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Review Article

A COMPREHENSIVE REVIEW ON IN SITU GELS

BUDUMURU PADMASRI[1,2*], RAVOURU NAGARAJU[1], DAMARASINGU PRASANTH[2]

[1]Department of Pharmaceutics, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam, Tirupati, Andhra Pradesh, India, [2]Sri Venkateswara College of Pharmacy, Srikakulam, Andhra Pradesh, India
Email: padmasripharma@gmail.com

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ABSTRACT

The current review on in situ gelling systems becomes one of the most popular and prominent. It had a tremendous potential advantage of delivery systems due to many benefits like easy to use simple manufacturing; improve both adherence and patient comfort by minimizing the frequency of drug administration by its unique characteristics feature of sol to gel transition. It also provides in situ gelling nanoemulsions, nanosphere, microspheres, and liposomes. The drawbacks associated with conventional systems of both solutions and gels, such as accurate dosing, ease of administration overcome by using in situ gelling systems. This review focused on definitions, types, advantages, disadvantages, polymers used, and suitable characteristics of polymers, including the preparation of in situ gels covered in the introduction. Approaches, applications, and evaluation of in situ gels were explained with examples.

Keywords: Gels, Hydrogels, In situ gel, Polymers, Gelling mechanism

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INTRODUCTION

A gel is a soft, stable, or solid-like material which consists of at least two components, one of them being a liquid, present in substantial quantity [1]. Gels are a transitional state of matter containing both liquid and reliable ingredients (semisolids or semi-liquids). Gels combine the cohesive properties of solids and the diffusive transport characteristics of fluids [2]. It consists of a three-dimensional, stable, and secure component network [3]. In gels, the polymer network is formed by the cross-linking of polymer chains either by the formation of covalent (chemical cross-linking) or non-covalent bonds (physical cross-linking). Based on nature, gels are classified into two types (i.e., physical and chemical). Physical gels have weak bonds like hydrogen, electrostatic, and Vander Waal bonds [4]. Due to toxicity concern, there is increasing interest in physically cross-linked gels—chemical gels when arising strong covalent bonds [5].

Hydrogels are the polymeric chains of three-dimensional (3-D) structures. So, they can be easily forms in various sizes and shapes [6]. These hydrogels have an excellent absorbing ability to transition between liquid-gel and itself; it is a type of hydrophilic preparations [7]. Hydro-gels consist of cross-links to serve to accommodate a considerable amount of air, and it retains enormous amounts of water and biological fluids to swell. Hydrogels are also classified into two types (i.e., preformed hydrogels and in situ gels) [8].

Preformed gels or preformed particle gels (PPG) are simple viscous solutions that can't undergo any modification after administration [9]. PPG is superabsorbent cross-linking polymers that can swell up to 200 times its original size and act as a fluid diverting agent to control conformance is a novel process designed to overcome some distinct drawbacks inherent in situ gelation system [10]. To overcome changes in gel composition, degradation, lack of gelation time control, and several weaknesses go with preformed gels. Still, dilution by water and it has a defect in ophthalmic dosage form, including less accurate dose, blurred vision, lacrimation, etc. preformed gels are formed on the surface before it is injecting through the reservoir [11]. Hence, no gelation occurs, and it needs to be considered, including pH, salinity, multivalent ions, hydrogen sulfide, temperature, and shear rate [10].

In situ gels are the solutions or suspensions that undergo gelation after reaching the particular site due to contact with body fluids or physicochemical changes such as pH, temperature, ionic concentration, UV radiation, presence of specific molecules, or ions, external triggers, etc. [12]. In situ gel produces a constant plasma drug profile in the body by extending the release of a drug, so it is attached and absorbed in gel form and is known to prolong the life of the drug in the mucosa [13]. The drug delivery systems having the properties, as mentioned earlier, of sol to gel transition can be widely used for sustained delivery vehicle preparation of bioactive molecules [14]. In situ gels, potentially used for oral, buccal, subcutaneous, transdermal, intraperitoneal, ocular, nasal, rectal, vaginal, and parenteral routes [15]. From a manufacturing point of view, less complicated and thus lowers the investment and manufacturing cost [16]. In the discovery phase, the gel formulations are used to enhance the local and systemic exposure of potential lead compounds, which is ideal for establishing animal models for various conditions quickly and cost-effective [17]. Despite the massive diversity of gels, a particular class of gels, namely smart polymer gels, are in the focus of pharmaceutical research during the last decades [18]. These intelligent polymers change their physicochemical properties in response to an altered environment. In recent advances, in situ gels have made it possible to exploit the changes in physiological uniqueness [19]. Comprehensive research has been carried in designing of in situ gels, emerged as one of the best novel drug delivery systems (NDDS) [20]. In this review, they mainly focussed on introduction, advantages, disadvantages, suitable polymer characteristics, approaches, applications, evaluation, and marketing products of in situ gels. It also focused on some reported studies as well as recent advancements of in situ gels.

