IN-SITU GEL: THE REVOLUTIONARY APPROACH FOR DRUG DELIVERY

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ABSTRACT:

In situ gels are innovative drug delivery systems that transition from a liquid to a gel state in response to specific physiological conditions, such as changes in temperature, pH, or ionic strength. These systems offer advantages over traditional methods including reduced dosing frequency, controlled drug release, enhanced bioavailability, improved patient compliance, and targeted therapy. These gel formulations are highly versatile and can be administered via various routes including, ocular, nasal, injectable, and transdermal. This review aims to provide an overview of the current advances in the design and applications of in situ gels in various therapeutic areas, including the materials used (such as natural and synthetic polymers), gelation mechanisms, and their potential in drug delivery. The article also discusses challenges such as stability, biocompatibility, and drug release profiles. It highlights recent advancements in formulation strategies and clinical applications, emphasizing the potential of in situ gels in modern drug delivery systems.

INTRODUCTION:

Over the past 25-30 years, greater attention has been gained on developing the controlled and sustained release system. Among that extensive research has been carried out to develop polymeric drug delivery systems. The attention on the In-Situ gel has been raised in the past few years. In the past few years, In-Situ gel formulations have been investigated and many patents for their use have been reported (1). This concept of In-Situ gel was proposed in the early 1970s. This type of system has been accepted by patients for its prominent effect and ease of use (2). The importance of the In-Situ gel was their no side effects because of low dose of the drug was required to produce the desired therapeutic effect. This was one of the best novel drug delivery systems which improved patient compliance through its special characteristics feature of “Sol to Gel”. This type of gelling system is a formulation that converts from liquid

phase to gel phase under various physiological conditions. The sol-to-gel transformation depends on various factors like temperature, change in pH, solvent exchange, UV radiation, and the presence of specific molecules or ions (3). Over the past 10 years, there has been progress made in the development of techniques that can efficiently define the rheological properties of the In-situ gelling system. The advanced method for evaluating the rheological properties of the In-Situ gel was oscillatory rheometry which can be paired with scanning calorimetry (4). In-Situ gels have been potentially used for the buccal, nasal, rectal, intraperitoneal, ocular, oral, parenteral, subcutaneous, vaginal, and transdermal routes. This formulation enhances the local and systemic exposure of potential lead compounds in starting of the discovery phase and animal models (5). Despite the massive diversity of the gels, a particular class of drugs namely smart polymer gels has been focused in pharmaceutical research during the last decades. These polymers change their physiochemical properties in response to an altered environment n in recent advances, In-Situ gels have made it possible to exploit the changes in physiological responses. A comprehensive study has been carried out on designing In-Situ gels emerged as one of the best novel drug delivery systems (6)

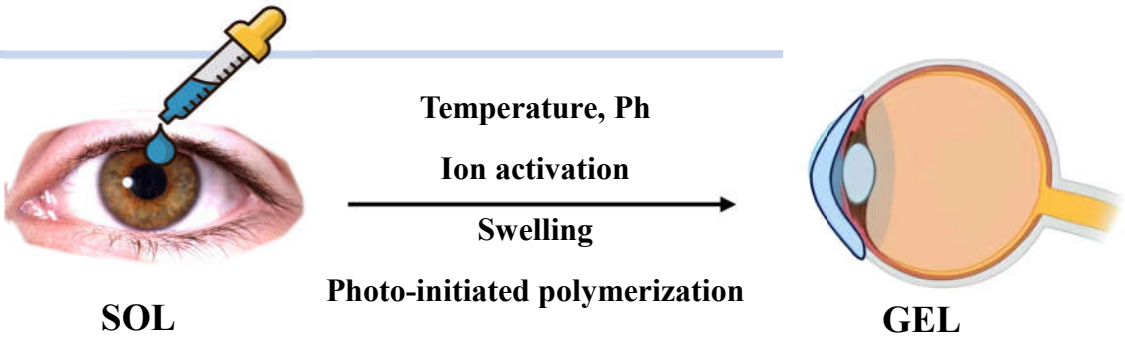


Fig 1: Diagrammatic representation of "sol to gel" transition.

ADVANTAGES:

1. Reduce drug wastage.
2. Sustained drug release is achieved.
3. Elute the in vivo circulation time of the delivery device providing vital stealth characteristics in vivo due to its hydrophilicity.
4. Reduces drug toxicity and drug accumulation does not occur due to these of a low dose.
5. Reduces local and systemic side effects.

6. Due to the formulation of the gel, the drug increases its contact with the tissues and the residence time of the drug improves.
7. It can be used on unconscious patients.
8. Minimize the frequency of administration.
9. The ophthalmic solution has a low bioavailability and this can be overcome with the gel instilled as eye drops.
10. Compared to the other pharmacological dosage forms, there are fewer side effects.
11. Improves bioavailability (6)(9).

DISADVANTAGES:

1. Due to chemical degradation, there may be a risk of stability problems.
2. A high fluid level is required.
3. It is in solution form, which is more susceptible to stability problems such as microbial and chemical degradation.
4. It must be stored properly if it is not stored properly, it will change the pH and cause stability problems.
5. After placing the medicine, eating and drinking is forbidden for several hours.
6. Due to the lower mechanical strength, this can lead to premature disintegration.
7. Only minimal doses can be administered. (11)(12).

POLYMERS USED AS IN-SITU GELLING AGENTS:

1. **Pectin**: Pectin is a type of complex carbohydrate that belongs to the polysaccharide group and its primary component of pectin is (1-4)-D galacturonic acid residues (14). Low methoxy pectin easily creates a gel when free calcium ions are present, which binds the galacturonic acid chains together in a way explained by the egg-the-box model. When a moderate amount of calcium is present, pectin stops a strong gel from forming (15). Since pectin dissolves in water, organic compounds can be removed from the formulation. When it is taken orally, the divalent cations present in the stomach induce the gelation of pectin (7).

2. **Carbopol:** The pH-dependent polymer is Carbopol, at acidic pH it stays in acidic form but at alkaline pH, it forms a low viscosity gel. HPMC is combined with carbopol to increase the viscosity of the Carbopol solution while simultaneously lowering its acidity (1). In comparison to other polymeric agents, Carbopol lacks the benefit of enhanced mucoadhesive properties (16).

