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INSITU GEL BY USING NATURAL POLYMER

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ABSTRACT

The Parenteral route of administration is the most common and efficient for delivery of active drug substances with poor bio-availability and the drugs with a narrow therapeutic index. But parenteral route shows rapid action and rapid decline in systemic drug level. It is desirable to maintain systemic drug level within therapeutically effective concentration range. It requires frequent injection, which ultimately leads to patient discomfort. To overcome all these disadvantages *in situ* gel forming parenteral drug delivery system has been developed. *In situ* gel forming injectable drug delivery system is the ability to inject a drug incorporated into a polymer to a localized site and have the polymer form a semi-solid gel drug depot has a number of advantages. Among these advantages is ease of application and localized, prolonged drug delivery. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner. It is an alternative to microspheres, liposomes and emulsion as parenteral depot systems. Hence *in situ* gelling polymeric delivery systems have been developed and investigated for use in delivering a wide variety of drugs including proteins. The production, packaging of such delivery system is less complex and hence reduce the manufacturing cost.

KEY WORDS: Insitu gel, Mechanism, Natural Polymer, Method of Preparation, Evaluation.

INTRODUCTION

In situ is a Latin phrase which translated literally as “In position “. *In situ* gel is drug delivery systems that are in sol form before administration in the body, but once administered,

undergo gelation *in situ*, to form a gel. Administration route for *in situ* gel oral, ocular, rectal, vaginal, injectable and intraperitoneal routes.

Ease of administration and reduced frequency of administration, improved patient compliance and comfort. Deliverance of accurate dose, i*n 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. Various natural and synthetic polymers are used for formulation development of *in situ* forming drug delivery system. *In situ* gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient or oral route, which can result in unacceptably low bioavailability and passes the hepatic first-pass metabolism, in particular of proteins and peptides. This is novel drug delivery system.[1]

Importance of in Situ Gelling System[2]

- The major importance is the possibilities of administrating accurate & reproducible quantities compared to already formed gel.
- In-situ forming polymeric delivery system such as ease of administration & reduced frequency of administration improved patient compliance & comfort.
- Poor bioavailability & therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of gel system that are instilled as drops into eye &undergoes a sol-gel transition from instilled dose.
- Liquid dosage form that can sustain drug release & remain in contact with cornea of eye for extended period of time is ideal.
- Reduced systemic absorption of drug drained through the nasolacrimal duct may result in some undesirable side effects.

Advantages[3,4,5]

- Ease of administration, comfort
- Reduced frequency of administration further
- Improved patient compliance
- Can be administered to unconscious patients
- Drug gets released in a sustained and controlled manner

- Natural polymers have inherent properties of biocompatibility, biodegradability, and biologically recognizable moieties that support cellular activities.
- Synthetic polymers usually have well-defined structures that can be modified to yield tailorable degradability and functionality.
- *In situ* gels can also be engineered to exhibit bioadhesiveness to facilitate drug targeting, especially through mucus membranes, for non-invasive drug administration.
- *In situ* gels offer an important "stealth" characteristic *in vivo*, owing to their hydrophilicity which increases the *in vivo* circulation time of the delivery device by evading the host immune response and decreasing phagocytic activities

Disadvantages[6,7]

- It is more susceptible to stability problems due to chemical degradation.
- It requires high level of fluids.
- It leads to degradation due to storage problems.

Approaches of In-Situ Gelling System[8,9,10,11]

Various approaches for in-situ gelling system,

A) Stimuli Responsive In-Situ Gelling System

1. Temperature induced in-situ gel system.

2. pH induced in-situ gel systems.

B) Osmotically Induced In-Situ Gelling System

C) Chemically Induced In-Situ Gelling System

1. Ionic cross linking.

2. Enzymatic cross linking.

3. Photo- polymerization.

A) Stimuli Responsive In-Situ Gelling System

Physical or chemical changes in response to small external changes in the environmental conditions.

1. Temperature induced in-situ gel system

Temperature is Temperature is the most widely used stimulus in environmentally responsive polymer systems. The change of temperature is not only relatively easy to control, but also easily applicable both in-vivo and in-vitro. These hydrogels are liquid at room temperature (20˚-25˚C) and undergoes gelation when in contact with body fluids (35˚-37˚C), due to

increase in temperature. The polymers which show temperature induced gelation are poloxamers or pluronics, cellulose derivatives (methyl cellulose).

