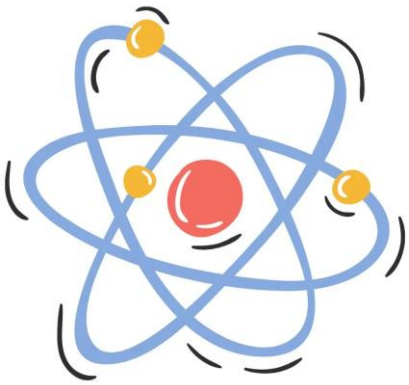

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IN-SITU GELS: A DETAILED OVERVIEW

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Abstract: ***Background-****In-situ gels have been providing a promising approach due to its sustained and controlled delivery of drug offering an incrementation in patient compliances due to target site specific release and reduction in dose frequency, as transition occurs in solution to gel under exposure of physiological condition depending on type of polymer used like temperature, pH, ionic providing a larger target section of diseases on ophthalmic, nasal, vaginal, oral.* ***Aim-*** *The review below aimed benefits of in-situ gel over conventional formulation with the current marketed formulation present.* ***Method-*** *In-situ gel formulation is dependent on the type of polymer used for specific purpose like thermo-responsive, ion-activated, pH-sensitive and the evaluation parameter depending on the methods and the targeted disease pH, gelation time, capacity, viscosity, in-vitro gelation studies using simulated physiological fluid (STF), rheological studies.* ***Future advancement****-.In-situ gel have been prepared by 3D printing a recent advancement in hybrid approach allowing the development of bio-links its advantages and application are clearly visible in field of tissue engineering, patient specific implant, wound healing, controlled drug delivery providing a localized therapeutic delivery ,showing a recent advancement in personalized medicine, and polymer science, causing development in stimuli-responsive gel having enhancement in biocompatibility, mechanical strength and drug loading efficiency hence this review below provides an comprehensive overview of different types of in-situ with formulation and mechanism diseases that can be targeted, evaluation parameters with recent and future advancement in-situ gel in personalized medicines.* ***Conclusion-*** *In-situ gel shows a modern and enhanced approach to drug delivery as compared to traditional/conventional formulation*

Keywords- *In-situ gels, thermos-responsive, ion-activated, pH sensitive, evaluation, 3D printing, nano delivery.*

1) Introduction-

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In-situ means “in place” pharmaceutical refer to a drug delivery system that is administered as a liquid or solution but transform into gel once inside the body. The in-situ gel causes increase in retention time also cause increase in localized and prolonged drug release by causing betterment in patient compliance and increment of therapeutic index as compared to conventional dosage form [1]. In- situ gels system works on mechanism either physical or chemical:

Physical mechanism includes: diffusion, swelling, degradation/erosion

Diffusion controlled release-Movement of drug through a polymeric gel matrix commonly seen in hydrophilic drug in loosely crosslinked gels or pores within gels, depending mainly on the factor like drug size, solubility, polymer conc., crosslinking density.

Swelling controlled release-Release of drug occurs in this method by absorbing of biological fluid transition of gel allowing release of drug phenomenon mainly in pH or ion-sensitive systems containing polymer like hydrogels like HPMC, Carbopol etc.

Degradation/erosion-controlled release- Phenomenon mainly seen in long-acting formulation that consisted biodegradable polymer like PLGA, chitosan, alginates, release of encapsulated drug occur by degradation and erosion of polymer overtime.

Chemical mechanism includes: Temperature(thermosensitive), pH -sensitive, Ion presence etc. [2]

1.1) Salient key feature of in-situ gels

- Administration of drug is easier
- Controlled drug in predetermined rate for a prolonged drug release (like gels)
- Increase in bioavailability
- Reduction in dosing frequency
- Bypass first metabolism to targeted/ localized effect (reduce systemic side effect). [3]

1.2) Merits and Challenges

Merits

- Increase in patient compliance (less frequent dosing)
- Better alternative for sensitive drug (e.g., Protein and Peptide)
- It also provides minimize invasive.

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- It also provides controlled and sustained release of dug for a predetermined rate for a prolonged period of time. [4]

Challenges/Limitation

- In-situ gels being of different type according to polymer used and sensitive to (temperature, pH, ion, solvent etc.) so, stability become issue before administration
- Technique of formulation of gels may vary and effect the formulation
- Amount, type of polymer may affect the gelation consistency and vary for different formulation. [5]

2)DISEASE TARGETTED FROM IN-SITU GELS

2.1) Ocular-Glaucoma, Conjunctivitis, Dry eye syndrome

- **Glaucoma-**Damages the optic nerve, caused by increased in intraocular pressure (IOP). In serious conditions causes vision loss, if untreated hence in- situ gel shows a promising approach by increase in contact time and increase in bioavailability due to its sustained and controlled release. [6]
- **Conjunctivitis-**Disease occurs in outer membrane of eyes, causes inflammation of conjunctiva by viral, bacterial and allergic reactions symptom constitute of redness, watery eyes, discharge irritation.