3. **Chitosan:** Chitosan is an ideal choice for ophthalmic formulations because of its biocompatibility, biodegradability, mucoadhesive properties, ability to enhance permeation, effectiveness in corneal wound healing, as well as its antimicrobial and antifungal activities. This polymer can create a gel on its own, without any additional substances. Chitosan can form in situ gels with negatively charged macromolecules, including proteins (such as gelatin), polysaccharides (like alginate, xanthan), or synthetic poly-anions (such as poly-acrylic acid) (17). It is a bio-compatible, positively charged polymer that stays dissolved in strong solutions as long as the pH is below 6.2. When the pH of an aqueous solution of chitosan is raised above 6.2, it forms a gel-like precipitate by absorbing water (13).

4. **Poloxamer:** Poloxamer is a type of co-polymer made of three blocks that dissolve easily in water. Poloxamer or Pluronics is composed of more than 30 different non-ionic surfactants. The in-situ formulation depends on temperature variations. Pluronic F217 produces a clear and colourless gel and is one of the most widely used polymers in pharmaceutical technology (18). A 20% weight concentration of Pluronic F217 is needed for gelation at 25ºC. At this temperature, the solution is a fluid, viscous liquid, but it transforms into semisolid, transparent gel at body temperature (37ºC) (19).

5. **Hydroxy propyl methyl cellulose (HPMC)**: Cellulose is made up of glucan chains that consist of repeating units of β-(1, 4)-D- glucopyranose unit. Cellulose increases in viscosity as the temperature drops, while its derivatives like HPMC and methyl cellulose, become more viscous when the temperature rises. At a low temperature of 30ºC, the solution remains in liquid form, but as temperature rises (40-50ºC), it undergoes gelation (19). HPMC is combined with Carbopol to enhance the viscosity of the Carbopol solution and to lower its acidity (20).

6. **Xanthan gum:** Xanthan gum is a high molecular weight polysaccharide that is generated outside of the cells through the fermentation of the gram-negative bacterium Xanthomonas

campestris (21). It dissolves in both cold and hot water and is compatible with acidic and alkaline environments, exhibiting good stability in alkaline conditions (22).

7. **Xyloglucan:** In other words, xyloglucan commonly referred to as tamarind gum, is a polysaccharide obtained from the endosperm of tamarind seeds. In other words, xyloglucan is composed of three different types of oligomers: heptasaccharide, octa saccharide, and nosaccharide. These oligomers vary in the number of galactose side chains they contain. In other words, just like Poloxamer, this material begins to form a gel when it is cooled to refrigerator temperatures or heated at a high temperature (23).

8. **Gellan gum:**

   Gellan gum is a negatively charged polysaccharide that dissolves in water and is sold under names like Phytagel and Gelrit (24). When it's exposed to certain temperatures or specific metal ions (like calcium), it can form gels. This happens because the molecules twist into double helices, which then link together to create a solid, network-like structure (25). This ability to form gels makes gellan gum useful as a thickener and stabilizer in foods (23).

APPLICATIONS:

1. **Oral drug delivery system:** Oral gel in situ, or environmentally sensitive gel, is a new dosage form that has recently been used in drug delivery. Compared with traditional formulations, in situ gels were administered as low-viscosity solutions, and in the sensitive environment, the polymer changed its conformation to produce a gel, so it could not only extend the time of contact between the drug and absorption sites in the stomach but also release the drug slowly and continuously, making it especially useful for chronically used drugs (27). Since the oral route is the most compatible and simple route of drug administration, in situ gelling system are so also formulated for oral administration. Formulations of different categories of drugs are presented. Examples include the antimicrobial drug clotrimazole, formulated as an in-situ gelation system with carbopol 934P, gellan gum, and HPMC as polymers showing zero-order kinetics release with 8 hours of sustained drug action. Paracetamol, an anti-inflammatory drug, was formulated as an in-situ gelation system with xyloglucans, a natural polymer that exhibits a diffusion-controlled release of the drug (10).

2. **Ophthalmic drug delivery system:** In the new drug delivery system, different approaches are used such as in situ gelation, the use of mucoadhesive polymers, polymer-

coated nanoparticles, and liposomal formulation. These delivery systems delay the elimination of the active ingredient from the eye and also improve the corneal penetration of the drug molecule (28). The low bioavailability and poor therapeutic response of conventional eye drops can be overcome by using a new ophthalmic drug delivery system. In the last two decades, several NODDS have been developed to improve the ocular bioavailability of drugs. These include aqueous gels, liposomes, nanoparticles, dendrimers, nano micelles, implants, contact lenses, and nanosuspensions (29).

3. **Nasal drug delivery system:** An in-situ gel system for the nasal administration of mometasone furoate was developed and evaluated for its efficacy in the treatment of allergic rhinitis. Gellan gum and xanthium gum were used as in situ gelling polymers. Animal studies were carried out with a model of allergic rhinitis and observed the effect of the gel in situ on the nasal systems induced by the antigen in sensitized mice. The in-situ gel was found to inhibit the development of nasal symptoms compared to the marketed formulation Nasonex [mometasone furoate suspension 0.05 percentage]. The aforementioned intact respiratory epithelium and normal appearance of goblet cells, demonstrated by histopathology of the nasal cavity of the mouse, showed that these formulations were safe for nasal administration. Thermoreversible gel formulations of flunarizine hydrochloride have been investigated to improve the residence time of the drug in the nasal cavity. The formulations thus prepared were in the liquid state at 4℃, but they turned into gel at the temperature of the nasal cavity. Poloxamer 407 was used as a polymer that showed phase transition behaviour. Inclusion complexes using B-cyclodextrin were prepared to increase the solubility of flunarizine in nasal secretions. The prepared formulations are characterized by drug loading, content uniformity, in vitro drug diffusion, adhesive strength, gel strength, viscosity, and gel point. The formulations showed a drastic increase in viscosity at 37℃, indicating their potential use as topical gel release, possibly due to the increased solubility and dissolution rate of flunarizine hydrochloride (30).