The intention of writing this review article to describes every aspect of in situ gels, which near the readers a specific feature and might contribute to research and development.

Advantages

- To decrease the wastage of drug [21]
- To ease of administration [22]
- It administered to unconscious and old patients [23]
- It helps to extended or prolonged release of drugs [24]
- It allows more patient comfort and compliance [25]
- Due to the low dose, there will be no drug accumulation and minimize the drug toxicity [26]
- It offers more bio-availability [27]
- By using natural polymers, provides biocompatibility and biodegradation [28].
- By using synthetic polymers usually well defined that can be modified to yield tolerable degradability and functionality [29]

➢ To Facilitate drug targeting primarily through mucus membranes, for non-invasive drug administration [30]

➢ It offers a vital stealth characteristic *in vivo*, owing to its hydrophilicity, which increases the *in vivo* circulation time of the delivery device [31]

➢ It exhibits bio-adhesiveness to facilitate drug targeting, primarily through mucus membranes, for non-invasive drug administration [32]

➢ To reduce the systemic absorption of drugs drained through the nasolacrimal duct [33]

Disadvantages

➢ Requires a high level of fluids [10, 25]

➢ The solution form of the drug is more susceptible to degradation [34, 35]

➢ Due to chemical degradation, there is a chance of stability problems [36]

➢ Eating and drinking restricted for a few hours after placing the drug [25, 36, 37]

➢ Only small doses administered [38]

➢ Due to low mechanical strength, it may result in premature dissolution [36, 39]

➢ Particularly for hydrophobic drugs, the quantity and homogeneity of drug loading into hydrogels may be limited [39, 40]

Suitable characteristics of polymers

An essential ingredient in the manufacture of in situ and preformed gel is a polymer**.** The suitable polymer characteristics for in situ gels given below [41-45]:

➢ It should be compatible

➢ It should not provide any toxic effects

➢ It should have good tolerance and optical clarity

➢ It should have pseudo-plastic behavior

➢ It should be capable of adhering to the mucous membrane

➢ It should be capable of decreasing the viscosity with an increase in shear rate

➢ It should influence tear behavior

Classification of in situ gelling polymers

Based on their origin, polymers can be classified or the mechanism of gelation. According to a source in situ, gelling systems are classified into two types [46-50].

i. Natural polymers (e. g., Alginic acid, carrageenan, chitosan, guar gum, gellan gum, pectin, sodium hyaluronate, xanthan gum, xyloglucan, etc.)

ii. Synthetic or semi-synthetic polymers (e. g., CAP, HPMC, MC, PAA, PLGA, poloxamers)

Sol-gel method

The starting materials, 'sol', are usually inorganic metal salts or organic compounds such as metal alkoxides. In the conventional 'sol-gel' method, the precursor goes through reactions of hydrolysis and polymerization or condensation to produce a colloidal suspension or solution. Complete polymerization along with subsequent loss of solvent directs conversion from 'sol' (liquid phase) to 'gel' (solid phase) [51].

Preparation of in situ gel

The polymer may differ based on the development of in situ gelling systems. The polymeric solution was prepared by dispersing required quantities of polymers and copolymers in distilled water using a magnetic stirrer until the polymers completely dissolve. After the preparation of an aqueous drug solution, transferred to a primarily prepared polymeric solution with continuous stirring until to get a homogeneous solution, and then add excipients based on the delivery system. Finally, make up the volume with distilled water [52].

