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A review on "in situ gel-forming system: an attractive alternative for improvement of bioavailability''

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ABSTRACT: Now a day's inventions have been made in the research and development of rate-controlled oral drug delivery systems by overcoming physiological adversities, such as short gastric residence times (GRT) and unpredictable gastric emptying times (GET). In situ gelling systems are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. In situ forming polymeric formulations are drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation in situ, to form a gel. Various biodegradable polymers that are used for the formulation of in situ gels include gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide) and poly-caprolactone. The in situ gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems and good patient compliance, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. Evaluation of In situ gel systems include in vitro drug release studies, sol-gel transition temperature and gelling time, gel strength, viscosity & rheology, texture analysis, clarity.. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing

KEY WORDS: In-situ drug delivery, Polymer, Gastro retention, sustained release, biodegradable polymers.

1. INTRODUCTION:

In-situ is a Latin word which means 'In its original place or in position'. There are many mechanisms which triggers the formulation of *in-situ* gels such as solvent exchange, ultra violet irradiation, ionic cross linkage, temperature modification, pH change and ionization. With the increased demand in techniques and recent developments in the field of polymer sciences various stimuli sensitive hydrogels like pH and temperature sensitive hydrogels are developed, which are used as chemotherapeutic agents to tumour regions. Prolonged and sustained release of the drug, reproducible, excellent stability, biocompatible and accurate quantities of administration makes the *in-situ* gel system more reliable. *In-situ* gel formulation applied for targeted delivery via ophthalmic, rectal, vaginal, nasal mucosa avoids the hepatic first-pass metabolism, especially for the proteins and peptides.[1] Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems.

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The goal in designing these systems is to reduce the frequency of dosing or to increase effectiveness of the drug by localization at the site of the action, decreasing the dose required or providing uniform drug delivery.

Polymers have historically been the keys to the great majority in drug delivery systems. Gels are an intermediate state of matter containing both solid and liquid components. The solid component comprises a three dimensional network of inter connected molecule or aggregates which immobilizes the liquid continuous phase. Gels may also be classified based on the nature of the bonds involved in the three dimensional solid network. Chemical gels network together and physical gels when hydrogen bonds and electrostatic and vander waals interaction maintain the gel network. The term network indicates the presence of cross-links, which help avoid the dissolution of the hydrophilic polymer in an aqueous medium.[2]

2. IN SITU GELLING SYSTEM:-

This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa. In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart

polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. The advantages of using biodegradable polymers in clinical applications are apparent. Various natural and synthetic polymers are used for formulation development of in situ forming drug delivery systems.[3]

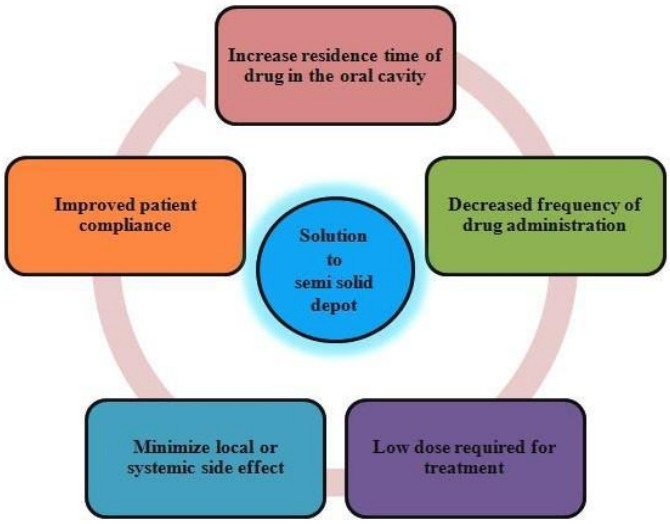

Fig no. 1: Applications of in situ gelling system.