4. **Vaginal drug delivery system:** The vagina is a convenient route of administration for drugs with local and systemic effects. Antimicrobials, hormones, spermicides, and anti-inflammatories are traditionally administered locally via the vaginal route (31). In addition, the vagina has many characteristics, such as an abundant blood supply, a large surface area, and the ability to bypass first-pass metabolism, thus creating a convenient vaginal route for the systemic administration of drugs such as calcitonin. On the other hand, the self-cleaning effect of the vaginal fluid is responsible for the leakage of many dosage forms,

resulting in a decrease in therapeutic effectiveness. The adoption of mucoadhesive and gel formulations in situ represents a strategy proposed in the last decades to increase the residence time of liquid vaginal formulations at the site of action/absorption. In the last decade, two approaches have been proposed to increase the viscosity of the formulation during administration. This allows the use of a mixture of thermosensitive polymers and mucoadhesive polymers that can interact with the proteins present in the vaginal cavity (8).

5. **Rectal drug delivery system:**

   a) **Antiepileptic drugs are administered rectally:** It is possible to prevent to some extent the metabolism of the first step of antiepileptic drugs. Diazepam solution is absorbed quickly and completely, with the maximum plasma concentration being reached in 5 to 15 minutes. Rectal formulations of carbamazepine, lamotrigine, levetiracetam, Phenobarbital, topiramate, and valproate are bioequivalent to oral tablets and suspensions.

   b) **Medicines against rectal pain:** Morphine controlled-release suppositories are used to a greater extent (higher AUC) than controlled-release oral tablets. It has also been used to study pharmacokinetic interactions and drug effects over time at sustained intensity (32).

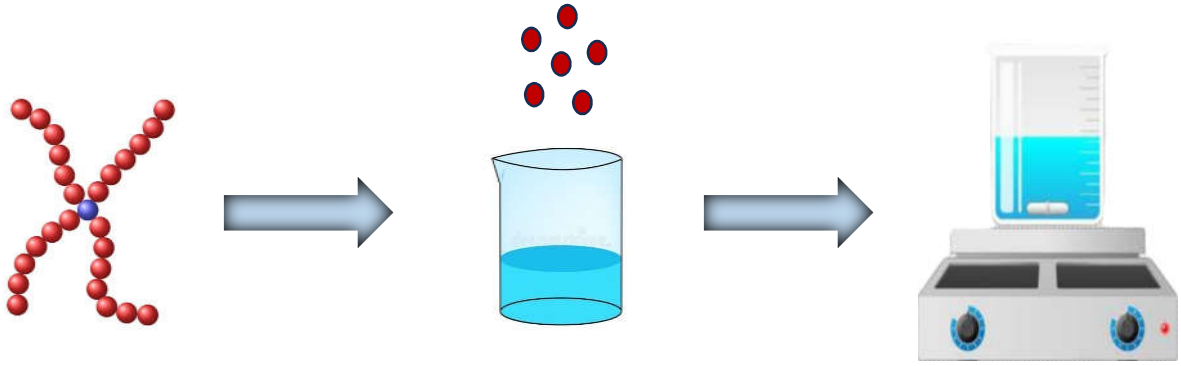
6. **Injection:** Simvastatin as a treatment for osteoporosis was prepared as a biodegradable gel in situ by subcutaneous administration using a chitosan polymer. Chitosan was used as a biodegradable polymer and the disodium salt of beta-glycerol phosphate as a buffering agent to carry out the gelation process at pH and body temperature.

   The results showed that the development of simvastatin gel administered in situ subcutaneously, was effective for the treatment of osteoporosis. Doxorubicin (DOX) with the addition of zein was converted into gel preparations in situ and administered by intratumoral injection.

   Doxorubicin is a drug commonly used to treat colorectal cancer, but what is it has many side effects. The test results showed that there was one effective accumulation of DOX gel in situ as an inhibitory agent in tumors with a low concentration of the drug in the blood and in normal organs, which potentially reduced the side effects of the drug (33).

7. **Dermal and transdermal drug delivery system:** Pluronic F127 in heat-release gel form was tested as a delivery vehicle for indomethacin. In vivo studies suggest that a 20% w/w aqueous gel can be used as an effective base for topical drug delivery. The combination of iontophoresis and chemical improvements led to the development of insulin penetration interaction (13).

PREPARATION OF IN-SITU GEL: (42)(48)(49)(50)

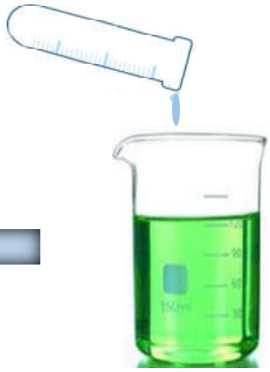


Select suitable polymers Based on desired properties (e.g., temperature and pH).

Disperse the selected Polymers in the Suitable solvent.

Dissolve using magnetic stirrer until fully dissolved.

Excipients are then added, if any and adjust the Ph of the solution to the desired range.



Excipients are then added, if any and adjust the Ph of the solution to the desired range.

Store the solution in the air tight container.

Flowchart: 1 method of preparation of the in-situ gel

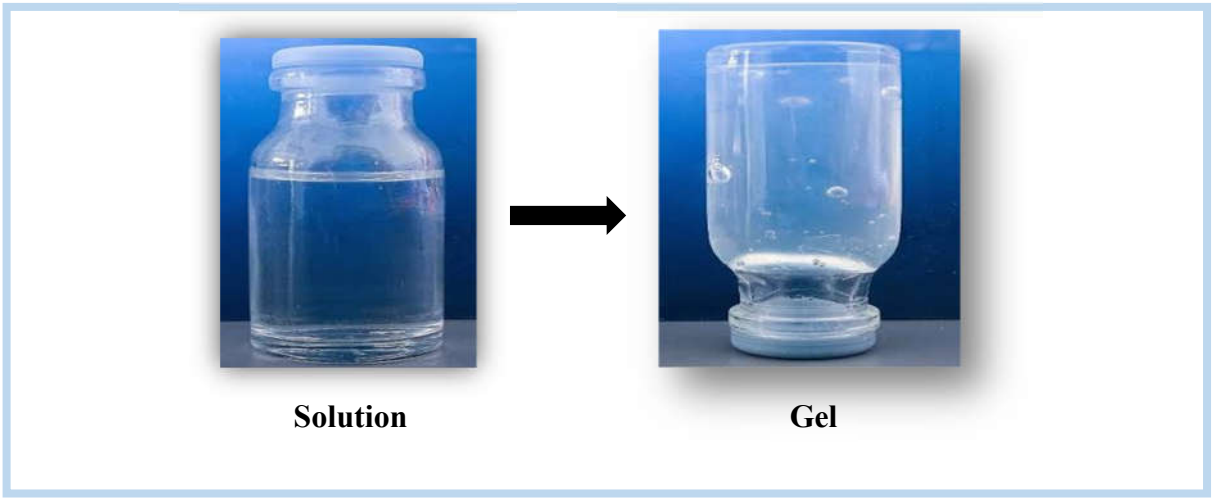


Fig 2: Diagrammatic representation of in-situ gel.