Approaches of in situ gels

There are four generally defined mechanisms used for triggering the in situ gel formation of biomaterial [15, 22, 34, 42, 53-55]:

A. Physiological stimuli (e. g., temperature and pH)

B. Physical stimuli (e. g., solvent exchange or diffusion and swelling)

C. Chemical stimuli (e. g., enzymatic, chemical and photo-initiated polymerization)

A. Physiological stimuli

Based on physiological stimuli classified into two categories [22]:

Temperature-induced in situ gelling systems or thermally triggered systems

In these systems, no external heat other than body temperature is required to cause gelation, and the mechanism showed in fig. 1 [43, 49]. These are the most widely used systems.

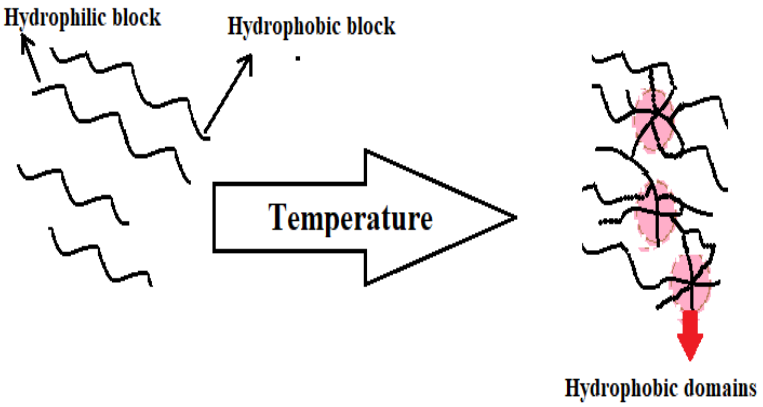

Fig. 1: Mechanism involved in temperature triggered system [56]

Three main strategies exist in the engineering of the thermo responsive sol-gel polymeric system. For convenience, this thermal-induced or thermal sensitive in situ gel system classified into three types [57-59]

i. Negatively thermo-sensitive type; e. g., poly-N-isopropyl acrylamide (PNIPAAm)

ii. Positively thermo-sensitive type, e. g., polyacrylic acid (PAA), poly (acrylamide-co-butyl methacrylate), or polyacrylamide

iii. Thermally reversibly type; e. g. poloxamer, pluronic (poloxamer), tetronics (poloxamines), poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide).

***pH* triggered systems**

In this approach, pH-responsive or pH-sensitive polymers to be used to form a gel. All pH-sensitive polymers contain acidic or alkaline ionizable functional groups and that either loose or let accept protons in comeback to change in pH. A huge number of ionizable groups are known as poly-electrolytes and mechanisms shown in fig. 2 [49, 60, 61].

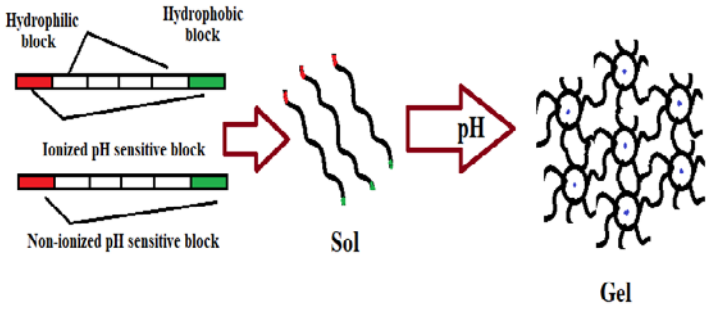

Fig. 2: Mechanism involved in the pH-sensitive system [56]

These poly-electrolytes cause an increase in external pH that leads to the swelling of hydrogels that leads to the formation of in situ gels—some of the anionic groups used as pH triggered systems. E. g., Cellulose acetate phthalate (CAP), polyethylene glycol (PEG), pseudo latexes, ploy methacrylic acid (PMC), carbomer, and its derivatives, etc., Mixtures of poly-methacrylic acid (PMA) and PEG also been used as a pH-sensitive system to achieve gelation. The most of anionic pH-sensitive polymers are PAA (carbopol, carbomer) or its derivatives [22, 62, 63].

B. Physical stimuli

When a material absorbs water from the surrounding environment, in situ formation may also occur and expand to the desired space [22, 42, 64].

