International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277 – 7873
REVIEW ARTICLE **V-1, I-2, 2012**

A Review on Polymers Used in In-Situ Gel Drug Delivery Systems

Shaikh RG*[1], Shah SV[1], Patel KN[1], Patel BA[1], Patel PA[1]
[1]*Shree Swaminarayan Sanskar Pharmacy College, Zundal, Gujarat-382421, India.*
Manuscript No: IJPRS/V1/I2/00021, Received On: 01/04/2012, Accepted On: 05/04/2012

ABSTRACT

In situ gel drug delivery systems are used in sol form before administration in the body, but once administered, undergo gelation *in situ*, to form a gel. The formation of gel depends on factors like temperature modulation, pH change, presence of ions and ultraviolet irradiation, electrical sensitivity, enzyme sensitive from which drug get released in a sustained and controlled manner. Typically, aqueous solutions of hydrogels used in biomedical applications are liquid at ambient temperature and gel at physiological temperature. The *in situ* gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems. From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost. This review stresses on the polymeric use of natural polymers and synthetic polymers.

KEYWORDS

Natural Polymers, Synthetic Polymers, Sol to gel transformation, *In-situ*, Drug delivery

INTRODUCTION

A main goal of pharmaceutical technology is the design of technologically optimal vehicles for the administration of drugs. Innovative processes allowed an enhancement in the organoleptic properties of the preparations and the maximization of the stability and bioavailability. However, a still existing drawback is the low solubility in the physiological aqueous environment of about 50% of the approved active molecules, resulting in limited gastrointestinal absorption and poor bioavailability. Paradoxically, oral administration of therapeutic agents is the preferred way to achieve the highest patient compliance.[1] Limited solubility also constitutes a hurdle in the development of parenteral and even topical formulations.

Since improved solubility usually correlates well with higher bioavailability.[2,3] Several nano-technological strategies are being pursued in order to guarantee the appropriate drug solubilization[4]. Among them, it is worth mentioning nanoparticle engineering.[5–8]

Hydrogels are polymeric networks that can absorb and retain large amounts of water and biological fluids and swell, still maintaining their three-dimensional structure. These polymeric networks contain hydrophilic domains that are hydrated in an aqueous environment, thereby creating the hydrogel structure. The term ***network*** indicates the presence of cross-links, which help avoid the dissolution of the hydrophilic polymer in an aqueous medium. Hydrogels have many advantages over other drug delivery systems such as good mechanical and optical properties and biocompatibility. The degradation products of hydrogels are usually nontoxic or have lower toxicity. Lower interfacial tension between the surface of the hydrogel and the physiological fluid minimizes protein adsorption and cell adhesion on the hydrogel's surface. The soft rubbery nature of hydrogels also can minimize

***Address for Correspondence:**
Rizvana G. Shaikh,
M.Pharm, Department of Pharmaceutics,
Shree Swaminarayan Sanskar Pharmacy College, Zundal,
Gandhinagar, Gujarat, India.
E-Mail Id: rizy.pharma09@gmial.com

mechanical irritation when used as *in vivo* implants. Currently two groups of hydrogels are distinguished, namely preformed and in situ forming gels. ***Preformed hydro gels*** can be defined as simple viscous solutions, which do not undergo any modifications after administration***. In situ gels*** can be defined as formulations, applied as solutions, sols or suspensions that undergo gelation after instillation due to physico-chemical changes inherent to the stomach.[9]

In-Situ Gel Delivery Systems[9]

In-situ gelation is a process of gel formation at the site of application after the composition or formulation has been applied to the site. In the field of human and animal medicine, the sites of application refers to various injection sites, topical application sites, surgical sites, and others where the agents are brought into contact with tissues or body fluids. As a drug delivery agent, the in-situ gel has an advantage related to the gel or polymer network being formed in-situ providing sustained release of the drug. At the same time, it permits the drug to be delivered in a liquid form. *In situ* is a Latin phrase meaning *in the place.*