2.1.1. ADVANTAGES OF IN SITU GELLING SYSTEM:-[4]

- In situ gel are advantageous for drugs absorbed through the stomach e.g. ferrous salts and for drugs meant for local action in the stomach and treatment of peptic ulcer disease e.g. antacids.
- When there is vigorous intestinal movement and a short transit time as might occur in certain type of diarrhoea, poor absorption is expected under such circumstances it may be advantageous to keep the drug in floating condition in stomach to get a relatively better response.
- Improved drug absorption as it remain at absobtion window for longer time.
- In situ gelling system is useful for Controlled delivery of drugs and Treatment of gastrointestinal disorders
- The major importance is the possibilities of administrating accurate & reproducible quantities compared to already formed gel.
- Poor bioavailability & therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of gel system.
- Sustained drug delivery reduced frequency of Dosing.
- Ease of administration and better patient compliance.

2.1.2. DISADVANTAGES OF FLOATING DRUG DELIVERY SYSTEMS:-[4-6]

- There are certain situations where gastric retention is not desirable. Aspirin and non-steroidal anti-inflammatory drugs are known to cause gastric lesions, and slow release of such drugs in the stomach is unwanted.
- Thus, drugs that may irritate the stomach lining or are unstable in its acidic environment should not be formulated in gastroretentive systems.
- Furthermore, other drugs, such as isosorbide dinitrate, that are absorbed equally well throughout the GI tract will not benefit from incorporation into a gastric retention system.

3. APPROPRIATE CANDIDATE DRUGS FOR IN SITU GEL SYSTEM:

- Drugs acting locally in the stomach: e.g. Antacids and Anti H. Pylori viz. Misoprostol.
- Drugs that are primarily absorbed in the stomach: e.g. Amoxicillin
- Drugs that is poorly soluble at alkaline pH: e.g. Furosamide, Diazepam, Verapamil, etc.
- Drugs with a narrow absorption window: e.g. Cyclosporine, Levodopa, Methotrexate
- Drugs which are absorbed rapidly from the GI tract: e.g. Metronidazole, tetracycline.
- Drugs that degrade in the colon: e.g. Ranitidine, Metformin.
- Drugs that disturb normal colonic microbes: e.g. antibiotics against Helicobacter pylori.

4. IDEAL CHARACTERISTICS OF POLYMERS:-[7]

A polymer used to in situ gels should have following characteristics-

- It should be biocompatible.
- It should be capable of adherence to mucus.
- It should have pseudo plastic behavior.
- It should be good tolerance & optical activity.
- It should influence the tear behavior.
- The polymer should be capable of decrease the viscosity with increasing shear rate there by

- offering lowered viscosity during blinking & stability of the tear film during fixation.

5. POLYMERS USED IN IN SITU GELLING SYSTEM

5.1.1 Gellan gum:-[8-9]

Gellan gum (commercially available as Gelrite TM or Kelcogel TM) is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucuronic acid residues.
It has the tendency of gelation which is temperature dependent or cations induced. This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three dimensional network by complexation with cations and hydrogen bonding with water. The formulation consisted of gellan solution with calcium chloride and sodium citrate complex. When administered orally, the calcium ions are released in acidic environment of stomach leading to gelation of gellan thus forming a gel in stomach.

5.1.2 Xyloglucan:-[10-13]

Xyloglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-D-glucan backbone chain, which has (1-6)-α-D xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. When xyloglucan is partially degraded by β- galactosidase, the resultant product exhibits thermally reversible gelation by the lateral stacking of the rod like chains. The sol-gel transition temperature varies with the degree of galactose elimination. It forms thermally reversible gels on warmingto body temperature. Its potential application in oral delivery exploits the proposed slow gelation time (several minutes) that would allow in-situ gelation in the stomach following the oral administration of chilled xyloglucan solution. Xyloglucan gels have potentially been used for oral, intraperitoneal, ocular and rectal drug delivery.

5.1.3 Alginic acid:-[14-15]

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1, 4-glycosidic linkages. The proportion of each block and the arrangement of blocks along the molecule vary depending on the algal source.
Dilute aqueous solutions of alginates form firm gels on addition of di and trivalent metal ions by a cooperative process involving consecutive glucuronic residues in the α- L glucuronic acid blocks of the alginate chain. Alginic acid can be chosen as a vehicle for ophthalmic formulations, since it exhibits favorable biological properties such as biodegradability and non toxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability togel in the eye, but also because of its mucoadhesive properties.