Solvent exchange or diffusion

This process solvent diffuses from the polymer solution into surrounding tissue and results in precipitation or solidification of the polymer matrix [25, 36]. The most commonly used polymer for this approach is N-methyl pyrrolidone (NMP) [65].

Swelling

The polar lipid or polymer swells from inside to outside and slowly releases the drug (i.e., to form lyotropic crystalline phase structures). It has some bio-adhesive properties and degraded *in vivo* enzymatic action [66]. E. g, Myverol 18-99 (glycerol-mono-oleate)

C. Chemical reactions

Chemical reactions that result in situ gelations may involve precipitation of inorganic solids by following processes.

Chemical polymerization of ionic cross-linking

Ion sensitive polymers induce gelation in the presence of ions like Na+, K+, Ca+2, and Mg+2. These ionic polymers undergo a phase transition to form a gel. Some of the polysaccharides fall into this class [67, 68].

Enzymatic polymerization or enzymatic cross-linking

In this approach, the gel was formed by cross-linking with the enzymes that are present in the body fluids and have some advantages over chemical and photochemical methods, and mechanism showed in fig. 3 [69, 70].

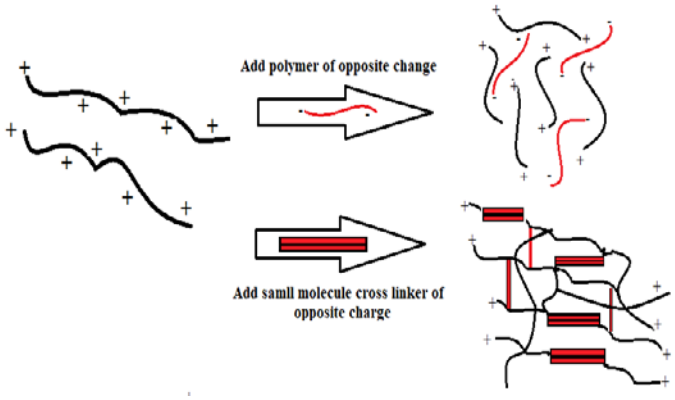

Fig. 3: Mechanism involved in ion activated system [56]

Photo-initiated polymerization

It is the most convenient and commonly used approach in the formation of in situ gels. Monomers or reactive micromere solutions and the initiators injected into a tissue site, and the application of electromagnetic radiation used to form a gel. Usually, long-wavelength ultraviolet (i.e., ketones) and visible (camphor-quinone and ethyl eosin) wavelength polymers were used (i.e., acrylates or other polymers)—short-wavelength polymers not used because they are biologically harmful [71].

Novel approaches of in situ gels

A variety of unique systems are used to extend the drug delivery by in situ gelling systems. These systems delay the elimination of active ingredients from the eye and also improved corneal penetration of a drug molecule [33, 72].

Nanoparticles incorporated in situ gel

Recently, nanoparticles were employed to address issues related to topical formulations. These represent promising drug carriers for targeting ocular tissues by remaining at the site of application and providing prolonged release by particle degradation or erosion drug diffusion or a combination of both [73].

Liposome incorporated in situ gel

It is also a tool for prolonged controlled delivery of a drug; in lipid vesicles, active ingredients were encapsulated and transport drug through the cornea [74].

In situ gelling ocular films or inserts

Ocular inserts or films are semisolid or solid consistency, usually composed of a polymeric vehicle containing the drug, whose size and shape are designed for ophthalmic application [75].

Nanoemulsified in situ gels

Nanoemulsions are widely using due to its intrinsic advantages such as the higher penetration into the deeper layers, sustained release of drugs to the cornea, and ease of sterilization [76].

Applications

Oral drug delivery systems

Gellan gum, pectin, xyloglucan, etc., are used for oral in situ gels. The pH-sensitive gels have potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract (GIT), and some of the reported polymers and drugs, including the route of administration of in situ gelling systems showed in table 1[12, 74].

