurred by four mechanism viz. electrostatic interaction, hydrogen bonding, hydrophobic interaction and inter diffusion. It is pH sensitive polymer which shows sol to gel transition in aqueous solution when pH is raised above 5.5 [2]. It required in high concentration to form stiff gel. At higher concentration it forms highly acidic solution which is not easily neutralized by buffer action of tear fluid and results in ocular irritation. Reduction in its concentration without affecting the gelling capacity and viscosity can be achieved by addition of viscosity increasing polymers such as HPMC [20, 22, 46]. It is included in US-FDA inactive ingredient guidelines and in non-parenteral medicines licensed in Europe.

2.1.4 Chemically induced in situ gel system

Ion activated systems

Ion activated gelling system is triggered by cations present in eye tear fluid like Na⁺, Ca²⁺ and Mg²⁺. Generally anionic polymers are used in the formation of ion sensitive drug delivery system. Polymers like sodium alginate, gelrite, tamarind gum, gellen gum are used in combination with other polymers like MC and HPMC to increase the effect. They provide sustain release of drug by providing mucoadhesiveness. This system based on the mechanism of ionic interaction of ions of polymer and divalent ions of tear fluid. As soon as anionic polymers come in contact with cationic ions they convert into gel [2, 29]. The concentration of Na⁺ in human tear is 2.6 g/l is particularly suitable to cause gelation of material when formulation administered topically [47].
2.1.5 Polymers used in ion activated in situ gelling system

Gellan gum

It is linear, anionic hetero polysaccharide secreted by the microbe Sphingomonas elodea (formerly known as Pseudomonas elodea) which is available with brand name as Gelrite TM, Kelcogel TM. It can be produced by aerobic fermentation and then isolated from the fermentation broth by alcohol precipitation. The polymer back bone consists of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1, 4-glycosidic linkages. Alginic acid

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1, 4-glycosidic linkages. The proportion of each block and the arrangement of blocks along the molecule vary depending on the algal source [49]. Ratio of β-D-mannuronic acid and α-L-glucuronic acid plays important role in determination of various properties of polymer such as mechanical strength, porosity etc. Alginate with a high guluronic acid content will improve the gelling properties and reduces concentration of polymer required to form stiff gel. The alginate forms 3-dimensional ionotropic hydrogel matrices by interaction with calcium ions and results in the formation of homogeneous gel. It is biodegradable, non-toxic polymer approved by several regulatory authorities [50].

Enzymatic cross linking

In this approach, in situ formation is catalysed by natural enzymes present in tear fluid. It has not been investigated widely but seems to have some advantages over other approaches like no need of any potential and harmful chemicals [1].

Table 2 contains some examples of the use of stimuli responsive polymers in ophthalmic pharmaceutical systems.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Drug</th>
<th>Polymers</th>
<th>Release extension</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Betoxolol</td>
<td>pluronic F-127 and HPMC-E 50 LV</td>
<td>upto 7 h</td>
<td>Geethalakshmi et al., 2013</td>
</tr>
<tr>
<td>Temperature</td>
<td>Timolol Maleate</td>
<td>Poly (N isopropylacrylamide) –chitosan (PNIPAAm–CS)</td>
<td>Upto 12 h</td>
<td>Cao, et al., 2007</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ciprofloxacin</td>
<td>Pluronic and Chitosan</td>
<td>upto 8 h</td>
<td>Varshoza, et al., 2008</td>
</tr>
<tr>
<td>Temperature</td>
<td>Pilocarpine hydrochloride</td>
<td>Xyloglucan and pluronic F-127</td>
<td>Upto 7 h</td>
<td>Miyazaki, 2001</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ketorolac tromethamine (KT)</td>
<td>methylcellulose (MC) and hydroxypropylmethyl cellulose (HPMC)</td>
<td>Upto 4 h</td>
<td>Manas BHOWMKI-2011</td>
</tr>
<tr>
<td>pH</td>
<td>Sinomenine HCl (SIN)</td>
<td>Carbopol 940 and HPMC K4M</td>
<td>Upto 8 h</td>
<td>Song et al., 2013</td>
</tr>
<tr>
<td>pH</td>
<td>Baicalin</td>
<td>Carbopol 974P and HPMC E4M</td>
<td>Upto 8 h</td>
<td>Wu et al, 2011</td>
</tr>
<tr>
<td>pH</td>
<td>Timolol Maleate</td>
<td>Carbopol/Chitosan</td>
<td>Upto 24 h</td>
<td>Gupta et al., 2010</td>
</tr>
<tr>
<td>pH</td>
<td>Ciprofloxacin</td>
<td>Carbopol 940 and Methocel E50LV</td>
<td>Upto 8 h</td>
<td>Srivdy et al., (2001)</td>
</tr>
<tr>
<td>Ion activated</td>
<td>Diclofenac Potassium</td>
<td>sodium alginate and HPMC</td>
<td>Upto 10 h</td>
<td>Subinol et al., 2013</td>
</tr>
<tr>
<td>Ion activated</td>
<td>Ketorolac tromethamine</td>
<td>Gelrite</td>
<td>Upto 6 h</td>
<td>Sirish V, et al., (2010),</td>
</tr>
<tr>
<td>Ion activated</td>
<td>Ofloxacin</td>
<td>Sodium alginate and HPC</td>
<td>Upto 7 h</td>
<td>Sindhu A, et al., (2009)</td>
</tr>
</tbody>
</table>