  Soumya Narayana et: al *Design and evaluation of ocular hydrogel containing combination of Ofloxacin and Dexamethasone for the treatment of Conjunctivitis* study showing viscosity of gel was depends on range and drug released 85% up-to 13 hours.[7]
- **Dry eyes syndrome-**Disease caused when tear evaporate too quickly or production is inadequate, condition may get worsen and chronic with age, increase in screen time or certain medications. Symptom causes irritation, gritty, and burning sensation.

  Deepika Modi, G. K Jain, Nazeer Hasan et: *al Formulation and development of tacrolimus Gellan gum nano-formulation for treatment of dry eyes syndrome* Tacrolimus showing Prolonged release up-to 12 hours high precorneal retention as compared to conventional dosage form (Eye drops/ solution).[8]

2.2) Nasal-Allergic Rhinitis, Nasal congestion, Sinusitis

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- **Allergic Rhinitis-**It is nasal disorder caused by reaction to pollen, dust, or allergens affecting nasal mucosa. It is caused seasonal (hay fever) or perennial, Symptoms including sneezing, nasal congestion in chronic condition, and itchy eyes
  Shi-lei Cao, Qi-Zhi Zhang et: al *In-situ gel based on Gellan gum as new carrier for nasal administration of Mometasone Furoate* as the formulation provides the promising approach for intranasal- delivery of mometasone furoate for increase in therapeutic effect than common conventional suspension composition of it.[9]
- **Nasal congestion-**Disease occur when nasal membrane gets blocked by swollen nasal membranes caused by cold, allergies and sinus infections tends to breathing difficulty.
- **Sinusitis-**Disease occur due to inflammation of sinuses, which can be acute and chronic depending of stages of infection and the detonatable symptoms are headache, nasal discharge and facial pain /pressure. [10]

2.3) Buccal-Mucositis, Oral ulcer, Gingivitis, Periodontitis

- **Mucositis-**Disease act as part of adverse effect of chemotherapy or radiotherapy usually affecting GI tract, mouth, throat causing painful inflammation and ulceration of mucous membrane. In chronic condition tends pain, risk of spreading infection, difficulty eating.[11]
- **Oral ulcer-**It occurs due to stress, autoimmune condition and nutritional deficiency produced a small and painful lesion in oral cavity.
  Nikita Harekrishna, Preena Shantinath et: al *Development and evaluation of in-situ gel formulation for the treatment of mouth ulcer* as the composition consisted of two polymer -Carbopol as mucoadhesive polymer for reducing dose and poloxamer 407 being formulation of thermo-responsive, propylene glycol and active ingredient as choline salicylate and borax, as the above article provide the patient compliance by minimizing dose frequency.[12]
- **Gingivitis-**Disorder also known bleeding gum produced inflammation in gums, due to accumulation of plaque can be cured if treated earlier denoted symptom are redness, swelling and may cause bleeding from gums while brushing
- **Periodontitis-**Disorder causes tooth loosing and is the advanced form of untreated gingivitis tends to destruction of supporting structure of teeth. including bone. [13]

2.4) Topical-Acne, Psoriasis, Wounds

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- **Acne-**Chronic skin condition presents as pimples, blackhead, cysts or whiteheads mainly occurs in adolescence but can also be adulthood caused due to clogging pores by sweat, dirt, bacteria, and inflammation. [14]
- **Psoriasis-**Disorder is an autoimmune occurs in elbows, knees, scalp and black causing rapid skin cell turnover, resulting in scaly, red plaques. Chronic condition flare up can be triggered by cold weather, stress or infection.
- **Wounds-**Condition occur when damage to the underlying tissue of skin by burn, injury, surgery or ulcer healing requires proper protection, moisture balance and control towards infection.

2.5) Vaginal-Bacterial vaginosis, Vaginal Candidiasis, Vaginal Dryness/Atrophic Vaginitis

- **Bacterial vaginosis-**A infection caused by excess growth of anaerobic bacteria, thereby counter changing the normal lactobacilli causes symptoms like thin gray/white discharge, irritation, fishy odor, itching. formulation of pH triggered gelation dur to difference occur in vaginal area. Formulation causing release of antibiotic locally hence causing reduction in dosing frequency and systemic side effect.
  Shital R. Nikhar, Kaka Saheb R. Mahadik et: al *Tinidazole in treatment of Bacterial vaginosis formulation and evaluation* providing a promising approach for bacterial vaginosis, and providing a sustained release of drug for 7 hours. [15]
- **Vaginal Candidiasis-** infection caused by fungus usually by *Candida Albicans* producing vaginal inflammation causing symptom like itching, burning, thick white discharge. Enzyme-triggered gelation can be formed using local anti-fungal like clotrimazole, fluconazole producing prolonged residence time, avoiding systemic toxicity. [16]
- **Vaginal Dryness/Atrophic Vaginitis-** Disorder occurred due to estrogen deficiency producing thinning and drying of vaginal walls during post-menopause causes symptom like painful intercourse, burning sensation, dryness. Thermosensitive in-situ gel can be formulated carrying estrogen or lubricants for longer duration causing mucosal healing and as it retains drug for longer duration and causing prolonger residence time hence reducing messiness than compared to traditional creams.