Table 1: Summary of reported drug and polymers of in situ gelling systems

Drug	Polymer used	Route of administration	Reference
Doxorubicin	Human serum albumin and tartaric acid derivative	Parenteral	[77]
Paracetamol and Ambroxo	Pectin	Oral	[78]
Pheniramine maleate and albumin FITC	Ploymethacrylic acid	Parenteral	[79]
Recombinant human interleukin-2	Physically cross-linked dextran	Parenteral	[80]
Testosterone	Poly-lactic acid and PLGA	Parenteral	[81]
Theophylline	Gellan gum	Oral	[82]

Gellan gum

Gellan gum has the tendency of gelation, which is temperature-dependent or cations induced. The in situ gelling systems consisted of a gellan solution with calcium chloride and sodium citrate complexes. When it's administered orally, the calcium ions were released in the acidic environment of the stomach leading to the gelation of gellan, thus form an in situ gel [83].

Pectin

Gelation of pectin will occur in the presence of H^+ions, a source of divalent cations; generally, calcium ions are required to produce the gels that are suitable as vehicles for drug delivery [22]. The main advantage is that it is water-soluble, so there is no need for organic solvents in the formulation. Divalent cations present in the stomach carry us the transition of pectin to gel state when it is administered orally [83]. Calcium ions in the compound form may include in the formulation for the induction of pectin gelation. Sodium citrate may be added to the above solution to form a complex with most of the calcium ions added. The fluid state ('sol') is maintained until the breakdown of the complex in the acidic environment in the stomach, where the release of calcium ions causes gelation [54]. The quantities of calcium and citrate ions may be optimized to maintain the fluidity. Before administration into the stomach, it is resulting in gelation when the formulation.

Xyloglucan

Xyloglucan is partially degraded by β-galactosidase, resultant product exhibits thermally reversible gelation by the lateral stacking of the rod-like chains or on warming to body temperature [84]. Depends on the degree of galactose elimination, sol-gel transition temperature also varies. Its potential application in oral drug delivery exploits the proposed slow gelation time that would allow in situ gelations in the stomach following the oral administration of chilled xyloglucan solution [83]. The gelation behavior of xyloglucan is similar to Pluronic F127, but it forms a 'gel' at a much lower concentration [22].

Ocular drug delivery systems

Conventional delivery systems often result in reduced bioavailability and therapeutic effect because of high tear fluids; dynamics cause rapid elimination of drugs [85]. Alginic acid, gellan gum, and xyloglucan most commonly used for ocular drug delivery. For locally acting drugs such as antimicrobial, anti-inflammatory, and autonomic drugs used to relieve intraocular tension in glaucoma [86]. Various water-soluble polymers and pH-induced in situ precipitating polymeric systems such as carbopol, HPMC, PMA-PEG [87].

Alginic acid

Aqueous solutions of alginates form gels on the addition of di-and trivalent metal ions by a cooperative process and involves consecutive glucuronic residues in the α-L-glucuronic acid blocks of the alginate chain. A prolonged pre-corneal residence of formulations containing alginic acid looked for, not only based on its ability to gel in the eye, and it includes mucoadhesive properties [22, 83, 88].

Carbopol

It is a well-known pH-dependent, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. In combination with HPMC, impart the viscosity of carbopol solution while reducing the acidity of the solution [22, 83, 89].

Gellan gum

Much of the interest in the pharmaceutical application of gellan gum has concentrated on its implementation of ophthalmic drug delivery. An aqueous solution of gellan dropped into the eye undergoes a transition into the gel state due to the temperature and ionic condition (Ca^{+2}) in the tear fluid. Drug release from these in situ gels prolonged due to longer pre-corneal contact times of the viscous gels compared with conventional eye drops [90, 91].

Xyloglucan

Xyloglucan chains are hydrophilic, dense, and having mucoadhesive strength, is a suitable candidate for increases in the corneal residence time of drugs. The increased drug absorption and the prolonged drug elimination were obtained with viscous in situ gel formulations [92].

Nasal drug delivery systems

Nasal drug administration has been considered as an alternative route for systems use of drugs restricted to intravenous administration. Nasal drug delivery can also provide a way of entry to the brain that circumvents the blood-brain barrier (BBB) because the olfactory

receptor cells are in direct contact with the central nervous system. Because of the large absorptive surface and low proteolytic activity, the nasal mucosa is considered an attractive site for the delivery of vaccines. Nasal vaccines will improve patient compliance and reduce production costs compared with parental products. Mostly protein and peptides can deliver by this route [93, 94].