5.1.4 Xanthum gum:-[16]

Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram negative bacterium Xanthomonas campestris. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β- D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid-α-D-mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain.

5.1.5 Chitosan:-[17-20]

Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell. Chitosan is abiocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pH of 6. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. The pH gelling cationic polysaccharides,solution are transformed into thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking by addition of polyol salts bearing a single anionic head such as glycerol, sorbitol, fructose or glucose phosphate salts to chitosan aqueous solution.

5.1.6 Carbopol:-[21-22]

Carbopol is a well known pH dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. HPMC is used in combination with carbopol to impart the viscosity to carbopol solution, while reducing the acidity of the solution. Various water soluble polymers such as carbopol systemhydroxypropylmethylcellulose system, poly (methacrylic acid)-poly (ethylene glycol) come under the category of pH-induced in-situ precipitating polymeric systems. Based on this concept, the formulation and evaluation of an ophthalmic delivery system for indomethacin for the treatment of uveitis was carried out. A sustained release of indomethacin was observed for a period of 8 h in vitro thus considering this system as an excellent candidate for ocular delivery. A pH induced in-

situ precipitating polymeric system (an aqueous solution of carbopol-HPMC system) was designed and developed by Ismail et al. for plasmid DNA delivery.

5.1.7 Pectin:-[23-24]

Pectins are a family of polysaccharides, in which the polymer backbone mainly comprises α-(1-4) D galacturonic acid residues. Low methoxy pectins (degree of esterification <50%) readily form gels in aqueous solution in the presence of free calcium ions, which crosslink the galacturonic acid chains in a manner described by egg-box model. Although the gelation of pectin will occur in the presence of H + ions, a source of divalent ions, generally calcium ions is required to produce the gels that are suitable as vehicles for drug delivery.

The main advantage of using pectin for these formulations is that it is water soluble, so organic solvents are not necessary in the formulation. Divalent cations present in the stomach, carry out the transition of pectin to gel state when it is administered orally. Calcium ions in the complexed form may be included in the formulation for the induction of pectin gelation30. Sodium citrate may be added to the pectin solution to form a complex with most of calcium ions added in the formulation.

5.1.8 Synthetic polymers:-[25-28]

Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactide-coglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations. Synthetic polymers are popular choice mainly for parenteral preparations. The trend in drug delivery technology has been towards biodegradable polymers, requiring no follow up surgical removal, once the drug supply is depleted. Aliphatic polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactide-coglycolide), poly (decalactone), poly ε-caprolactone have been the subject of the most extensive recent investigations. Various other polymers like triblock polymer systems composed of poly(D,L-lactide)-block-poly(ethylene glycol)-block poly(DL-lactide), blends of low molecular weight poly(D,L-lactide) and poly(ε-caprolactone) are also in use. These polymers are mainly used for the injectable in situ formulations. The feasibility of lactide/glycolide polymers as excipients for the controlled release of bioactive agents is well proven. These materials have been subjected to extensive animal and human trials without evidence of any harmful side effects. When properly prepared under GMP no evidence of inflammatory response or other adverse effects upon implantation. Another type of synthetic polymeric system includes the in situ cross linked system, where the polymers form cross linked networks by means of free radical reactions that may occur by means of light (photopolymerizable systems) or heat(thermo setting systems). Thermosetting systems are in the sol form when initially constituted, but upon heating, they set into their final shape. This sol-gel transition is known as curing. But if this cured polymer is heated further, it may lead to degradation of the polymer. Curing mainly involves the formation of covalent cross links between polymer chains to form a macromolecular network. Dunn et al. designed a thermosetting system using biodegradable copolymers of DL-lactide or L-lactide with ε-caprolactone for prosthetic implant and slow release drug delivery systems. This system is liquid outside the body and is capable of being injected by a syringe and needle and once inside the body, it gels. In in situ precipitating polymeric systems, the polymer precipitation from solution may lead to gel formation in situ and this precipitation can be induced by conditions from purified monomers, the polymers exhibit change in temperature (thermosensitive systems), solvent removal or by change in pH. An important example of thermosensitive polymer is poly-(N-isopropyl acrylamide), [poly (NIPAAM)], which is used for the formation of in situ gels. It has lower critical solution temperature phaseseparation at about 32. The polymers such as poly (DLlactide), poly (DL-lactide-co-glycolide) and poly (DLlactide- co-Ξ-caprolactone) form solvent-removal precipitating polymeric systems.