MECHANISM OF IN SITU GEL:

In-situ gel formation occurs through three primary mechanisms:

1. Physical mechanism
2. Chemical mechanism
3. Physiological stimuli

1. Physical mechanism:

In situ, gel formation based on physical mechanism consists of the following,

A) Diffusion:

In-situ gel formulation relies on diffusion to create a gel at the site of administration. When a polymer solution is introduced into the body, the solvent within the solution gradually moves out into the surrounding tissues or body fluids. As the solvent diffuses away, the polymer becomes less soluble and starts to solidify, forming a gel at the location of administration. This

gel acts as a reservoir for the drug, gradually releasing it in a controlled way over time. This method is beneficial for drug delivery because it allows for localized and sustained drug release without requiring invasive procedures. The performance of the in-situ gel depends on various factors, such as the properties of the polymer, the solvent used, and the environmental conditions (like temperature and pH). These factors influence how the gel forms and how effectively it controls drug release (34)

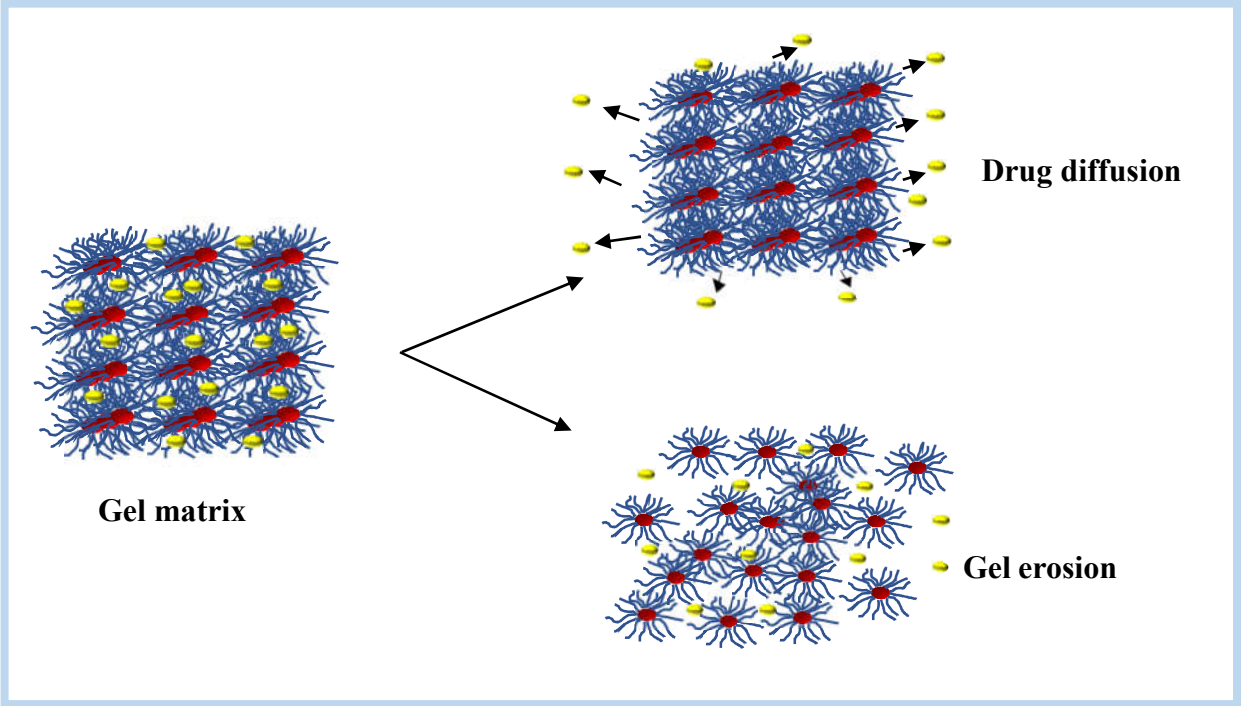


Fig 3: In-situ gel formation through diffusion mechanism.

B) Swelling:

Swelling is another significant physical approach used in in-situ gel formulation, where the polymer absorbs fluids from the surrounding environment, leading to its expansion or swelling. As the polymer swells, it gradually forms a gel-like structure, and the drug is released slowly over time from within the swollen polymer matrix. This method is useful for achieving sustained and controlled drug release.

In this context, Myverol (glycerol mono-oleate) plays an essential role as a polar lipid. When it comes into contact with water, it swells and transitions into a lyotropic lipid crystalline phase. These phases have highly ordered structures that can trap and release drugs in a controlled manner. Myverol's bio-adhesive properties allow the gel to adhere to biological tissues, improving drug retention at the site of action.

Additionally, because Myverol can degrade in vivo by enzymatic action, it ensures that the polymer is eventually broken down and biodegradable material for drug delivery. This swelling mechanism is useful in various drug delivery applications, such as topical, oral, and injectable systems, where localized and prolonged drug release is required (8).