While table 3 contains FDA approved marketed in situ gel. This clearly indicates that formulation of ophthalmic in situ gel is feasible on lab scale as well as on large scale. Also it has ability to sustain the release of drug.

Table 3: List of FDA approved oculair in situ gel

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Product Name</th>
<th>API</th>
<th>Polymer</th>
<th>Type of in situ gel</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azasite</td>
<td>Lidocaine HCl</td>
<td>Poloxamer 407</td>
<td>Temperature sensitive</td>
<td>Bacterial conjunctivitis</td>
</tr>
<tr>
<td>2</td>
<td>Pilopine HS</td>
<td>Pilocarpine HCl</td>
<td>Carbopol 940</td>
<td>pH sensitive</td>
<td>Glaucoma</td>
</tr>
<tr>
<td>3</td>
<td>Timoptic XE</td>
<td>Timolol Maleate</td>
<td>Gellan gum</td>
<td>Ion activated</td>
<td>Glaucoma</td>
</tr>
</tbody>
</table>
3. Combined approaches with in situ gel for enhanced ocular bioavailability

3.1 Ophthalmic formulations with two or more stimuli responsive polymers

The ophthalmic formulations containing different stimuli responsive polymers to obtained improved sustained or controlled drug release explore an excellent strategy. The main idea is that formulation contains combination of polymers which undergoes phase transition in response to simultaneous variations in at least two physical parameters such as pH, temperature and ionic strength [27].

Use of two stimuli responsive polymers together gives excellent gelation properties. Also, single polymer based systems requires high concentration of polymer to achieve desired viscosity of in situ gelling system. This may creates stability related issues of formulation e.g. Carbopol. It is pH sensitive polymer which shows sol to gel transition in aqueous solution when pH is raised above 5.5. It is polyacrylic acid (PAA) which is required in high concentration to form stiff gel. At higher concentration it forms highly acidic solution which is not easily neutralized by buffer action of tear fluid [20, 22, 46].

Lin and Sung [27] developed ophthalmic in situ gelling system of pilocarpine hydrochloride consist of carbopol, pluronic and combination of carbopol / pluronic at different concentration level. Formulations were evaluated for various parameters viz. rheological properties, in vitro release as well as in vivo pharmacological response. It was found that mixture of 0.3% carbopol and 14% pluronic solutions showed a significant enhancement in gel strength at the physiological condition; this gel mixture was also found to be free flowing at pH 4.0 and 25°C. Both the in vitro release and in vivo pharmacological studies indicated that the carbopol / pluronic solution had the better ability to retain drug than the carbopol or pluronic solutions alone. The results demonstrated that the carbopol / pluronic mixture can be used as an in situ gelling vehicle to enhance the ocular bioavailability.

Basaran and Bozkir [61] developed and evaluate ophthalmic in situ gelling systems of ciprofloxacin hydrochloride with Carbopol 934 and Poloxamer 407. Hydroxypropyl-β-cyclodextrin was used in order to increase the stability of ciprofloxacin hydrochloride. The developed formulations were found therapeutically efficient and provided sustained release of the drug over an 8 h period.