2. PH inducing in-situ gelling system: Polymers containing acidic or alkaline functional groups that respond to changes in pH are called pH sensitive polymers. The pH is an important signal, which can be addressed through pH-responsive materials. Gelling of the solution is triggered by change in pH. At pH 4.4 the formulation is free from is a free running solution which undergoes coagulation when the pH is raised by the body fluid to pH 7.4. The polymers which shows pH induced gelation are cellulose and its derivatives polyvinyl acetate, polyethylene glycol.

B) Osmotically Induced In-Situ Gelling System: In this method, gelling of the solution instilled is triggered by changes in the ionic strength. It is assumed that the rate of gelation depend on the osmotic gradient across the surface of the gel. The aqueous polymer solution forms a clear solution forms a clear gel in the presence of the mono or divalent cations. The polymer which shows osmotically induced gelation is gellan gum, alginates.

C) Chemically Induced In-Situ Gelling System: The chemical reaction which forms in-situ gel systems are ionic crosslinking, enzymatic cross linking and photo-polymerization.

1. Ionic cross linking: Ion sensitive polysaccharides such as carragenan, gellan gum, pectin, sodium alginate undergo phase transition in presence of various ions such as k+, Ca2+, Na+. These polysaccharides fall into the class of ion-sensitive ones. For example, Alginic acid undergoes gelation in presence of divalent cations example-Ca2+ due to the interaction with guluronic acid block in alginate chains.

2. Enzymatic cross linking: In-Situ formation catalyzed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and physicochemical approaches. For example an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators.

3. Photo polymerization: Photo polymerizable systems when introduced to the desired site via injection get photo cured in-situ with the help of fiber optic cables and then release the drug for prolonged period of time. A photo polymerization, biodegradable hydro gels as a tissue contacting material and controlled release carrier.

Mechanism of in Situ Gel

***In situ* formation based on physical mechanism**

Swelling: *In situ* formation may also occur when material absorbs water from surrounding environment and expand to desired space. One such substance is myverol (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some bioadhesive properties and can be degraded *in vivo* by enzymatic action.[12]

Diffusion

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methyl pyrrolidone (NMP) has been shown to be useful solvent for such system.[13]

***In situ* formation based on chemical reactions mechanism**

Chemical reactions that results *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

Ionic crosslinking

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones**.** While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, i-carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite is an anionic polysaccharide that undergoes *in situ* gelling in the presence of mono- and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low methoxypectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e.g. Ca2+ due to the interaction with glucoronic acid block in alginate chains.[14]

Enzymatic cross-linking

In situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. 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.

Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation.[15]

Photo-polymerisation

Photo-polymerisation is commonly used for *in situ* formation of biomaterials. A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photoinitiator. Typically long wavelength ultraviolet and visible wavelengths are used. Short wavelength ultraviolet is not used often because it has limited penetration of tissue and biologically harmful. A ketone, such as 2,2 dimethoxy-2-phenyl acetophenone, is often used as the initiator for ultraviolet photo- polymerization, where as camphorquinone and ethyl eosin initiators are often used in visible light systems. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence in vivo. Photopolymerizable systems when introduced to the desired site via injection gel. Photocured *in situ* with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature. Furthermore, the systems are easily placed in complex shaped volumes leading to an implant formation.[16]

Polymers used as *in situ* gelling agents

Guar gum

Properties

Guar gum is a naturally occurring gum which is also called as guaran which is obtained from the endosperm of the seed. Guar gum is soluble in water but insoluble in hydrocarbons, fats, esters, alcohols and ketones. It shows its dispersibility in both hot and cold water that is it is soluble in both hot and cold water to form colloidal solution at low amount. Guar gum has derivatives that are used in targeted delivery systems in the formation of coating matrix systems, nano-microparticles and hydrogels. Guar gum also has derivatives such as graft polymers like polyacrylamide grafted guar gums that have good colon targeting properties. Guar gum can also be used as a polymer in matrix tablets which shows controlled release. The semi synthetic form of guar gum is carboxy methyl guar(CMG) which is anionic in nature that are used in formulation of transdermal drug delivery systems because it shows good release rate profile, safety and stability. Guar gum is also available in various cross