6. APPROACHES OF IN SITU GEL DRUG DELIVERY:-[29]

Different approaches utilized in producing the in situ gel formation are as follows

- Based on producing physical changes
- Based on producing chemical changes
- Based on physiological stimuli
- Dilution-sensitive.
- Electrical signal-sensitive.
- Light-sensitive.
- Glucose-sensitive

6.1. In situ Gel Formation by Physical Changes:-[30]

This approach involves either swelling or diffusion phenomenon. In swelling, polymer in the system absorbs water from the surrounding environment and swells to

form a viscous gel (e.g. glycerol mono-oleate). In diffusion, solvent in which the drug and polymer is dissolved or dispersed, diffuse into the surrounding tissues causing the precipitation of the polymer to form gel (e.g. N-methyl pyrrolidone).

6.2. In situ Gel Formation Based on Chemical Changes or Stimuli: Change in the chemical environment of system may lead to gel formation by producing polymeric cross linking.

6.2.1. Ionic cross linking:-[31-33]
In presence of the various ions present in the body fluids, e.g. Na+, K+, Ca2+, Fe3+ etc., ion sensitive polysaccharides, e.g. carragenan, gellan gum, pectin etc., undergo transition in phase due to development of the polymer cross linking, e.g. Sodium alginate undergoes gel formation in presence of calcium chloride.

6.2.2. Enzymatic cross-linking:-[34]
Enzymes present in the body fluids may also cause cross linking to form a polymer network and is considered, as most convenient mode of gel formation.

6.3. In situ Gel Formation by Physiological Stimuli:-
Physiological stimuli that can induce gel formation include change in temperature and change in pH of the system.

6.3.1. In situ gel formation depending on change in temperature: - [30, 34]
In this approach, temperature dependent phase transition from less viscous solution to comparatively high viscosity gel is seen. Change in temperature causes abrupt change in the solubility of polymer within system and polymer polymer interaction occurs to form a solvated macromolecule of hydrophobic nature. Temperature sensitive polymers are the most studied class for producing the *in situ* gel characteristics, e.g. Polyacrylic acid, polyacrylamide etc.

6.3.2.In situ gel formation due to change in pH of system:-[35]
Polymers, such as polyacrylic acid and its derivative (carbopol), polymethacrylate etc., undergo gel formation because of change in the pH, due to presence of various ionizable groups in the chemical structure of the polymer. Polymer with anionic groups leads to increase in swelling with increase in the pH, while polymer with cationic groups shows a decrease in the swelling.

6.4. Dilution-Sensitive
This type of hydrogel contains polymer that undergoes phase transition in presence of higher amount of water. By having a system undergoing phase transition as a consequence of dilution with water a higher amount of polymer can be used. E.g:-Lutrol F68

6.5. Electrical Signal-Sensitive
Hydrogels sensitive to electric current are usually made of polyelectrolytes such as the pH-sensitive hydrogels. Electro-sensitive hydrogels undergo shrinking or swelling in the presence of an applied electric field. Chitosan gels as matrices can be used for electrically modulated drug delivery.

6.6. Light-Sensitive
Light-sensitive hydrogels can be used in the development of photo-responsive artificial muscle or as the *in situ* forming gels for cartilage tissue engineering. Polymerizable function groups and their initiator like ethyl eosin and camphor Quinone can be injected in to tissue and applied electromagnetic radiation used to form a gel by enzymatic processes. For that long ultraviolet wavelengths are used.

6.7. Glucose-Sensitive
Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Another approach is based on competitive binding of insulin or insulin and glucose to a fixed number of binding sites in concanavalin A, where insulin is displaced in response to glucose stimuli, thus functioning as a self-regulating insulin delivery system. An alternative route through phenylborate-poly (vinyl alcohol) polymers is also possible.