2.6) Rectal-Inflammatory Bowel Disease (IBD) e.g., Ulcerative Colitis, Hemorrhoids

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- **(IBD) Inflammatory Bowel Disease** –It is immune mediated causes chronic inflammation of colon and rectum disease causing symptom like pain, bloody diarrhea, urgency. pH- sensitive & enzyme responsive formulation can be prepared as the inflammation increase enzyme activity and trigger drug release with the local corticosteroids and 5-ASA causes reduction in systemic exposure and inflammation.
  Satish Kumar Ramadass, Sathyamurthy Perumal, Sugin Lal Jabaris et: al *Prepared and evaluate mesalamine collagen in-situ rectal gel for the treatment of ulcerative colitis* by sustained release of mesalamine over the period of 12 hours than the conventional dosage (suspension). [17]
- **Hemorrhoids –** Disease occur due to swollen veins in anus by constipation causes symptoms like itching, swelling, pain, bleeding. Ion activated or thermosensitive in-situ gel can be formulated by use of analgesic, corticosteroids causing minimization in leakage increase in comfort mucoadhesive action produces prolonged drug release in rectal mucosa.

2.7) Gastrointestinal Disease

- **Helicobacter pylori (*H. pylori*) infection-** *H. pylori* is bacterial infection which reduces mucus layer of stomach and linked with peptic ulcer, gastric cancer, gastritis. In this type of infection in-situ gel is promising approach, because conventional dosage form has less gastric residence time hence, in-situ gel causing prolonged contact with gastric mucosa, localizing delivery of antibiotics and reduction of systemic side effect, increments of bacterial eradication. Commonly seen example of Amoxicillin loaded in-situ gel by using sodium alginate and addition of calcium carbonate to gel in acidic stomach and float.
  P.S Rajinikanth, B. Mishra et: *al Floating in-situ gelling system for stomach site specific drug delivery of clarithromycin to eradicate H. pylori* formulation having sustained drug release from the gels over the period at least 8 hours than conventional dosage form. [18]
- **Gastric/ peptic ulcer-** Peptic ulcer is caused due to erosion of stomach lining due to increase in acid concentration in stomach either by *H. pylori* or NSAIDS, hence in-situ gel being a promising approach due to its localize effect and prolonged drug release at ulcer site, causing mucosal adhesion and protective effect drug that can be used in conventional as well as in formulating in-situ gel proton pump inhibitor, H_2 blocker,

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antacids. Some common examples sucralfate in-situ gel forms a protective layer of gel over ulcers. Ranitidine and omeprazole gels for sustained acid suppression. [19]

- **Gastroesophageal reflux disease (GERD)-** GERD is caused when the stomach acid refluxed into the esophagus producing sensation like heartburn and inflammation. In-situ gel has advantages of controlled released of acid- suppressing drugs and formulation of floating gels can lead to raft-like structure acts as barrier to acid reflux and floats on gastric content, drugs that are commonly used in cure of disease are antacid, proton pump inhibitors, h_2 blockers, common example of alginate-based raft-forming systems with antacids (like calcium carbonate, sodium bicarbonate).

  Table number 1 describes the comparison between the conventional dosage form and in-situ gel with marketed formulation of both available

Table number 1: Comparison Between Conventional Dosage Form and In-Situ Gel

PARAMETERS	CONVENTIONAL DOSAGE FORM	INSITU GEL
STATE OF ADMINISTRATION	Traditional dosage form that are available in the market in form of tablets, capsule, solution, suspension emulsions	Involves advancement in polymer science present in liquid form in normal state and undergoes transition into gel under influence of physiological condition like (temp., pH, ions, solvent)
RESIDENCE TIME/RELEASE PROFILE	Low retention/residence time due to barrier cleared and dissolved quickly especially seen in case of eyes (Nasolacrimal drainage, mucosa, GI. Immediate release until it modifies as seen in cases of gastro-retentive drug delivery system (GRDDS)	High retention/residence time after transition occur from solution to gel stays on targeted site for longer duration as compared to conventional dosage form (seen in case of ophthalmic, nasal, gastric, mucosal). Sustained and controlled release of drug due to longer