The in-situ gelation compositions using ionic polysaccharides have been disclosed in U.S. Pat. No. 5,958,443, which discloses compositions comprising a drug, a film forming polymer and a gel forming ionic polysaccharide (such as an alginate). These compositions employed two separately applied components, one being a solution of cross linking cations, which is applied to the site, and a second liquid component comprising the drug, film forming polymer and an ionic polysaccharide, which is then applied to react with the cross linking ions and form a gel. Various other synthetic and natural polymers have also been used in drug delivery formulations that may or may not have formed cross linked gels, including starches and modified celluloses, gellan, chitosan, hyaluronic acids, pectins, and the like.

Polymers used in in-situ gelling systems

The ***sol phase*** is defined as a flowing fluid, whereas the ***gel phase*** is non-flowing on an experimental time scale, while maintaining its integrity. Above the critical concentration (Critical Gel Concentration - CGC) of a polymer, the gel phase appears. The CGC is most often inversely related to the molecular weight of the polymer employed. The development of physical junctions in the system is regarded as one of the prerequisites in determining gelation, which must be sufficiently strong with respect to the entropically driven dissolving, forces the solvent. The gelation of organic or aqueous polymer solutions occurs have been reviewed extensively and summarized.[10-11]

Materials that exhibit sol to gel transition in aqueous solution at temperatures between ambient and body temperature is of interest in the development of sustained release vehicles with in situ gelation properties. Polymers capable of in-situ gelation include Poloxamer, Pluronics, various copolymers such as PEO-PLLA and PEG-PLGA-PEG, Cellulose acetophalate latex, Pectin, Gelrite, Gellan gum, Alginate, Carbopol, Chitin and Matrigel. The gel formation is induced by temperature change (Poloxamer, Pluronics, PEO-PLLA diblock copolymer, PEG-PLGA-PEG triblock copolymer, and Matrigel), pH change (Cellulose acetophalate latex and Carbopol), or reaction with mono- or divalent cations (Gelrite). Some of the most important polymers used as in-situ gelling agents are describes as follows.

NATURAL POLYMERS AND DERIVATIVES

Many natural polymers have been shown to exhibit gelation upon temperature change. Researchers have used them alone or in combination with synthetic polymers to fabricate thermally responsive hydrogels with desired properties.

POLYSACCHARIDES

CELLULOSE DERIVATIVES

Thermoreversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic

examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation.[12,13] Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10%wt), their aqueous solutions are liquid at low temperature, but gel upon heating. Methylcellulose (Fig. 1A) and hydroxypropyl methylcellulose (HPMC) (Fig. 1B) are typical examples of such polymers. Methylcellulose solutions transform into opaque gels between 40 and 50°C.[14,15], whereas HPMC shows phase transition between 75 and 90°C.[15] These phase transition temperatures can be lowered by chemical or physical modifications. For example, NaCl decreases the transition temperature of methylcellulose solutions to 32–34°C. Similarly, by reducing the hydroxypropyl molar substitution of HPMC, its transition temperature can be lowered to 40°C.

Gelation of methylcellulose or HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated, and there is little polymer–polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity. Eventually, when sufficient but not complete dehydration of the polymer occurs, polymer–polymer associations take place, and the system approaches an infinite network structure, as reflected experimentally by a sharp rise in relative viscosity.[14]

This sol–gel transformation has been exploited to design in situ gelling systems. Tate et al.[16] evaluated methylcellulose-based constructs as potential tissue engineering scaffolds for the repair of brain defects. These systems exhibited low viscosity at 23°C and formed soft gels intracerebrally at 37°C. The gels were biocompatible both in the presence of cultured cells and in the injured rat brain.