Parenteral drug delivery systems

Chitosan is a biocompatible pH-dependent cationic polymer, which remains dissolved in an aqueous solution, pH exceeding 6.2 leads to form a hydrated gel-like precipitate [22, 83]. The main problem with chitosan is its non-biodegradability, and the following procedure could overcome this. The pH gelling cationic polysaccharides solution is transformed into thermally sensitive pH-dependent, without any chemical modification or cross-linking by the addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose, glucose phosphate salts to chitosan aqueous solution [34, 83]. This transformation has solved the non-biodegradability problem of chitosan. Synthetic polymers are a trendy choice for parenteral preparations. The trend in drug delivery skills has been towards biodegradable polymers, requiring no follow up surgical removal once the drug supply is depleted. Aliphatic polyesters such as PLA, PGA, PLCG (poly-lactide-co-glycolate), PDL (poly-deca-lactone), PCL (poly-ε-caprolactone) have been the subject of the most extensive recent investigations and various tri-block polymers also used in injectables [95, 96]. The feasibility of lactide or glycolide polymers as excipients fort the controlled release fo bioactive agents is well proven. These materials are subjected to extensive animal and human trials without evidence of any harmful side effects. When under GMP conditions from a purified monomer, the polymers exhibit no evidence of inflammatory response or other adverse effects upon implantation [97, 98]. The photoreactions provide rapid polymerization rates at physiological temperatures; furthermore, the systems are placed in complex-shaped volumes leading to an implant formation. Thermosetting systems are in the 'sol' form when initially constituted, but upon heating, they set into their final shape ('gel'). This sol-gel transition is known as curing [42, 54]. Curing mainly involves the formation of covalent cross-links between polymer chains to form a macromolecular network. But if this one is heated further, it may lead to degradation. In situ precipitating polymeric systems, the solution may lead to form a gel, and this precipitation induced by a change in temperature (thermosensitive), solvent removal, or by a change in pH [22, 83]. A notable example of a thermosensitive polymer is NIPAAm, which has a lower critical solution temperature phase separation at 32 °C. It is unique concerning the sharpness of its almost discontinuous transition, which is usually observed only with ionizable polymers. Some tri-block copolymers like pluronic or poloxamers consist of POE and POP units that undergo solubility changes with temperature [99].

Dermal and transdermal drug delivery systems

Skin is considered an essential route of administration of drugs for both local and systemic effects. Common preparations for topical and dermatological administration of drugs have certain limitations like poor adherence, reduced permeability, and compromised patient compliance. *In vivo* studies suggest that 20 % w/w aqueous gel maybe it is used as a base for topical administration of the drug. The combination of iontophoresis and chemical enhancers results in a synergistic enhancement of insulin permeation [100].

Rectal drug delivery systems

Although the oral route is the most convenient route for drug administration, this is not possible from either a clinical or pharmaceutical perspective. In these cases, the rectal way may represent a practical alternative and can be used to administer drugs for both local and systemic effects. The environment in the rectum is considered relatively constant and stable and has low enzyme activity in comparison to other sections of the GIT. However, the rectal cavity can be challenged by erratic drug absorption due to the low adhesion to the rectal membrane and the potential expulsion of the dosage form. These can prevent dosage form leakage, which is familiar with rectal suspensions and enemas [33, 101, 102].

Vaginal drug delivery systems

It is a relatively unexploited site other than the treatment of vaginal infections, contraception, local menopausal symptoms, and labor induction. It is a potential route for a therapeutic portfolio, including vaccine delivery and chemotherapy. Blood supply of the vaginal epithelium and the large surface area can allow smooth systemic drug delivery. Thermoreversible, mucoadhesive gels and pessaries studied as formulation platforms for the delivery of antiretroviral and antimicrobial compounds, labor-inducing hormones, and even for intra-vaginal vaccine delivery [103, 104].

Summary of some reported studies by in situ gels showed in table 2, and some Marketed products of *in situ* gelling systems shown in table 3.

Evaluation of in situ gels

In situ, gels evaluated characterized for the following parameters:

Physical evaluation

Compatibility studies

Compatibility studies carried out for a physical mixture of interaction between drug and excipients by a suitable method such as Fourier Transform Infra-Red Spectroscopy (FTIR) or Differential Scanning Calorimetry (DSC) [105].