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		contract time /gel matrix releasing drug.
BIOAVAILABILITY	Low bioavailability as it undergoes rapid clearance	High compared to conventional dosage form localized absorption to target site and narrow absorption window having longer contact time
PATIENT COMPLIANCE	Low patient compliance due to high dosing frequency (multiple times in a day)	High patient compliance due to low dosing frequency because of sustained release
MARKETED FORMULATION		
	CONVENTIONAL DOSAGE FORM	**IN-SITU GEL**
GLAUCOMA	**Timoptol® / Isopto Carpine® (eye drops)** It is present in simple aqueous eye drop solution having short residence time (as it is removed by tears turnover clear 2-3min) also require high dosing frequency 2 to 3 time daily (less patient compliance). Immediate release of drug, dose absorbed only 1-3% have low bioavailability **ADR-** Possible irritation, causing cardiovascular effect due to systemic circulation. [20]	**Timoptic-XE® / Pilopine HS® (gel-forming)** It undergoes transition from solution to gel by mechanism of ion-activated and pH triggered having long residence time and retain corneal surface for hours. Sustained /controlled release of drug due to which it requires, less dosing frequence (once daily or less frequency than conventional eye drop (high patient compliance). High bioavailability due to longer corneal contact causing better absorption

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		ADR- Lower adverse effect than conventional dosage form due to Localized retention causes lower systemic exposure. [21]
PERIODONTITIS	**Oral doxycycline tablets / mouth rinses** Its route of administration is (systemic oral) low retention time due to which requires multiple dosing daily having moderate to low patient compliance **ADR-** Gastro-Intestinal upset resistance. [22]	**Atridox® (doxycycline hyclate in ATRIGEL system)** Its application is local (sub-gingival placement) targeting to direct periodontal pocket. High retention time due to sustained/ controlled release up to 7days causing high patient compliance **ADR-** Minimal adverse effect seen due to localized/targeted effect. [23]
OCULAR	**Ciprofloxacin or Loteprednol eye drops** It is also present in aqueous solution as other conventional dosage form for eyes having low residence time washed rapidly by tears. Increased in dosing frequency 3-4 times a day hence having low patient compliances. Its therapeutic effect is for short period of time.	**Ciprofloxacin / Loteprednol in-situ ocular gel (research/marketed in some regions)** Transition from solution to gel adheres longer to eyes surface. Less dosing frequency compared to conventional dosage form. Once or twice a day providing better comfort (less leakage) causing high patient compliances, due to sustained release of both

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		antibiotics, anti-inflammatory provide therapeutic effect is high. [24]
DIABETIC TYPE 2	**Metformin tablets** Conventional dosage available in (systemic solid form) having dose frequency 2-3 times a day, immediate release of drug (rapid dissolution), having low patient compliance and its absorption window is upper small intestine and bioavailability might fluctuate because of gastric emptying time. [25]	**Gastro-retentive metformin in-situ gel (research)** Transition from solution to floating gel in the stomach, having low dosing frequency once daily(potentially) sustained release (8-12 hours retention) having high patient compliance and its absorption window is maintained in stomach hence causing better absorption and bioavailability is more consistent plasma level. [26]
VAGINAL	**Miconazole / Clotrimazole creams, suppositories** Conventional dosage present in form in cream, semi-solid or solid pessary. Low retention time leakage, messy application. Release of drug quick having short residence time moderate irritation risk having moderate to poor patient compliance (due to frequent application). [27]	**Miconazole or Clotrimazole mucoadhesive in-situ gel** Transition of phase from liquid to gel while getting in contact with vaginal fluid. High retention time minimal leakage, strong mucoadhesive. Release of drug sustained and controlled release rate for (24-72 hours). Lower

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		irritation risk, improved patient compliance (1-2 application /week). [28], [29]

3) Method of preparation

3.1) Thermo-responsive/Temperature triggered in-situ gels

Mechanism-These gels undergo transition phase of sol. to gel in response to, typically body temperature (37^0C).

Polymers used – Poloxamers (e.g., Pluronic F127), Methyl cellulose, HPMC, Chitosan with beta-glycerophosphate. Manas Bhowmik *et :al Study of in-situ gel for ocular delivery* use 1% methyl cellulose, 6% of ORS solution without dextrose reduce the gel temperature physiological temperature and 1% of HPMC to increase in viscosity at 37^0C , and its main active ingredient (API) constitute of Ketorolac tromethamine for treatment of seasonal allergies causing itching, swelling, inflammation in eyes hence increase in release time of drug up-to 4 hours than conventional dosage form causing reduction in nasolacrimal drainage absorbed in duct

Reason for using above suggested polymer- Polymer reveals their transition from solution to gel as being temperature dependent like poloxamer 407 is liquid in room temperature and undergoes into gel transition at body temperature cause of micelle formation

Formulation method –

- Accurately weighing the drug and dissolve it in suitable buffer or cold distilled water.
- Now addition of polymer (e.g., Poloxamers) slowly with continuous stirring at cold temperature (4-8^0C) in the above solution to avoid lumping
- Now leave the above solution in refrigerator for allowing polymer to hydrate overnight
- Addition of co-polymers or viscosity enhancer (e.g., HPMC) and adjustment of pH accordingly to target site
- Lastly sterilize the formulation by filtration or autoclaving (if heat stable) [30]

Example of use: Injectable formulations, ophthalmic, rectal, or nasal in- situ gels.