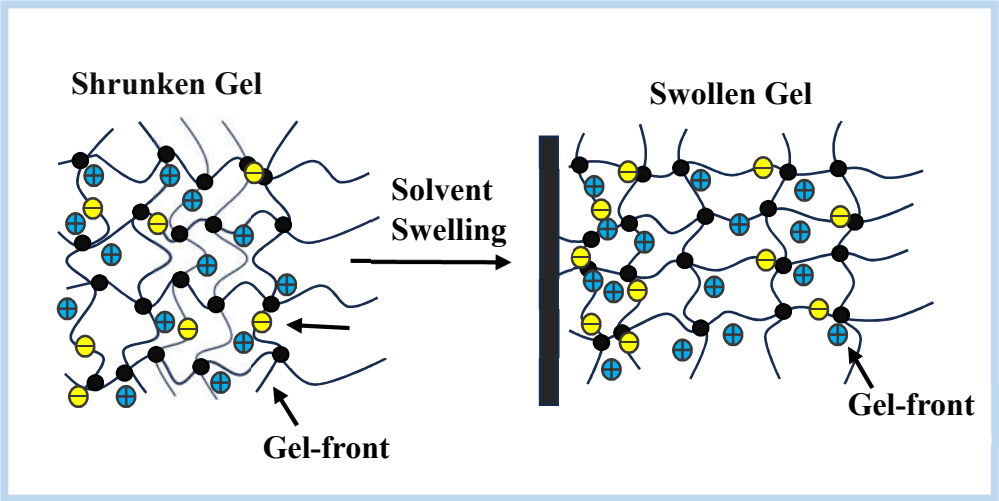


Fig 4: Swelling of in-situ gel.

2. Chemical mechanism:

A) Enzyme cross-linking:

Enzyme-catalyzed in-situ gel formation is an exciting and promising approach that offers several advantages over traditional chemical or photochemical methods. One of the key benefits is that enzymatic processes occur under physiological conditions—meaning they work in the body's natural environment—without the need for potentially harmful chemicals like monomers, initiators, or UV light. This makes enzyme-based systems more biocompatible and suitable for sensitive applications, such as drug delivery. A notable application of this approach is in the development of intelligent, stimuli-responsive drug delivery systems, such as those for insulin release. These systems are designed to respond to physiological triggers, like changes in blood glucose levels.

For instance, cationic, pH-sensitive polymers can be combined with immobilized insulin and glucose oxidase. When blood glucose levels rise, the polymer swells, triggering the release of insulin in a pulsatile manner—mimicking the body's natural insulin regulation process. Another advantage of enzyme-catalyzed systems is that the gelation process can be finely controlled by adjusting the enzyme concentration. This allows the polymer solution to stay in a liquid form

during injection, making it easier to administer, while still ensuring that the gel forms at the right time once it reaches the desired site in the body. Overall, enzyme-catalyzed gel formation offers a more controlled, biocompatible, and responsive mechanism for drug delivery, particularly in applications that require real-time adaptation to changes in physiological conditions, such as managing diabetes. (7).

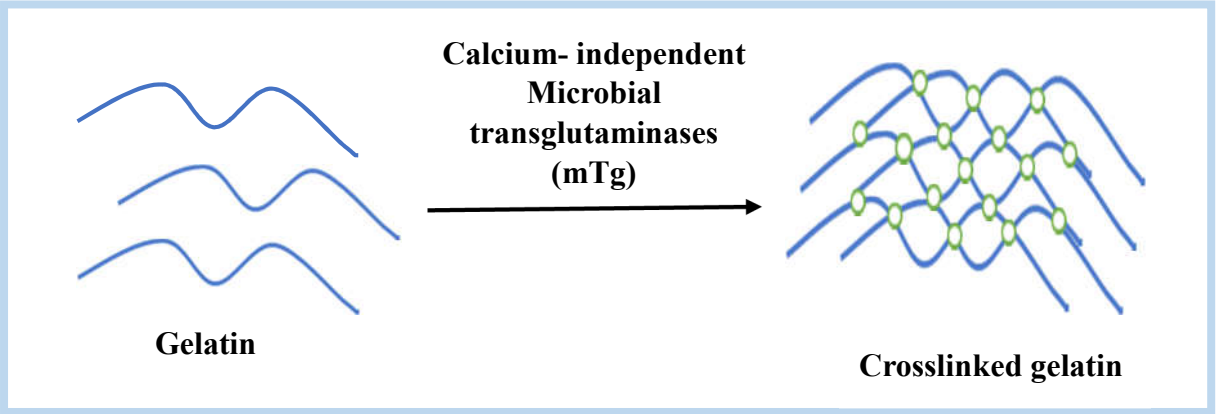


Fig 5: Gelatin crosslinked by transglutaminase enzyme.

B) Photo-polymerization:

Photo-polymerization is a versatile and widely used technique for the in-situ formation of biomaterials. In this method, a solution containing monomers or reactive macromers along with a photo-initiator is injected into the target tissue, and electromagnetic radiation, typically in the ultraviolet (UV) or visible light spectrum, is applied to initiate the polymerization process, forming a gel.

Advantages of Photo-Polymerization:

- **Rapid polymerization:** Photo reactions provide fast polymerization at physiological temperatures, allowing the quick formation of hydrogels or implants.
- **Customizable for complex shapes:** This method is particularly useful for forming implants in irregularly shaped tissue spaces, as the polymer solution can conform to complex geometries before it is cured.
- **Prolonged drug release:** Once the polymer has cured in situ, it can release the encapsulated drug in a controlled manner over an extended period.

- **Biodegradability:** Certain photo-polymerizable systems, like those reported by Sawhney, involve biodegradable hydrogels. These materials degrade over time, which makes them excellent candidates for use as tissue-contacting materials and controlled-release carriers.
- This technique is widely applicable in tissue engineering, wound healing, and localized drug delivery, offering precise control over gel formation and the release kinetics of therapeutic agents (9).

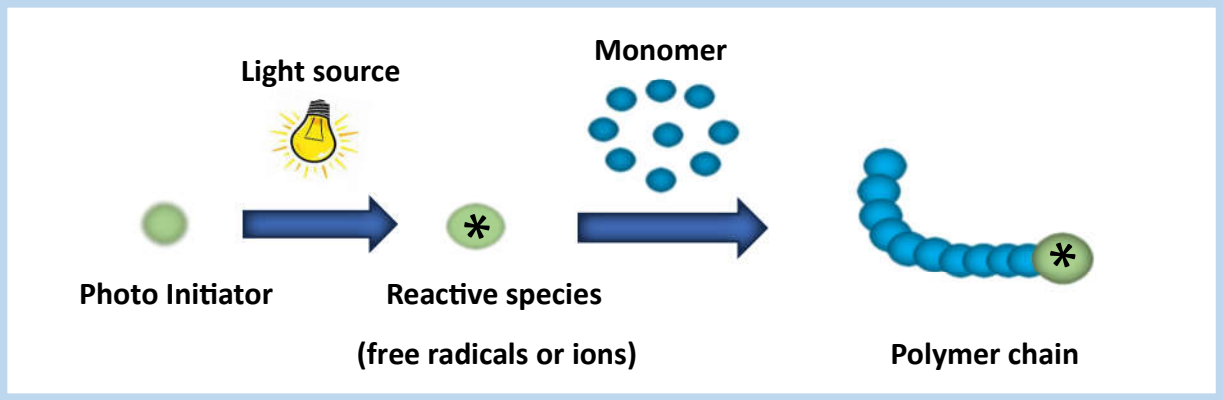


Fig 6: Representation of photo-polymerization

C) Ionic cross-linking:

Ion-sensitive natural polysaccharides, such as Carrageenan, Gellan gum, Pectin, and Sodium alginate, exhibit phase transitions in the presence of specific ions (e.g., K^+, Ca^{2+}, Mg^{2+}, Na^+). These polysaccharides can transition from a solution to a gel state when exposed to these ions, making them ideal candidates for in-situ gelation in drug delivery and tissue engineering.

For example, Alginic acid undergoes gelation in the presence of divalent or polyvalent cations, such as Ca^{2+}. This gelation occurs due to the interaction between the cations and the glucuronic acid blocks in the alginate polymer chains. The calcium ions crosslink with the carboxyl groups on the glucuronic acid, leading to the formation of a three-dimensional gel network. This process, commonly referred to as the “egg-box” model, creates a stable gel structure (5).

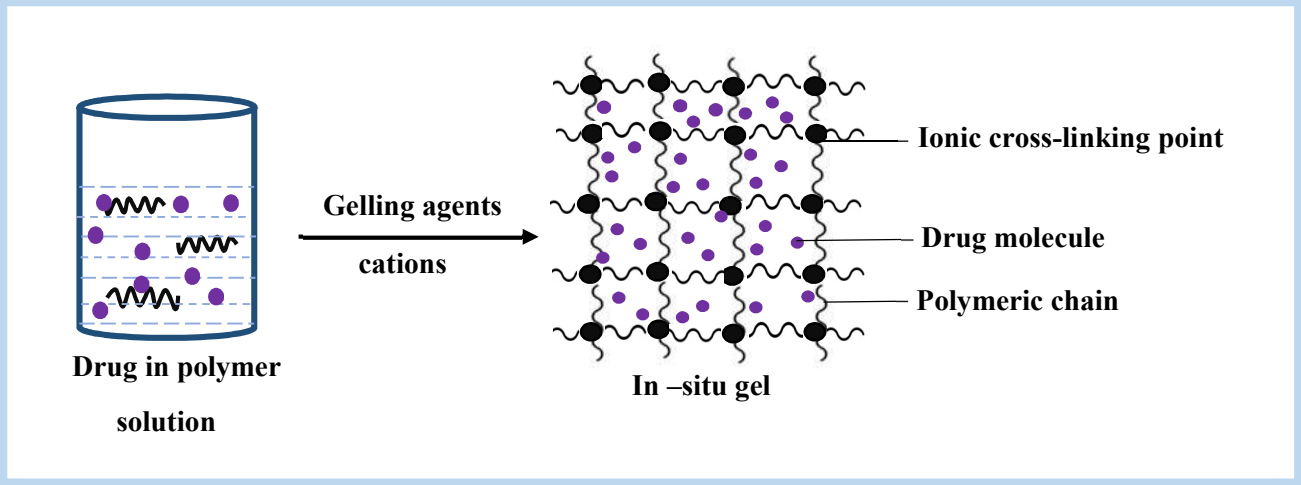


Fig 7: Sol-gel transformation via ionic cross-linking.

3. Physiological stimuli:

A) Temperature-triggered in-situ gel:

These systems are particularly favoured in drug delivery because temperature is easy to control and applicable both in vitro and in vivo. In this system, the polymer solution remains in a liquid state at room temperature (20-25°C) and forms a gel when exposed to body temperature (35-37°C), making it ideal for in-situ drug delivery applications. Upon injection into the body, the solution undergoes gelation due to the increase in temperature, allowing for sustained drug release without the need for external heating. The Ideal temperature range for these systems is around ambient and physiological temperatures, ensuring that clinical control is facilitated and no external heat source beyond the body is required to trigger the gelation process.

Three Main Types of Thermo-Sensitive Systems:

I. Negatively Thermo-Sensitive Polymers:

These polymers contract upon heating. They have a Lower Critical Solution Temperature (LCST), and when the temperature exceeds the LCST, the polymer undergoes phase separation and forms a gel. Examples include PNIPAAm (poly N-isopropyl acrylamide.

II. Positively Thermo-Sensitive Polymers:

These polymers contract upon cooling. They possess an Upper Critical Solution Temperature (UCST), and below this temperature, they form a gel. An example is polyacrylamide or polyacrylic acid.

III. Thermo-Reversible Gels:

These gels undergo reversible phase transitions between liquid and gel states based on temperature changes. Examples include Poloxamers (Pluronic®) and cellulose derivatives such as hydroxypropyl methylcellulose (HPMC), methylcellulose, and xyloglucan.

These temperature-sensitive systems are widely utilized in drug delivery, particularly for injectable formulations, ophthalmic preparations, and tissue engineering, as they offer easy administration, controlled drug release, and non-invasive application methods (13).