7. FORMULATION DESIGN:-
The design of in-situ gel formulation depends on the physicochemical properties of the drug molecule, the diseased condition for which treatment is required, the patient population and the marketing preference. Physicochemical factors include molecular weight, lipophilicity and molecular charge; an anatomical and physiological factor includes membrane transport, pH of tissue fluid, and mucocilliary clearance (as in case of nasal administrations). While formulation factors include clarity, pH, gelation temperature, viscosity, osmolarity, spreadability.

8. APPLICABILITY OF IN SITU GEL DRUG DELIVERY SYSTEM:-

8.1. Oral drug delivery system:-[36]

The pH-sensitive hydrogels have a potential use in site specific delivery of drugs to specific regions of the GI tract. Hydrogels made of varying proportions of PAA derivatives and crosslinked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium or showed gastroprotective property. Cross-linked dextran hydrogels with a faster swelling under high pH conditions, likewise other polysaccharides such as amide pectins, guar gum and insulin were investigated in order to develop a potential colon-specific drug delivery system.

8.2. Ocular drug delivery system:-

In ocular delivery system natural polymers like gallan gum, alginic acid & xyloglucan are most commonly used. For local ophthalmic delivery system various compounds like antimicrobial agent, anti-inflammatory agent & autonomic drugs are used to relieve intra ocular tension in glaucoma.

8.3. Vaginal drug delivery system:-

Formulations based on a thermo-plastic graft copolymer that undergo in situ gelation have been developed to provide the prolonged release of active ingredients such as nonoxynol-9, progestins, estrogens, peptides and proteins[

8.4. Rectal drug delivery system:-

The rectal route may be used to deliver many types of drugs that are formulated as liquid, semisolid (ointments, creams and foams) and solid dosage forms (suppositories).

8.5. Nasal drug delivery system:-

In nasal in-situ gel system gallan gum & xanthan gum are used as in-situ gel forming polymers Momethasone furoate was evaluated for it's efficacy for the treatment of allergic rhinitis.[

8.6. Injectable drug delivery system:- [37]

One of the most obvious ways to provide sustained release medication is to place the drug in delivery system and inject or implant the system into the body tissue. Thermoreversible gels mainly prepared from poloxamers are predominantly used.

8.7. Dermal and transdermal drug delivery system:-[38]

Poloxamer 407 gel was found suitable for transdermal delivery of insulin. The combination of chemical enhancers and iontophoresis resulted in synergistic enhancement of insulin permeation.

Table 1: List of drugs developed as in-situ gel drug delivery system

Drug	Polymer	Route of administration
Clotrimazole	Carbopol 934P, gellan gum, HPMC	Oral
Paracetamol	Xyloglucan	Oral
Doxycycline hyclate	Poloxamer 188,Gellan Gum, HPMC	Ocular
Moxifloxacin Hydrochloride	HPMC Sodium Alginate	Ocular
Itraconazole	Poloxamer 407, 188, HPMC	Vaginal
Acetomenophen	Polycarbophil, polaxamer F188, 407	Rectal
Curcumin	Capryol 90, solutol HS15, transcutol HP	**Nasal**
Bupivacaine HCl	Poly(D,Llactide), poly(D,L-lactide-co-glycolide) (PLGA)	Parenteral

9. EVALUATION AND CHARACTERIZATION OF IN SITU GELLING SYSTEM:-

In-situ gel evaluated & characterized by the following parameters-

9.1. Clarity:-[40]

The clarity of formulated solution is determined by visual inspection under black & white Background.

9.2. Texture analysis:-[40]

The consistency, firmness &cohesiveness of in situ gel are assessed by using texture profile analyzer which mainly indicated gel strength & easiness in administration in vivo higher value of adhesiveness of gel are needed to maintain an intimate contact with mucus surface.

9.3. pH of gel:-[41]

pH can be determined formulation is taken in beaker & 1ml NaOH added drop wise with continuous stirring. pH is checked by using pH meter

9.4. Sol-Gel transition temperature and gelling time:-[39]
For in situ gel forming systems incorporating thermo reversible polymers, the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. Gelling time is the time for first detection of gelation as defined above.