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3.2) pH triggered in-situ gels

Mechanism- This gel undergoes the Gelation when exposure to pH change (e.g., from acidic formulation to neutral pH of the body)

Polymer used– Carbopol (polyacrylic acid)934p or 940, Polyvinyl acetate, Cellulose acetate phthalate, Chitosan (soluble in acidic pH, gel at neutral/alkaline pH). Swati Gupta, Suresh Vyas et: al *Carbopol/ Chitosan based pH- triggered in-situ gelling system for ocular delivery of Timolol Maleate* as timolol maleate in conventional dosage eye drops cause the rapid drainage or nasolacrimal in formulation of in-situ gel increase the release behavior over 24 hours period shown by Fiskian diffusion type.

Reason for using above suggested polymer- Polymer already consisted of ionizable groups that undergoes physical mechanism (swell) and converting it in to gel phase in response to change in pH, by taking an example of Carbopol remain solution from in acidic pH and transition occur in gel at neutral pH like eyes or vaginal cavity.

Formulation method-

- Primarily weight the drug accurately and dissolve it in acidic aqueous solution (typically pH 4-5)
- In above prepared solution addition of pH- sensitive polymer (e.g., Carbopol 934) and by stirring to ensure complete dissolution
- By use sodium hydroxide or triethanolamine for neutralizing formulation or for attaining desired pH according to treatment site
- For improvement of rheological properties of formulation addition of co- polymer or viscosity modifier is introduction in it
- Successfully preparation of formulation by above method now sterile it with filtration or autoclave depending on stability of it [31]

  Example of use: ophthalmic, vaginal, buccal delivery system.

3.3) Ion Activated (Ion -sensitive) in-situ gels

Mechanism- In this form of a gel the gelation undergoes when triggered by cations (e.g., sodium, calcium, magnesium) present in the fluid like tear or mucosal secretions

Polymer used –Gellan gum (e.g., Gel rite), Alginate, Pectin, Xanthan gum

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Reason for using above suggested polymer- Polymer undergoes ion-exchange or crosslinking mechanism transition of solution to gel, by taking common example of alginates crosslinks with calcium ions in transition of gel in the body especially in case of ophthalmic or nasal drug delivery because of natural presence of calcium ion in it. J. Balasubramaniam, J.K. Pandit et: al *Ion -activate din-situ gelling system for sustained ophthalmic delivery of ciprofloxacin hydrochloride* as conventional dosage form of ciprofloxacin hydrochloride have poor bioavailability and therapeutic response due to precorneal elimination of drug. In-situ gel is promising approach as it sustained release of above drug use it sustained release of above drug use in formulation up-to 8 hours period in-vitro.

Formulation method –

- Weighing accurately the drug and addition of the drug in distilled or deionized water.
- In the above prepared solution addition of ion- sensitive polymer (e.g., sodium alginate/ Gellan gum) and stir it till the time it is completely dissolve.
- Now we can add chelating agent like sodium citrate in above solution formulated for preventing premature gelation
- For viscosity modulation we can add co-polymer (e.g., HPMC or Carbopol)
- For its application adjust it pH according to its target site and sterile accordingly with sensitivity by method like autoclave or filtration
- Upon administration, the formulation gels in contact with cations in the biological fluid [32]

Examples of use: Ophthalmic, nasal, buccal, and rectal drug delivery.

3.4) Enzyme Triggered in-situ gels

Mechanism-Enzyme -triggered gelation occurs by converting a precursor to gelling agent depending upon site of administration

Polymer used- Succinylated gelatin, peptide-based polymer, certain synthetic-based co-polymers responsive too enzymes like phosphatase

Reason for using above suggested polymer- Polymer act as the substrate and modified by the enzymes act as crosslinking or degradation causing gelation

Formulation method –

- Firstly, prepare solution containing enzymes-sensitive polymer precursor by (w/v)

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- Now weight the drug and add it to the above solution mix well under aseptic condition
- Now apply it on site of administration for catalyze reaction causing gelation (e.g., Dephosphorylation)

  Examples of use: Injectable system for localized drug delivery (e.g., Tumor site).