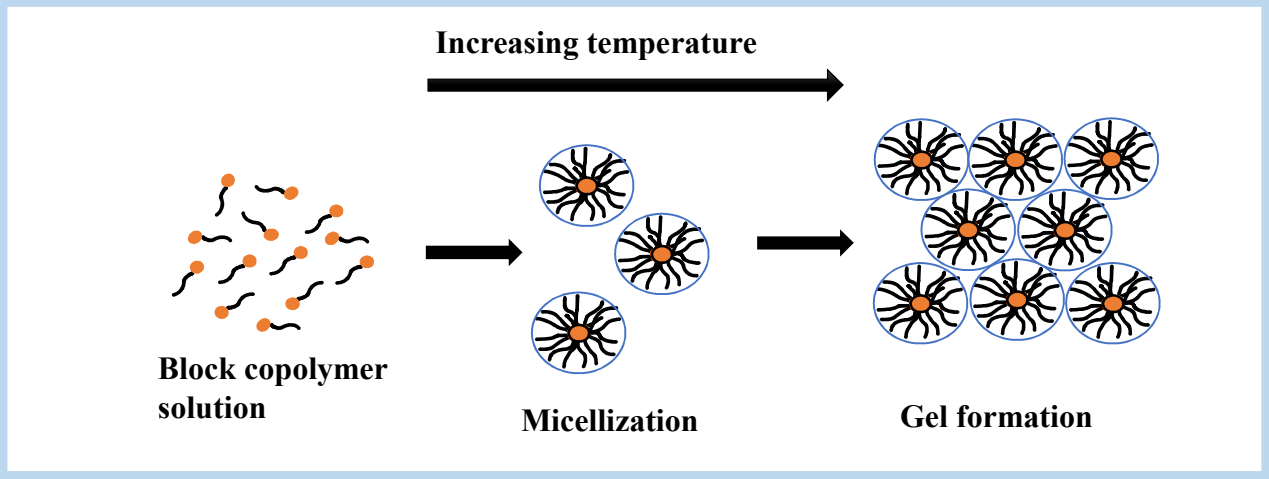


Fig 8: Thermo-sensitive system.

B) pH-sensitive in-situ gel:

pH-sensitive polymers are a class of stimuli-responsive materials that exhibit significant changes in their properties based on the pH of the surrounding environment. These polymers contain ionizable functional groups, typically weakly acidic or basic moieties, that respond to changes in pH by altering their charge state. This results in changes in solubility, conformation, swelling behaviour, or even phase transitions (from solution to gel or vice versa).

Key Characteristics of pH-Sensitive Polymers:

I. Weakly Acidic Polymers:

Polymers with weakly acidic groups, such as polymethacrylic acid, contain carboxylic acid (-COOH) groups on their backbone. At pH levels above the polymer's pKa, these groups become deprotonated (losing H^+), acquiring a negative charge.

This deprotonation increases electrostatic repulsion between polymer chains, which can lead to physical transitions, such as swelling or dissolution. At alkaline pH, this results in the formation of polyelectrolytes, where repulsion between negatively charged polymer chains leads to the formation of viscous solutions, dependent on factors like molecular weight and the ionic strength of the solution.

At acidic pH (below the pKa), the carboxylic acid groups remain protonated (uncharged), promoting polymer-polymer interactions (such as hydrogen bonding between -COOH groups), which can lead to gel formation or shrinkage of the polymer.

II.Weakly Basic Polymers:

Polymers with weakly basic groups, such as amines ($-NH_2$), exhibit the opposite behaviour. These groups become protonated (gaining H^+) at acidic pH levels below their pKa. In this state, the polymer chains are positively charged, leading to electrostatic repulsion and changes in the polymer's physical structure (e.g., swelling).

At alkaline pH, the amine groups become deprotonated (uncharged), leading to polymer collapse or gel formation, depending on the system.

pH-Responsive Behaviour and Gel Formation:

pH below pKa: Promotes polymer-polymer interactions, such as hydrogen bonding (e.g., between carboxyl groups), which are necessary for gel formation.

pH above pKa: This leads to polymer-polymer repulsion due to the ionization of acidic groups, resulting in the formation of polyelectrolytes and typically viscous solutions rather than gels.

For polymers with weakly basic groups, the reverse is true, with protonation occurring at low pH, leading to swelling or gelation, while at high pH, the polymer may collapse or exist in a soluble state.

Designing pH-Responsive Systems:

In addition to ionization-based transitions, chemical reactions can be exploited to create pH-sensitive systems. For example, covalent bonding between polymer chains may occur or break down depending on the pH, leading to changes in the polymer's structure, such as sol-gel transitions or swelling/shrinking behaviour.

These pH-sensitive polymers are commonly used in drug delivery, especially for targeted release in areas of the body where the pH varies (e.g., in the stomach, intestines, or tumor environments). They allow for controlled and site-specific drug release, reducing systemic side effects and improving therapeutic outcomes (35).

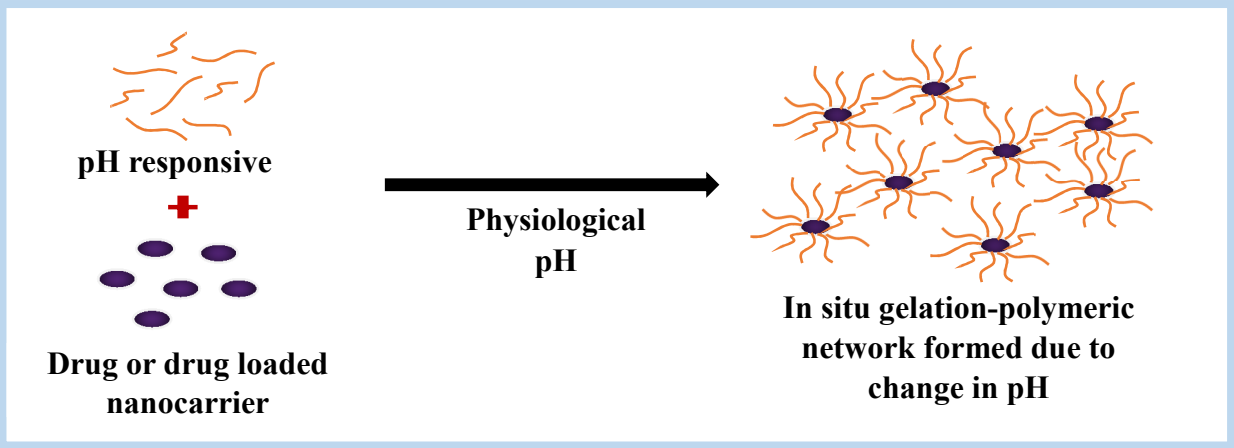


Fig 9: pH-sensitive in-situ gel

EVALUATION AND CHARACTERIZATION OF IN-SITU GELLING SYSTEM:

1. Physical appearance:

The physical appearance of the preparation was visually evaluated (36).

2. Optimal pH:

pH is critical for the solubility and stability of drug formulations. The pH of the formulation was measured right after preparation using a calibrated digital pH meter (37). It's important for the formulation to stay stable at the intended pH and to be non-irritating when administered (38).