3.1.1 Nano in situ gel

Now a day’s nano-technology is the most emerging concept in pharmaceutical field [62]. Several new preparations based on nano-technology have been developed to protract ocular residence time as well as enhanced bioavailability. Use of nano-particles has led to the solution of various solubility related problems of poorly soluble drugs especially BCS class II & IV [63]. However, nanoparticles are non-muco adhesive, so are drained out of eyes quickly [64]. This problem can be overcome by suspending fabricated nano-particles in an in situ gelling vehicle which undergoes sol to gel phase transition upon exposure to physiological conditions [33,65]. This results in increasing the pre-corneal residence time of the nanoparticles and enhancing ocular bioavailability. This combined approach of in situ gel with nanoparticles is termed as “nanoparticle laden in situ gel” [64].

Nagarwal et al. [33] investigated a novel nano in situ gel forming system of 5-Fluorouracil (5-FU) for its potential use for conjunctival/corneal squamous cell carcinoma (CCSC). The study was conducted in two steps, in the first step PLA nanoparticles were prepared and characterized which were dispersed in sodium alginate solution yielding the modified nano in situ system. In vitro and in vivo study of prepared nano in situ gel was conducted in simulated tear fluid and in rabbit eye respectively. In vitro experiments indicated a diffusion controlled release of 5-FU from modified nano in situ system upto 8 h with high burst effect. Modified nano in situ gel system (MNS) showed significant increased the Cmax and AUC in aqueous humor as compared to 5-FU solution and PLA nanoparticles.

Gupta et al. [65] developed sparflloxacin poly-lactic-co-glycolic acid nanoparticle and incorporated in chitosan in situ gel for ophthalmic delivery. The formulation was tested for various physicochemical properties. Gamma Scintigraphy showed good retention over the entire pre-corneal area.

Sathyavathi et al. [66] prepared brimonidine tartrate niosomal in situ gels for glaucoma treatment. Niosomes formulated with span 60 showed highest entrapment efficiency. In situ gelling of niosomal drops was formulated by using Carbopol 940 and HPMC K 15 M. Antiglaucoma activity of the prepared gel formulations showed more significant and sustained effect over period of 480 min than marketed and niosomal drops. Overall in situ gel can be summarised by using Fig. 1.
4. Evaluation of in situ gel

Ocular in situ gel can be tested for various parameters in order to ensure that prepared formulation satisfy safety guidelines for ODDS.

4.1 Visual appearance and clarity

Visual appearance and clarity of prepared in situ formulation is checked for presence of any particulate matter under fluorescent light against a white and black background [67].

pH

pH affects both solubility as well as stability of drug in ophthalmic formulations. It should be such that the formulation will remain stable at that pH at the same time there would no irritation to the patient upon administration. It is measured by digital pH meter [68].

Gelling capacity

Gelling capacity of formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and time taken for its gelling is noted [6].

Tonicity

Isotonicity is important characteristics of ophthalmic formulation which has to be maintained to prevent any tissue damage or irritation to the eye. It refers to the osmotic pressure exerted by salts in aqueous solution. Ophthalmic formulation must possess osmotic pressure within the range of 290-310 mOsmol/kg. Tonicity is measured by using osmometer [7, 69].

4.2 In vitro drug release study

In vitro drug release study is done by using Franz diffusion cell. In receptor compartment freshly prepared ATF is placed. Dialysis membrane is placed in between receptor and donor compartments. Whole assembly is kept on the thermostatically controlled magnetic stirrer to simulate in vivo conditions and temperature of medium is maintained at 37°C ± 0.5°C. Medium is continuously stirred at 20 rpm. 1ml of formulation is placed in donor compartment. Sample (0.5ml) is withdrawn at predetermined time interval and same is replaced by ATF. Samples are analysed either on UV spectrophotometer or HPLC [16, 51].

4.3 Rheological studies

Viscosity determination of in situ formulation is carried out on Brookfield viscometer having small volume adapter. Viscosity is measured before and after gelation by increasing angular velocity gradually from 0.5 to 100 rpm [61].

4.4 Texture analysis

The consistency, firmness, and cohesiveness of in situ gel are assessed by using texture profile analyzer. This mainly indicates gel strength and easiness in administration. Texture analysis provides information on hardness, compressibility and adhesiveness which can be correlated with various parameters like ease of removal from container, good spreadability on corneal surface and adherence to mucous layer in order to prolong residence time [5].