linked forms that are used in various novel formulations i.e, glutyraldehyde cross linked guar gum, hydroxyl ethyl guar gum, poly acrylic acid conjugate guar gum, hydroxyl methyl gum; 4-vinyl pyridine conjugated guar gum. The modified guar gum has potential to prevent cancer by inhibiting carcinogen activating enzymes and promoting the carcinogen detoxification enzyme glutathione-s-transferase.[17]

Mechanism

As guar gum has the capability of forming high viscous solution at low concentrations, the galactose side chains that are attached to mannose backbone interact with water molecules that are present in the solution leading to the formation of inter molecular chain which causes entanglement of gaur gum molecules that are present in the aqueous phase causing the formation of gelling or thickening of the solution. As guar gum is soluble in both hot water and cold water, temperature plays a key role in the formation of gelling in the solution. So, increase in temperature causes reduction in gelling property of guar gum. As the temperature reduces and causes the formation of sol. So, temperature causes reversible change in gelling of gaur gum.[18]

Method of Preparation

***In-situ* forming drug delivery systems (ISFD)**

Injectable *in-situ* forming implants are classified into five categories, according to their mechanism of depot formation

i. Thermoplastic pastes

ii. *In-situ* cross linked systems

iii. *In-situ* polymer precipitation

iv. Thermally induced gelling system

v. *In-situ* solidifying organogels.

Thermoplastic pastes (TP)

Thermoplastic pastes are semisolid polymers, it is injected as a melt and form a depot upon cooling to body temperature. They are considered as having a low melting point or glass transition temperature in the range of 25-65°C and an intrinsic viscosity in the range of 0.05-0.8 dl/g. Below the viscosity of 0.05 dl/g, no delayed release could be observed, whereas above 0.8 dl/g the ISFD was no longer injectable using a needle. At injection temperature above 37°C but below 65°C these polymers perform like viscous fluids which solidify to highly viscous depots. Drugs are combined into the molten polymer by mixing without the

application of solvents. Bioerodible thermoplastic pastes could be prepared from monomers such as E-caprolactone, glycolide, D, L-lactide, dioxanone and orthoesters. Polymers and copolymers of this monomer have been widely used in surgical sutures, ocular implants, soft tissue repair etc. Zhang et al designed a thermoplastic ABA triblock polymer system composed of poly (D, L-lactide)- poly(ethylene glycol)-poly(D,L-lactide) and blend of ABA triblock copolymer and polycaprolactone (PCL) delivery of Taxol within tumor resection sites. Both provide release of Taxol for more than 60d but the rate of release was very slow. Additional drawback associated with this polymeric system was the high melting temperature of thermoplastic pastes requiring injection temperature at least 60°C. Poly (orthoesters) POE have well matched properties for TP due to their good biocompatibility, relatively low softening temperatures in the range of 35-45°C and degradation by surface erosion.

In-situ cross linked polymer systems

The formation of a cross-linked polymer network is beneficial, to control the diffusion of the hydrophilic macromolecules. Cross-linked polymer network can be establish *in-situ* by free radical reactions initiated by heat (thermosets) or ionic interactions or absorption of photon between small cation and polymer anions. Dunn et al, used biodegradable copolymers of L-lactide or D, Llactide with E-caprolactone to formulate a thermosetting system for prosthetic implants and slow release drug delivery systems it requires free radical producing agents such as benzoyl peroxide into the body which may induce tumor promotion Hibbell *et al*. defined a photopolymerizable biodegradable hydrogel as a tissue contacting material and controlled release carrier. This system involved of a macromer, PEG (polyethylene glycol) -oligo-glycol-acrylate, using a photo initiator, such as eosin and visible light. These hydrogel are restricted to surgical sites accessible to a light source as they form with difficulty after injection into the body. Ion-mediated gelation has been described for a number of polymers, e.g. chitosan/phosphate ions or alginates/calcium ions.