3.5) Solvent Exchange induced in-situ gels

Mechanism- In this method gelation undergoes the intake and uptake (exchange) of solvent (e.g., NMP or DMSO) with water, leading to polymer precipitation and gel formulation

Polymer used-(PLGA), (PCL) polycaprolactone. Xiaowei Zhag, Liqun Yang, Chong Zhang et: *al Effect of polymer permeability and solvent removal rate on in-situ forming implants: Drug burst release and microstructure* as the formulation consisted of appropriate amount of polymer dissolved in organic solvent 15% (w/w) of polymer solution stir up-to 24 hrs. at room temperature (20-27^{0}C) until clear and transparent, now add ostiole in appropriate amount until complete final concentration of 40mg/ml.

Reason for using above suggested polymer- Polymer used are basically hydrophobic in nature dissolved in a biocompatible solvent (e.g., NMP). After injecting them at the target site the diffusion of solvent outwards and intake of water prompt polymer precipitation undergoes transition of solution to gel.

Formulation method –

- Weight the drug properly dissolves the drug and mix it with polymer in a water-miscible organic solvent (e.g., NMP)
- Injected into the body where solvent, water diffusion exchange occurs, leading to phase separation and gel formulation

This method is mainly used for injectable depot formulations [33]

Example of use: long-acting subcutaneous or intramuscular drug delivery.

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Figure1. provide the general method of formulation of in-situ gels concluding the all methods.

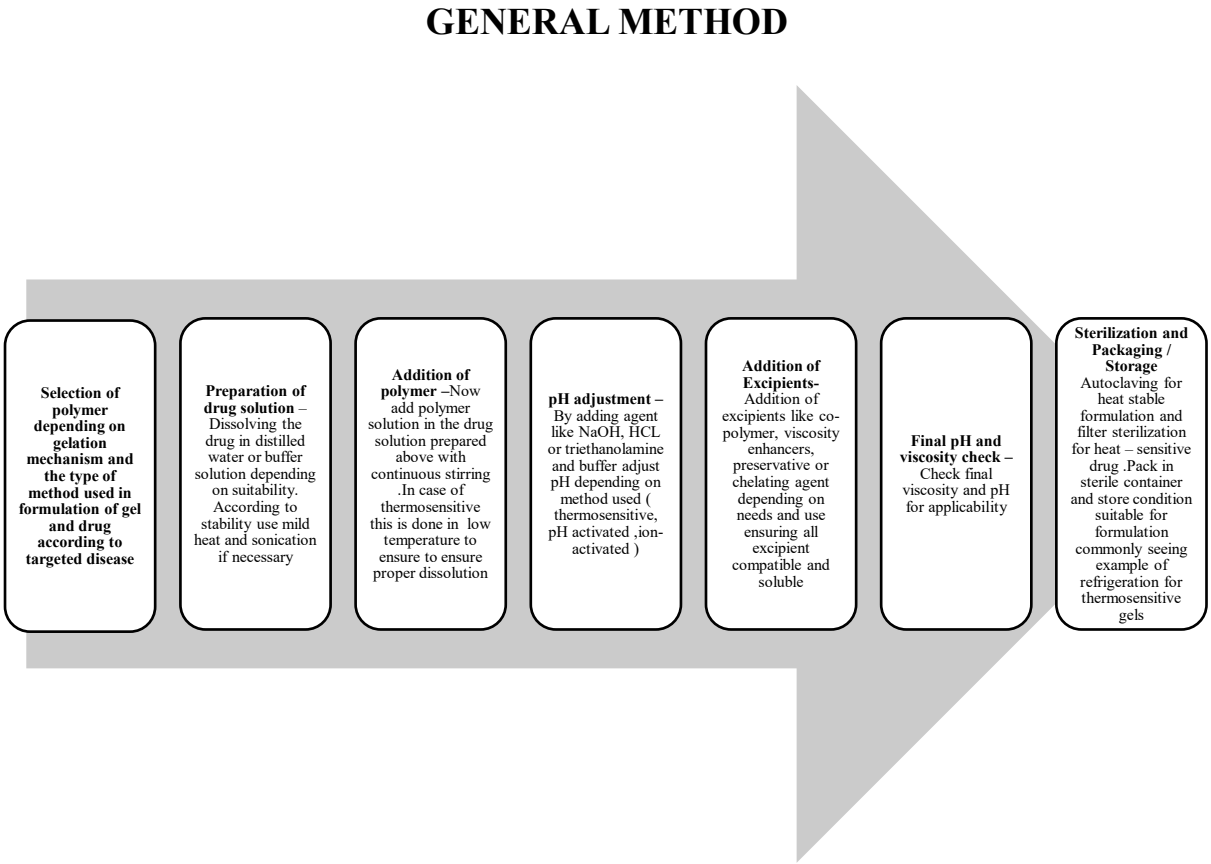

Figure no. 1: General method of formulation of In-situ gel

4) EVALUATION

- **Gelation Temperature/Gelation time-**

Involves “Test-Tube Inversion method”.