4.5 Transcorneal permeability study

Transcorneal permeability of drug is evaluated by using goat eye cornea. The fresh whole eyeball of goat is obtained from local butcher’s shop and transported in laboratory in normal saline solution (4°C). Cornea is then

*Fig. 1: Overview on in situ gel*
carefully excised along with 2-4 mm of surrounding sclera tissue and wash with saline solution. Excise cornea is place in between donor and receptors compartment of Franz diffusion cell in such a way that epithelial surface face the donor compartment. Receptor compartment is filled with freshly prepared ATF. Whole assembly is placed on thermostatically controlled magnetic stirrer, temperature (37°C ± 0.5°C) as well as stirring rate (20 rpm) is maintained. 1ml prepared formulation is placed in donor compartment. Samples (0.5ml) are withdrawn at predetermined time interval of 1hr to 5hr and same volume is replaced by ATF. Samples are then diluted upto 10ml and analysed on either UV spectrophotometer or HPLC [70, 71].

4.6 Ocular irritation study

As there is ban on Draize study in many countries ocular irritation study of in situ formulation can be performed by one of the following method.

4.7 Histological study

To evaluate effect of in situ formulation on corneal structure and study the irritation potential, corneas are removed from the eyes of freshly sacrificed goat and incubated at 37°C for 5 hrs in formulation. Sodium dodecylsulfate (SDS) solution in phosphate buffer saline (PBS) 0.1% (w/w) is used as the positive control. After incubation, corneas are washed with PBS and immediately fixed in formalin (8%, w/w). Tissues are dehydrated in an alcohol gradient, placed in melted paraffin and solidified in block form. Cross sections are cut, stained with haematoxylin and eosin (H&E). Cross sections are observed microscopically for any modifications [72].

4.8 Hen’s Egg Test-Chorioallantoic Membrane (HET-CAM)

HET-CAM test is performed by incubating the eggs for 10 days at 37°C and relative humidity of about 70% with automatic turning once per hour. After incubation period, a portion of each egg shell is removed and a drop of water is placed onto the air sack membrane to avoid capillary damage during its removal. The CAM is then carefully exposed to 0.1 ml or 0.1 gm of test substances, which is washed-off with normal saline solution after 30 sec of exposure. Simultaneously, CAM is exposed to saline solution (negative control) and 1% SDS solution (positive control). Each CAM is observed microscopically after 5 minutes for haemorrhage, lysis and coagulation. An irritation score (IS) is calculated for each CAM by using following formula:

\[
IS = \frac{(301 - \text{Hemorrhage})}{300} \times 5 + \frac{(301 - \text{Lysis})}{300} \times 7 + \frac{(301 - \text{Coagulation})}{300} \times 9
\]

Irritation score is given according to following scheme; 0 = no reaction; 1 = slight reaction; 2 = moderate reaction; 3= severe reaction [73, 74].

4.9 Accelerated stability study

A stability study for in situ formulation is carried out as per ICH guidelines to determine the physical stability of the formulation under accelerated storage conditions. Formulation is subjected to elevated temperatures and humidity conditions of 25±1°C/ 60%RH, 30±1°C/ 65%RH and 40 ± 2°C/ 75 ± 5 % RH. Samples are withdrawn at the end of 0, 30, 60 and 90 days and then evaluated for active drug content [76].

5. Conclusion

In situ gelling system is novel and technically superior to existing technologies. It solves various problems of conventional drug delivery systems including poor bioavailability, systemic side effect and poor patient adherence to therapy. It can be used for commercial manufacturing. In situ drug delivery systems can be breakthrough in treatment of number of ocular diseases. This new avenue of research would ultimately lead to a wide variety of practical therapeutic applications.

However, these systems have proved their effectiveness theoretically and much more efforts yet required producing such systems on commercial scale. In situ gel system is more idyllic approach for treatment of ocular diseases. Several promising in vitro and in vivo results have been reported so far with different types of in situ gelling systems. In future, more study is required for reconnoitring in situ formulation in treatment of various ocular chronic diseases.

References

U. D. Laddha and H. S. Mahajan / An insight to ocular in situ gelling systems