Appearance

Preferably, the gels should be transparent. The formulations were observed for a general appearance by the naked eye, such as color, odor, and the presence of suspended particulate matter [106].

Clarity test

The clarity of the product checked using a black and white background [107].

pH

The pH was checked by using a calibrated digital pH meter immediately after preparation. In the case of ocular preparations, the pH preferably near to ocular pH to avoid eye irritation and enhance patient compatibility and tolerance [108].

Homogeneity

By placing the preparation between two glasses, then observe particle roughness under the light [109].

Isotonicity

The formulation is mixed with few drops of blood, observe under a microscope, and compare with standard ophthalmic preparations. For all ophthalmic preparations, maintenance of isotonicity must need to prevent tissue damage and irritation to the eye [110].

Sol-gel transition temperature

The temperature of the phase transition of 'sol' meniscus was noted first and then heated at a specified rate. 'Gel' formation is indicated by a lack of movement of the meniscus on tilting the tube and note down the temperature [22, 83].

Gelling time

Gelling time is the time required for the first detection of gelation, as defined in sol-gel transition temperature [111, 112].

Texture analysis

The cohesiveness, consistency, firmness of in situ gels assessed using a texture profile analyzer, which mainly indicates the syringe ability of 'sol' so the formulation can be quickly administration via *in vivo* [113].

Spreading coefficient

The device consists of a ground glass slide fixed on the wooden block. Each formulation weighing about 2 grams was placed and studied on this ground slide. Gel preparation was then sandwich

between this slide and second slide having some dimension as that of the fixed glass slide. The second slide was provided with a hook. Weight of 1 gram placed on top of the two slides for 5 min. to expel air and provide a uniform film of gel between two slides. Measured weight is placed on a pan attached to the pulley with the help of a hook. The time required by the top slide to separate from a ground slide was noted. A shorter interval indicates a better spreading coefficient (S) [114, 115].

$$S = \frac{M X L}{T}$$

M = Weight tied to upper slide

L = Length of glass slides

T = time taken to separate the slides

Gelling strength

The rheometer determined the gelling strength, and it depends on the mechanism of the gelling agent, a specified amount of 'gel' prepared in a beaker, from the 'sol' form. This 'gel' containing beaker to be raised at a specific rate, so pushing a probe slowly through the 'gel' and the load on the probe is measured by the depth of the immersion of the 'gel' surface [93, 116].

Gelling capacity

Method 1

By placing a drop of a freshly prepared formulation with a vial containing 2 ml of stimulated tear fluid (STF) and note down the time taken for the 'gel' formed or 'gel' to dissolve in 7.4 pH phosphate buffer and its used for determination of the suitable polymer concentrations or gelling agent to form in situ gelling systems [117].

Methods 2

They were using water-soluble dyes such as amaranth, Congo red, indigo blue, etc., used by dissolving 1 g in distilled water and mixed with the prepared in situ gel. *In vitro,* gelling capacities of the formulations were measure by placing 5 ml of gelation solution (STF) in the glass test tube and maintained the temperature at 37±0.5 °C. It immediately converted into a stiff gel-like appearance. *In vitro*, gelling capacities are evaluated by the presence of the stiffness of the gel. And the time for which the gel converted into thick gel remains as such. Further, the color added to give a visual appearance to the gel. Based on the three categories *in vitro,* the gelling capacity period was calculated [118].

+'gel' forms after a few minutes disperse rapidly

++immediately gelation occurs, remains for a few hours

+++immediately gelation occurs, remains for an extended period of time

Viscosity and rheology

At room (i.e., 25 °C) and body temperatures (i.e., 37±0.5 °C), observe the viscosity using Brookfield viscometer. Rheology was observed due to the thixotropic behavior of the gel. In situ gel preparations should show pseudo-plastic and Newtonian flow before and after the gelation process. Before and after gelling, it should be 5-1000 m Pas ('sol') and after 50-50,000 m Pas ('gel'), respectively. The gel formulation in situ should be well-formulated, so administration to the patient is proper, especially in ocular administration. However, these agents have the disadvantage of making blurred vision and leaving residue on the eyelids; due to high viscosity can cause difficulties in screening [119, 120].