Aqueous solutions of ethyl (hydroxyethyl) cellulose (EHEC) (Fig. 1C) also exhibit thermosensitive behavior. However, their viscosity decreases with temperature, which is not appropriate for the preparation of in situ-forming implants. At the end of the 1980s, Carlsson et al.[17,18] reported that the addition of an ionic surfactant, like sodium dodecyl sulphate or cetyl triammonium bromide, to semi dilute (1–4 wt %) EHEC solutions completely changed their thermal behavior. These systems underwent sol–gel phase transition upon heating from room temperature to 30–40°C, resulting in the formation of stiff and clear gels. The rheological properties of such gels were further investigated by Nystrom et al.[19]. The surfactant was found to interact with EHEC by a strongly cooperative process implying the formation of micelle-like surfactant clusters on the polymer.[18,20,21] Binding increased with rising temperature, and gelation was attributed to the ability of micelle-like clusters on the polymer chain to couple with segments on other chains.

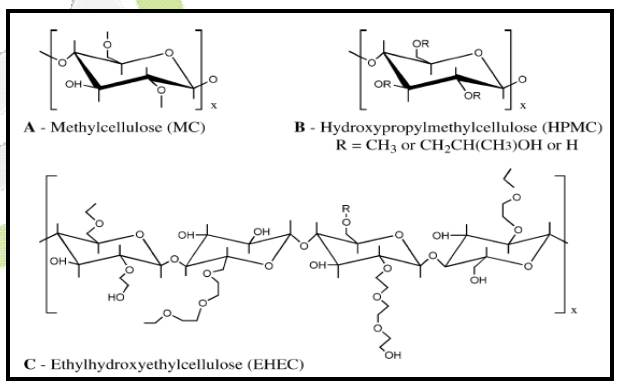


Figure: 1 Chemical structure of cellulose derivatives (A) Methylcellulose (B) Hydroxypropylmethylcellulose (C) Ethylhydroxyethylcellulose

Scherlund et al.[21] evaluated the EHEC/surfactant system for the local delivery of anesthetic agents to the periodontal pocket. They incorporated small amounts of lidocaine and prilocaine into the solution without affecting gelation behavior. The tested formulations showed sustained drug release over a minimum of 60min, making them interesting for short-term pain control. From a toxicological point of view, the need for

inclusion of an ionic surfactant in such a formulation may impair its clinical development.

XYLOGLUCAN

Xyloglucan is a cytocompatible polysaccharide and has exhibited thermally responsive behavior when more than 35% of its galactose residues are removed. Xyloglucan (Fig. 2), a polysaccharide derived from tamarind seed, forms thermoresponsive gels in water, under certain conditions. Xyloglucan is composed of a (1-4)-β-D-glucan backbone chain (GLU) which presents (1-6)-α-D-xylose branches (XYL) partially substituted by (1-2)-β-D-galactoxylose (GAL). Tamarind seed xyloglucan is composed of three units of xyloglucan oligomers with heptasaccharide, octosaccharide and nanosaccharide, which differ in the number of galactose side chains. When xyloglucan is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions. Such behavior does not occur with native xyloglucan.[22]

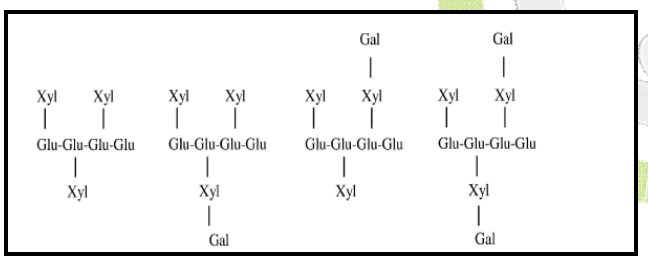


Figure: 2 Units structure of Xyloglucan

Xyloglucan gels have been used as a drug delivery vehicle for various applications due to non toxicity and lower gelation temperature.[23] However, there are not many data on the rheological and morphological characteristics of these hydrogels. Nisbet et al.[24] have examined the gelation properties of xyloglucan hydrogels as well as their morphology under physiological conditions. The gelation process seemed to be influenced by the presence of ions in PBS as compared to deionized water. As to the optimum concentration, it was found that 3%wt xyloglucan in aqueous media possesses an elastic modulus that is significantly higher than other natural or synthetic hydrogels. Moreover, this concentration yielded a gel that could be freeze-dried and examined with scanning electron microscopy. The images showed a macroporous, interconnected, three-dimensional network.