3. Clarity:

The visual clarity of formulation should be assessed before and after gelling. Prepared in-situ gel is examined visually against both white and black backgrounds, and by swirling the mixture to detect the presence of unwanted particles or any turbidity (37).

4. Homogeneity:

Place the sample between two glass slides and observe the surface roughness under the light (46).

5. Viscosity and rheology:

Viscosity and rheological properties are vital for in-situ gels in drug delivery systems. These can be measured using instruments such as the Brookfield rheometer or Ostwald's viscometer (39). The in-situ gel formulation will be initially placed in a sample tube with a viscosity ranging from 5 to 1000 mPas. Once the gelation process is activated by the addition of ions by the eye, the viscosity will increase significantly, reaching a range of approximately 50 to 50000 mPas (9). It's important to ensure that the viscosity is suitable for easy administration, particularly for parenteral and ocular routes. High viscosity may complicate injection or application, while low viscosity can result in poor drug retention and release. Achieving an optimal viscosity is crucial for effective and convenient drug delivery (39).

6. Texture analysis:

The In-situ gel's consistency, firmness, and cohesiveness can be evaluated with a Texture profile analyzer (TPA). This evaluation determines the gel's strength and its ease of administration in vivo. For optimal performance, the gel should have a high adhesiveness value which ensures the gel maintains good contact with mucus surfaces, enhancing its effectiveness (9).

7. Gel strength:

A gel is created from its liquid form in a beaker. As the beaker is raised slowly, a rheometer probe is pushed into the gel the gel. By measuring the force on the probe as it penetrates deeper into the gel, we can analyze how the gel's viscosity and flow properties change with depth. This data provides insights into the gel's rheological behaviour, including its resistance to flow and any yield stress characteristics (1)(40).

8. Fourier transform infrared spectroscopy and thermal analysis:

Fourier transform infrared spectroscopy (FTIR) helps determine if ingredients are compatible by detecting how they interact on a molecular level, based on their unique infrared absorption signals. Differential scanning calorimetry (DSC) looks at how the thermal properties of a mixture change, such as shifts in melting points or heat flow, by comparing the mixture's

thermal behaviour to that of the individual ingredients. Any changes in these properties indicate that the ingredients are interacting with each other, helping us understand how they might affect the final formulation (1)(41)(2).

9. Isotonicity:

Isotonicity is crucial for ophthalmic preparations to avoid inflammation, tissue damage, and eye irritation. To evaluate isotonicity, formulations are mixed with a few drops of blood and examined under a 45x magnification microscope, comparing the results with standard ophthalmic preparation (47).

10. Sol-gel transition temperature and gelling time:

In-situ gel forming systems using Thermoreversible polymers exhibit a solution-gel transition at a specific temperature. This transition is identified when there is an initial observable change in the meniscus of the sol during heating at a controlled rate. Gel formation is confirmed when the meniscus no longer moves upon tilting the tube, signifying the presence of a gel. The gelling time refers to the time taken to first observe this gelation (39).

11. Gelling capacity:

a) Method 1

Place 2ml of stimulated tear fluid (STF) in a vial at 37°C. Introduce a drop of the freshly prepared formulation into the STF and start the timer. Note the time it takes for the gel to form and the time for it to dissolve in a 7.4 pH phosphate buffer (37)(42).

b) Method 2

Dissolve 1g of a water-soluble dye (e.g. Amaranth, Congo red, indigo blue) in distilled water. Incorporate the dye solution into the prepared in-situ gel formulation. The gelling capacities of the formulations were measured by placing 5ml of the gelation solution (STF) into a glass test tube and maintaining the temperature at 37±0.5°C. Note the time it takes for the solution to transform into a stiff gel-like consistency. Evaluate the stiffness of the gel and record how long it retains this thick gel state. Use the dye to provide a clear visual indication of the gel's

formation. Based on these observations, classify the gelling capacity into three categories, (37)(43).

+ Gel forms after a few minutes and disperses rapidly (43).

++ Immediately gelation occurs and remains for a few hours (37)

+++ Immediately gelation occurs and remains for an extended period (43).

12. In vitro drug release studies:

In vitro, drug release studies for in-situ gel formulations intended for oral, ocular, or rectal administration typically utilize a plastic dialysis cell composed of donor and receptor compartments separated by a cellulose membrane. The solution form of the formulation is placed in the donor compartment, and the cell is shaken horizontally in an incubator. At designated intervals, samples from the receptor solution are withdrawn and replaced with fresh media to maintain volume, allowing for the analysis of drug release using appropriate analytical techniques (41)(2)(7).

For injectable in-situ gels, the formulation is taken in vials with receptor media and agitated in a water bath at controlled temperature and speed. Samples are periodically collected to assess drug release over time. This setup helps in assessing the release kinetics and behavior of the formulation under simulated physiological conditions (41)(7).

13. Drug content Estimation:

The formulation must contain the required amounts of active substances while ensuring there is no chemical breakdown or adverse reactions with the polymers or other components (44). Ensuring uniform drug distribution is essential for achieving optimal bioavailability. The drug content is measured using a simultaneous method with a UV-visible spectrophotometer. The procedure involves diluting 1ml of the formulation in 100ml of ATF solution at Ph 7.4. next, 1ml of this dilution is taken and further diluted to 10ml with ATF solution. The concentration is then determined using the UV-visible spectrophotometer (2)(45).

14. Accelerated stability studies:

A stability study of the in-situ formulation was performed by ICH guidelines to assess its physical stability under accelerated storage conditions (46). The formulation was filled into the glass vials, and sealed with gray butyl rubber closures and aluminium caps. The vials were stored in a stability chamber at 40 ± 2°C and 75 ± 5% relative humidity for one month. Weekly

samples were taken and analyzed for drug content, visual appearance, clarity, pH, gelling capacity, and the amount of active drug remaining (42).

CONCLUSION:

In conclusion, in-situ gel systems represent a significant advancement in drug delivery, offering advantages such as controlled release, enhanced bioavailability, and improved patient compliance. Their unique sol-to-gel transition allows for prolonged drug retention at the site of action, making them suitable for various applications, including ocular and nasal delivery. Despite challenges like stability and mechanical strength, ongoing research and clinical studies are essential to fully realize their potential. Future innovations in polymer design and formulation techniques could further enhance the efficacy and applicability of in situ gels in therapeutic settings.

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