Stability studies

Stability testing aimed to know the time of storage and the use of the material as per the International Conference on Harmonization (ICH). Place the sample in a climatic chamber at 40±2 °C temperature and 75±5% RH for approximately one month. After a few months, the sample analyzed associated clarity, pH, viscosity, drug content, rheological, and *in vitro* dissolution. The storage conditions and the length of the study chosen should be sufficient to cover the storage, shipment, and subsequent use [121, 122].

Drug release studies

***In vitro* drug release**

In vitro release study of in situ gelling systems can be carried out by using Franz diffusion cell to check the duration [44, 123].

***In vivo* drug release**

Evaluation of drug preparation is one drug release in the body (*in vivo*). By knowing the time devastated and the polymer components used, we can design the drug as per the needs of pharmacotherapy [94, 124].

Microbiological evaluation

Sterility testing

This testing is done with the aseptic transfer technique to avoid contamination of the environment. Sterility testing is an essential parameter for all ophthalmic preparation, and it must perform for aerobic, anaerobic bacteria and fungi by using suitable media under aseptic conditions. As per Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP), mostly direct inoculation method used to test sterility. Initially, inoculate the sample into liquid media (thioglycollate medium and soybean digest medium). After that, incubate for 7-14 d at different temperatures; for thioglycollate medium (30-35 °C) and soybean digest medium (20-25 °C), then identify microbial growth [125, 126].

Irritation studies

Albino rabbits are using for the Draize irritation test, a single drop of 0.04 ml formulation instilled into rabbit eyes (the lower conjunctive cul-de-sac). The test eye and by the eyelids can be held together for several seconds after installation. Rabbit eyes were observed periodically at 1, 24, 48, 72 h, one week after exposure. Ocular changes were graded by a scoring system that includes rate any alterations to eyelids, conjunctiva, cornea, redness, swelling, watering, and iris [127, 128].

Antifungal studies

Initially, sabouraud dextrose dissolves in hot water (i.e., media), after 15-20 min. of autoclaving transfer, the organisms such as *Candida albicans, Aspergillus fumigatus,* etc., in media in order and put a sample test with a micropipette and let set aside for 30 min. After 24 h incubation at 25 °C, the diameter of the zone of inhibition or zones was measured, finally compared with positive and negative controls [129].

Antibacterial studies

This test was conducted to find out the effectiveness of antibacterial of active antibiotic substances, the concentrations that are referred to as antibacterial. Finally, the results of the growth of bacteria samples could compare with standard antibiotics [130].

Statistical evaluation

Based on obtained data, a multivariate test was used to analyze mucoadhesive strength and release studies. To get a significant difference by using various SPSS software and considered significant at $P<0.05$. To storage conditions of the test, solutions can use one-way analysis variance (ANOVA) of viscosity data using prism with significance value ($P>0.05$). Still, there are no general rules regarding this statistical test [131-132]. And some of the marketed products of in situ gelling systems showed in table 2 [133].

Table 2: Summary of some of the marketed products of in situ systems

Manufacturing company	Name of the marketed product	Drugs used in the formulation	Reference
Akten	Akten ™	Lidocaine hydrochloride	[134]
Alcon Laboratories Inc.	Pilopine HS	Pilocarpine hydrochloride	[135]
Insite vision	Azasite	Azithromycin	[136]
Macromed	Cytoryn	Interleukin-2(IL-2)	[42]
Macromed	Regel Depot Technology	Human Growth Hormone	[137]
Merck and Co. Inc	Timoptic-XE	Timolol maleate	[138]
Spectrum Thea Pharmaceuticals	Virgan	Ganciclovir	[135]

CONCLUSION

The utilization of in situ gels providing various advantages over conventional dosage forms. The use of biocompatible, biodegradable, and water-soluble polymers for the in situ gel formulation can make excellent and excellent drug delivery systems. In recent years researchers have drawn interest, and there is a scope to provide an advanced technique in drug delivery. A novel carrier can incorporate in these systems to obtain sustained drug delivery in a much improved and extreme manner. These systems, as they can administer in solution form, undergo gelation at the site of action. Finally, in situ, gels are easy to apply and offer patient comfort and compliance.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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