9.5. Gel-Strength:-[39]
This parameter can be evaluated using a rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface.

9.6. Gelling capacity:-[42]
In-situ gel is mix with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25µl & normal volume of tear fluid in eye is 7µl) to find out gelling capacity of ophthalmic product. The gelation assessed visually by noting the time for & time taken for dissolution of the formed gel.

9.7. Rheological studies:-[42-43]
The viscosity measured by using Brookfield viscometer, cone & plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity 5-1000 mPas, before gelling & after ion gel activation by eye will have viscosity of from about 50-50,000 mPas.

9.8. Isotonicity evaluation:-
Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic Formulation.

9.9. Swelling studies:-[44-45]
Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature .The cell contains artificial tear fluid (composition – 0.67g Nacl, 0.20g NaHCo3, 0.008g Cacl2.2H2o & distilled water q.s to 100g). swelling medium equilibrating at 370c one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship.

Formula No 1.

$$\% St = (Wt - W0)\ 100/W0$$

Where,
St = Swelling at time't'.
Statistical analysis:-
Analysis of variance (ANOVA) is used the testing the difference between calculated parameters using SPSS statistical package. Statistical difference yielding $P \leq 0.05$ is considered. Duncan multiple comparison is applied when necessary to identify which of the individual formulations are significantly different.

9.10. High performance liquid chromatography:-[46]
The HPLC system is used in reversed phase mode. Analysis is performed on a Nova pack C18 packed column (150 mm length X 3.9 mm i.d).

9.11. Fourier transformer infra red:-[46-47]
The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBR pellets.

9.12. Thermal analysis:-[48]
Thermo gravimetric analysis can be conducted for in situ forming polymeric system to quantitative the percentage of water in hydrogel. Different scanning calorimetry is used to observed, if there are many changes in thermograms as compared with pure ingredients used thus indicating the interaction.

9.13. In vitro drug release studies:-[49]
In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated tear fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 µm poresize). The whole assembly is placed on thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at $37^{o}c \pm 0.5^{o}c$. 1ml sample is withdrawn at predetermined time interval

of 1hr for 6hrs the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism of their kinetics.

9.14. Ocular irritancy studies:-[50-51]

Ocular irritancy studies are performed on male albino rabbits, weighing 1-2 kg. The modified Draize technique is used for ocular irritation potential of ophthalmic products. The formulation is placed in lower cul-de-sac & irritancy is tested at time interval of 1hr, 2hrs, 48hrs, 72hrs, & 1 week after administration. The rabbits are observed periodically for redness, swelling, & watering of eyes.

9.15. Antimicrobial activity:-[52-53]

Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms. This is determined in agar diffusion medium employing 'Cup Plate Techniques'. The microbial growth of bacteria is measured by conc. Of antibiotic & compared with that produced by known conc. Of standard preparation of antibiotic & carried out the microbial assay serial dilution method is employed.

9.16. Sterility testing:-[54]

Sterility testing is carried out as per the IP 1996. The formulation is incubating for not less than 14 days at 300-350c in the fluid thioglycolate medium to find the growth of bacteria & at 200-250 c in Soya bean casein digest medium to find the growth of fungi in formulation.

9.17. Accelerated stability studies:-[55]

Formulation is replaced in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at 40± 20 c & 75 ±5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution.

9.18. Histopathological studies:-[56]

Two mucosa tissue pieces (3 cm2) were mounted on in vitro diffusion cells. One mucosa was used as control (0.6mL water) and the other was processed with 0.6 mL of optimized organogel (conditions similar to in vitro diffusion). The mucosa tissues were fixed in 10% neutral carbonate formalin (24 hours), and the vertical sections were dehydrated using graded solutions of ethanol. The subdivided tissues were stained with hematoxylin and eosin. The sections under microscope were photographed at original magnification ×100. The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged.

10. CONCLUSION:-

In conclusion, the basic requirement of a successful controlled release product focuses on improving patient compliance which the *in situ* gel offers. Exploitation of polymeric in- situ gel for controlled release of various drugs provides a number of advantages over conventional dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems

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