Apparatus required- Water bath, thermometer, test tubes, formulated in-situ gel

Procedure-Fill the test-tube with in-situ gel placing the test-tube in water bath at low temperature (approx. 20^0C) gradually increase the temperature by(1^0C/min) tilting the test-tube horizontally by increase of every 1^0C. Recording temperature at which sol. to gel

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conversion occur (i.e. the liquid no longer flows) is known as gelation temperature depending on application site. Nasal-32^{0}C, ocular-34 to 35^{0}C. [34]

- **Spread ability-**

Performed by "Glass Slide method".

Procedure-Firstly taking two slides and placing 1g of gel between them. Putting 500g weight for 1min. Calculate diameter of spread of gel. [35]

- **pH Measurement**

pH can be calculated by using digital pH meter.

Procedure-Primarily taking 1ml of soln. of in-situ gel of formulation prepared, dipping electrode into formulation hence recording the pH . Formulation pH should match with application site pH like ocular-7.4, nasal -6.4, vaginal-4 to 5.

- **Viscosity Measurement-**

Measured by "Brookfield viscometer /Rotational Rheometer".

Procedure-Involves by placing the sample of in-situ gel in the beaker at the temp. of 25^{0}C. Now observing and recording the viscosity by means of spindle used in viscometers with the proper RPM, raise the temperature to 37^{0}C and repeat it. Increase in viscosity after gelation. [36]

- **Drug Content Uniformity-**

Measured by "UV-Visible Spectrophotometry or HPLC".

Procedure- Weigh accurately 1ml of gel. Use suitable solvent for dilution, now filter it and analyze using UV/Vis or HPLC at drug specified wavelength

- **Gel Strength-**

Can be analyzed or measured by "Texture Analyzer/Custom Method ".

Procedure- Transferring the gel into a cylindrical container. Probe attached to the texture analyzer is lowered and placed in the cylindrical container into gel. Force required to penetrate the probe through the gel is recorded

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- **Mucoadhesive Strength-**

Is calculated by "Modified Balance method".

Procedure-Attachment of fresh mucosal tissue to the platform, placement of gel between the mucosa and weight is applied. Now observe and record the weight required to separate the two surfaces

- **In-vitro Drug Release Study-**

Measured by "Franz Diffusion Cell/Dialysis Bag [37]

Procedure-

Dialysis Bag- Use of pre-treated dialysis bag and place the gel inside it, which is suspended in buffer and rotate it at 50-100rpm in room temperature. Now analyze and withdraw the sample overtime.

Franz Diffusion Cell- Addition of gel to the donor chamber. Maintain the receptor chamber with the help of buffer at the room temperature. Mounting cell with synthetic / natural membrane. Now analyzation of sample by withdrawing it in different intervals.

- **Sterility Testing-**

Can be done by "Membrane Filtration/Direct Inoculation".

Procedure- Observation of microbial growth in the sample within 14 days by incubating them in thioglycolate medium and soya-bean casein digest medium.

- **Stability Studies-**

Involves two methods according to the ICH guidelines and its duration can be up-to 3- 6 months. Parameter includes: appearance, pH, viscosity, drug content, gelation behavior. [38]

Long term -Temperature (25^0C+2^0C, 25^0C-2^0C)

RH-(60%+5%, 60%-5%)

Accelerated-Temperature (40^0C+2^0C, 40^0C-2^0C)

RH-(75%+5%,75%-5%).

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Table number 2 provides the glimpse of evaluation parameters of In-situ gels with the possible method reported and its purpose of performing them.

Table number 2: Evaluation parameter of in-situ gels

PARAMETER	PURPOSE	Reported methods
GELATION TEMP/TIME/GELATION STRENGTH	To determine how fast the transition occurs from solution to gel at physiological fluid condition depending on triggered polymer used in it (temp, pH, ion) and to evaluate how strong and stable the gelation occur i.e., mechanical strength and the residence time.	Done by dropwise addition of stimulated tear fluid (STF), GEL STRENGTH- Textured analyzer.
SPREADABILITY	It is done for ocular in-situ gel to determine and evaluate the spread ability of sol. form in application site.	Parallel plate method, slip and drag tests, rheological studies.
pH MEASUREMENT	To determine compatibility with tissue/fluids for avoiding irritation.	Done by digital pH meter.
VISCOSITY MEASUREMENT /RHEOLOGICAL STUDIES	To determine flow behavior (shear thinning, pseudoplastic) in both sol. and gel state, gel stability	Done by Brookfields viscometer, Rotational rheometer.
DRUG CONTENT UNIFORMITY	To determine and evaluate that drug is uniformly loaded, no segregation within acceptable range.	Done by UV-Vis spectrophotometer /HPLC
MUCOADHESIVE STRENGTH	To determine and evaluate assess adhesion of gel to the mucosal tissue.	Done by modified balance method.
IN-VITRO DRUG RELEASE STUDY	To determine how the drug is released from gel under condition mimicking	It is done by Franz diffusion cell or