The concentrations of the counter ion available under physiological situations are usually lacking for cross-linking of the above mentioned polymers. Even with these applications, there are two important factors which limit the use of calciumalginate. The first factor is their potential immunogenicity and the second one is longer time *in-vivo* degradability.

In-situ polymer precipitation

A water-insoluble and biodegradable polymer is dissolved in a biocompatible organic solvent to which a drug is added forming a suspension or solution after mixing. When this

formulation is injected into the body, the water miscible organic solvent dissolves and water penetrates into the organic phase. This leads to precipitation and phase separation of the polymer forming the depot at the site of injection. This method has been developed as Atrigel TM technology, which used as a drug carrier for Eligard TM, contains the leuteinizing hormone releasing hormone (LHRH) agonist leuprolide acetate (7.5, 22.5 or 30mg) and poly(lactide-co-glycolic acid)(PLGA) 75/25 dissolved in N-methyl-2-pyrrolidone (NMP) in a 45:55 (m/m) polymer: NMP ratio. This system led to suppression of testosterone levels in dogs for approximately 91d. One of the problems with this system is the possibility of a burst in drug release especially during the first few hours after injection into the body. In order to control the burst effect, four factors have been investigated, the concentration of polymer in the solvent, the molecular weight of the polymer, the solvent used and the addition of surfactant. Also the drug burst is directly related to the dynamics of the phase inversion. Himmelstein and joshi studied that polymer complex of PEG, polyacrylic acid (PAA), and polymethacrylic acid (PMA) is stable below pH≤5.7, the complex is insoluble in water but dissolves in a hydroalcoholic solvent to yield a clear viscous solution. After injection the diffusion of ethanol from the liquid transforms the system into a gel form upon contact with physiological situation. The gel disappears from the site of application with time due to complex dissociation into water soluble and low molecular weight component, which can be eliminated by glomerular filtration. Carbopol is a pH dependent polymer, which forms a low viscosity gel in alkaline environment e.g. pH-7.4 and stays in solution in acidic pH. The addition of HPMC, a viscosity prompting agent, to carbopol reduces the carbopol concentration and hence the solution acidity while conserving the viscosity of the *in-situ* gelling system. This system gels upon an increase in pH when injected.

Thermally induced gelling system

Many polymers undergo rapid changes in solubility as a function of environmental temperature. The thermo sensitive polymer, poly (Nisopropylacrylamide) [poly (NIPAAM)] exhibit sharp lower critical solution temperature, LCST at about 32°C, which can be shifted to body temperature by formulating poly NIPAAM based gels with salt and surfactant. Unfortunately, poly NIPAAM is not suitable for biomedical applications due to its well-known non-biodegradability and cytotoxicity (activation of platelets). Triblock poly (ethylene oxide)-poly(propylene oxide)- poly(ethylene oxide) copolymer, pluronics or poloxamers, have shown gelation at body temperature when highly concentrated polymer solution >15% w/w were injected. These polymer concentration shown drawback of changing the osmolarity

of the formulation, kinetics of the gelation, and causes discomfort in ophthalmic applications due to vision blurring and crusting. Macromed produced thermo sensitive biodegradable polymers based on ABA and BAB triblock copolymers. Where A is hydrophobic polyester block and B denotes the hydrophilic PEG block. The aqueous polymer solution of PEG-PLAPEG is loaded with drug at 45°C after injected into animal form a gel at body temperature, which constantly releasing hydrophilic model substances fluorescein isothiocyanate dextran (FITC-dextran), over 10-20d.An aqueous solution of low molecular weight PEGPLGA- PEG (550-2810-550) triblock copolymers becomes gel at body temperature.