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	target site. Fitting in kinetic models (i.e., zero order kinetic, first order kinetics, Higuchi plot) to be done.	dialysis membrane method.
STERILITY TESTING	To determine and check sterility observing the microbial growth to prevent irritation.	It is done by Draize test.
STABILITY STUDIES	To determine and evaluate changes in pH, clarity, viscosity and drug content over storage condition.	ICH guidelines (25^0C/60%RH, 40^0C/75%RH).

5) Advances in in-situ gel system:

It includes improvement in broader application routes, ISG formulations enhancement of drug penetration into inner eyes compartments. Other advancement includes combination of different mechanism (dual or multiple), formulation of in-situ gel by advanced technology (3D printing). Carmen C. Pira's, Alasdair G. Kay, Paul G. Genever, Juliette Fitremann and David K. Smith et: al *Self-assembled gel tubes, filaments and 3Dprinting with in situ metal nanoparticle formation and enhanced stem cell growth* in the above article it involves two strategy first strategy tubular core-shell gel structures based on LMWG (DBS-$CONHNH_2$) with the mixture of calcium alginate. Second strategy include gel filament based on (DBS-$CONHNH_2$) alone prepared by wet-spinning at elevated concentration by means of solvent - switch method causing extended in 3D -printing method as LMWG object show stability for at least a week in water. LMWG retains unique ability for in-situ gel with previous metal reduction (Au) nano-particle as exposure of filament or tubes $AuCL_3$ solution. Since high loading of (DBS-$CONHNH_2$) in gel filament more loading of AuNPs. Cytotoxicity and viability studies shows that (DBS-$CONHNH_2$) and (DBS-$CONHNH_2$)/alginate hybrid gel loaded with AuNPs are biocompatible as it enhances stem cell metabolism results indicates 3D printing considerable potential for use in tissue engineering application. [39]

Rouging Li, Heying Zhao, Zhiqiang Zheng, Yangyang Liu, Shuri Song, Lei Song, Jiannan Ren, Jing Dong and Peigi Wang et: *al Bioinks adapted for in situ bioprinting scenarios of defect sites: a review* this article shows bioprinting in-situ provide a approach toward problem of in-vitro tissue culture and vascularization by printing tissue directly to the site of target or defect

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mutating the printed tissue using natural cell microenvironment in-vivo ,as above review consisted scenario according to three different aspect

1) In-situ design strategy of bio-links- (I) Design consideration in bio-link (ii) Role of hydrogels

2) Selection of commonly used bio-material- Alginate, gelatin, collagen, peptides, chitosan, silk fibroin, hyaluronic acid.

3) The application of bio-printing to different treatment scenarios- bone/cartilage defects, skin defects, other defects.

An emerging field is based on computer assisted scanning results, of target sites directly at the site with biomaterials, bioactive factors and material necessary for prefabricated graft used in traditional traditional/conventional in-vitro 3D printing method. [40]

Research is going on brain targeting (via nasal, ISG [42]) multi-stimulus gels polymer advancement and innovation, embedding of nano/microparticles. Avinash Takada, Pratiksha Ghodke, Ashvini Patange, Pravin Patil et: al *Nanostructured cubosomal in situ nasal gel for the treatment of migraine* as this article provide the research for the treatment of migraine for the enhancement of bioavailability of *sumatriptan succinate* than conventional *intravenous* (I.V) and the formulation consisted of two phases firstly: Cubosomal dispersion by using Glycerol mono-oleate, secondly: Gel preparation by use of sodium alginate (ion activated gelling mechanism) and kolliphor 188 (thermos-responsive gelling mechanism) therefore by use of dual mechanism ,*samanea saman seed* gum for mucoadhesive agent as the result shows the higher concentration of *sumatriptan succinate* in brain as compared to (I.V) *intravenous* traditional dosage form , hence providing potential promising approach. [41]

6) Conclusion:

In conclusion, as seen in above article in-situ gel provide a promising approach as compared to conventional / traditional dosage form cause of advancement in polymer science, incrementation in patient compliances due to less dosing frequency and minimal side effect. The transition occurs in solution to gel form provide longer contraction time to the target site and sustained and controlled release of drug produces effect of prolonged release of drug as well as good stability, bioavailability and efficiency due to increase in market and patient acceptability the potential of such dosage form is encouraging with the new development of product with new novel technology might emerge in future.

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