***In-situ* solidifying organogel**

This organogels are composed of water insoluble amphiphilic lipids, which swell in water and forms various types of lyotropic liquid crystals. The amphiphilic lipids studied for drug delivery are glycerol monooleate, glycerol monopalmitostearate, glycerol monolinoleate, sorbitan monostearate (SMS) and different gelation modifiers (polysorbates 20 and 80) in various organic solvents and oils. These compound forms a cubic liquid crystal phase upon injection into an aqueous medium which is gel like and highly viscous. Sorbitan monostearate organogels having either w/o or vesicular in water in oil (v/w/o) emulsion were examined *in-vivo* as delivery vesicles for vaccines using albumin (BSA) and haemagglutin (HA) as model anigens. Intramuscular administration of the v/w/o gel yielded the extended depot effect for 48hrs. Controlled releases of contraceptive steroids ethinyl estradiol and levonorgestrel were achieved by Gao *et al*. In these work biodegradable organogel formulations prepared from glycerol palmitostearate (precirol) in derivatized vegetable oil, show *in-vitro* release of levonorgestrel up to 14d74, while subcutaneous injection into rabbits demonstrated an estrus blockage for up to 40d75.[19]

Evaluation and Characterization of *In-Situ* Gel System

Physical parameters

Physical parameters to be tested for *in-situ* gel solution are clarity, pH, gelling capacity, and drug content estimation.

Gelling capacity

The gelling capacity test was done by placing a drop of the prepared formulation in a vial containing 2.0 ml of freshly prepared buffer solution and visually observe.[20]

Drug content

The drug content was determined by accurately placing 10gm of formulations in a volumetric flask and suitably diluted with buffer solution to obtain a concentration of 10µg/ml. By using UV-Visible spectrophotometer the drug concentration was determined.[21]

Viscosity

The viscosity and rheological properties of the polymeric formulations, either in solution or in gel made with artificial tissue fluid (depending upon the route of administrations) were determined with different viscometer like Brookfield viscometer, Cone and Plate viscometer.

Texture analysis

The consistency, firmness and cohesiveness of formulation are measured using texture analyzer which mainly shows the syringe ability of solution so the formulation can be easily administered *invivo*. To achieve the intimate contact with surfaces like tissues the gel should be in higher values of adhesiveness.

Sol-Gel transition temperature and gelling time

For *in-situ* gel forming systems, the sol-gel transition temperature and pH should be determined. Gelling time is the time required for first detection of gelation of *in-situ* gelling system. Thermo sensitive *in-situ* gel should be checked for *in-situ* gelling at body temperature.

Gel-Strength: The gel strength can be evaluated by using a remoter. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the solution 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.

Drug-polymer interaction study and thermal analysis

This study can be performed by using Fourier Transform Infra-Red (FTIR) spectroscopy. During gelation process, the nature of the interacting forces can be evaluated using the technique by employing KBr pellet method. Thermo gravimetric Analysis (TGA) can be conducted for *in-situ* forming polymeric system to quantitate the percentage of water in hydrogel. Differential Scanning Calorimetry (DSC) conducted to observe if there are any changes in thermo grams as compared with pure active ingredients used for gelation.[22]

Syringe ability Study

This study can be performed by taking a disposable syringe, with desirable amount of formulation in it, and then pass the formulation through 21-gauge needle. The formulations that passed easily from the needle it pass the syringe ability test.

***In-vitro* dissolution study**

In-vitro release profile was studied using USP apparatus II at 37° ± 10° C with a rotating speed of 100 rpm in dissolution media which containing Ph 7.4 buffer. During the study, 5 ml of aliquots were removed at fixed time intervals (0.5, 1, 2, 4, 6, 8, 10, and, 24 hr) from the dissolution medium and replaced with fresh buffer to ensure sink condition and drug content can be determined by spectrophotometrically.

***In-vitro* diffusion studies**

In-vitro diffusion test was determined by using Franz diffusion cell. In Franz diffusion cell, cellophane membrane is sandwiched securely between donor and receptor compartment with the epidermis site facing the donor compartment. The receptor compartment is filled with buffer solution, which is continuously stirred and maintained the temperature at 37°C ±1°C throughout the experiment. Before starting the experiment the donor cell was sealed with paraffin film and covered with aluminum foil to prevent exposure to light. At predetermined time interval (0.5, 1, 2, 4, 6,8, 10, and 24 hr) 5ml of aliquots are withdrawn and are replaced with an equal volume of fresh buffer to maintained sink condition and drug content can be determined by spectrophotometrically.[23]

REFERENCE

1. Amruta B. Kumbhar, Ashwini K. Rakde, P.D. Chaudhari. In situ gel forming injectable drug delivery system. IJPSR, 2013; 4(2): 597-609.
2. Parekh Hejal B, Rishad Jivani, Jivani NP, Patel LD, Makwana Ami, Sameja Krunal. Novel insitu polymeric drug delivery system: a review. Journal of Drug Delivery & Therapeutics, 2012; 2(5): 136-145.
3. Calfrs J, Edsman K, Peterson R. Rheological evaluation of Poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci., 2000; 6: 105.
4. Rathore KS, Nema RK. Formulation & evaluation of ophthalmic films for timolol maleate. Planta indica, 2008; 4: 49-50.
5. Gurny R, Ibrahim H, Buri P. The development & use of in situ formed gel triggered by pH. In Biopharmaceutics of ocular drug delivery. ed. Edman, 1993; 81-90.

6. S. Cohen, E. Lobel, A. Trevgoda, Y. Peled. A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J. Control. Release, 1997; 44: 201-208.
7. B. Srividya, R.M. Cardoza, P.D. Amin. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J. Control Release, 2001; 73: 205–211.
8. N.G.N.Swamy, Zaheer Abbhas. Mucoadhesive In-Situ gels as nasal drug delivery systems: A Review. Asian Journal of Pharmaceutical Sciences, 2012; 7(3): 168-180.
9. Saneesh V Ganga, Sujith Abraham. A Review: In-Situ Drug Delivery System. International Journal of Universal Pharmacy and Bio-Sciences, July-Aug 2014; 3(4): 135-149.
10. Sanjay Dey, Beduin Mahanti, Bhaskar Mazumdar, Ananya Malgope and Sandeep Dasgupta. Nasal drug delivery: An Approach of drug delivery through Nasal Route. Pelagia Research Library, 2011; 2(3): 94-106.
11. J.U Kute, A.B Darekar, R.B Saudagar. A Review: In-Situ Gel-Novel approach for nasal delivery. World journal of pharmacy and pharmaceutical sciences, 2014; 3(1): 187-203.
12. Geraghaty P, Attwood D, et al. An investigation of parameters influencing the Bioadhesive properties of Myverol 18-99/ water gels. Biomaterials, 1997; 18: 63-7.
13. Motto F, Gailloud P, et al., In-vitro assessment of new embolic liquids prepared from preformed polymers and water miscible solvents aneurysm treatment. Biomaterials, 2000; 21: 803-11.
14. Guo J-H, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring water-soluble polymers. Pharm Sci & Technol Today, 1998; 1: 254-61.
15. Podual K, Doyle III FJ, Peppas NA. Dynamic behavior of glucose oxidase-containing microparticles of poly (ethylene)-grafted cationic hydrogels in an environment of changing pH. Biomaterials, 2000; 21: 1439-50.
16. Sawhney AS, Pathak CP, Hubbell JA, Hill JL, Desai NP. Photopolymerizable biodegradable hy). drogels as tissue contacting materials and controlled release carriers.US Patent 5410016. 1995.
17. Kokate C.K., Purohit A. P., Gokhale S.B. Pharmacognocy. 14th Ed. Published by Nirali Prakashan, 2008; 137, 141, 146, 152.
18. Duke J.A. Handbook of medicinal herb. 2th Ed, published by CRC press, 2002; 4.
19. Hitesh Bari. A prolonged release parenteral drug delivery system An-overview, International Journal of Pharmaceutical Sciences Review and Research, 2010; 1(3): 1-11.

20. Sarika Meshram, Santosh Thorat. Ocular insitu gels: development, evaluation and advancements, Sch. Acad. J. Pharm, 2015; 4(7): 340-346.
21. Ramanjit Saini, Seema Saini, Gurpreet Singh, Angshu Banerjee. In-situ gels- a new trend in ophthalmic drug delivery systems, IJPSR, 2015; 6(5): 886-890.
22. Aman Kant, Suchetha Reddy, Shankraiah M M Venkatesh J S, Nagesh C. In-situ gelling system a-overview, Pharmacology online, 2011; 2(1): 28-44.
23. Hari Kumar SL, Kulwant Singh, Harpreet Kaur. Design and development of sustained release injectable in-situ gel of cytarabine, Pharmacophore, 2013; 4(6): 252-67.