Xyloglucan gels have been evaluated for the rectal delivery of indomethacin in rabbits.[25] They provided a broader absorption peak and longer residence time than commercial suppositories. Moreover, morphological studies of rectal mucosa after a single administration showed no evidence of tissue damage. Intraperitoneal administration of mitomycin C in a 1.5%wt xyloglucan gel to rats resulted in a broad concentration–time profile, as opposed to a narrow peak and rapid disappearance from the peritoneal fluid and plasma when the drug was given as a solution.[26] In two other studies, the gels were investigated as vehicles for the oral delivery of indomethacin[27] and theophylline.[28] The bioavailability of indomethacin from xyloglucan gels was increased approximately three-fold compared to the control suspension. Likewise, theophylline bioavailability was 1.7–2.5 times higher than that of the commercially available oral, sustained-release liquid dosage form. Xyloglucan formulations were also assessed for ocular delivery of pilocarpine, using poloxamer 407 as a positive thermosensitive control.[29] The 1.5%wt xyloglucan formulation enhanced the miotic response to a degree similar to that of a 25%wt poloxamer 407 gel.

As for cellulose derivatives, xyloglucan solutions gel at low concentrations (1–2%wt), and this may be advantageous from a toxicological viewpoint as the amount of administered polymer is low. In addition, xyloglucan is approved for use as a food additive. However, its relatively low transition temperature (22–27°C) makes handling at room temperature problematic. The gelation behavior of xyloglucan is similar to that observed with Pluronic F127, with a sol-gel transition on heating from refrigerator temperature or cooling from a higher temperature.[30] But the important difference between the gelation properties of the xyloglucan and Pluronic F127 from a

formulation view point is that xyloglucan forms gels at much lower concentration.[31]

DEXTRAN

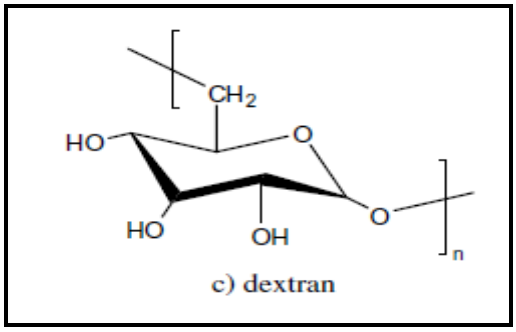


Figure: 3. Chemical formula of Dextran

A modified precursor of the enzymatically biodegradable dextran (Dex) was formed by reaction with maleic anhydride (MA) and the Dex-MA polysaccharide was given thermoresponsive properties by photocrosslinking it with NiPAAm. The resulting hydrogel was partially biodegradable and exhibited a higher (Lower Critical Solution Temperature) LCST than pNiPAAm due to the hydrophilic and biodegradable nature of Dex-MA.

Additionally, the carboxylic end groups of Dex-MA render the hydrogel pH sensitive.[32] Another approach based on a dextran polysaccharide was reported by Huang et al.[33], A dextran macromer containing oligolactate and 2- hydroxyethyl methacrylate units (Dex-lactate-HEMA), which has hydrolytically degradable blocks, was copolymerized with NiPAAm. This hydrogel showed an LCST close to that of pNiPAAm (approximately 32^{o}C). Its swelling and degradation in phosphate buffered saline (PBS) were studied at 25 and 37^{o}C. At 25^{o}C, which is below the LCST, the hydrogels had disintegrated within 2 weeks, with the rate of dissolution depending on their composition. At 37ºC, the degradation was much slower due to increased hydrophobic effects. Interestingly, when the hydrogel was tested for drug delivery, it was shown that a low molecular weight drug (methylene blue) was released slower at 25ºC than at 37ºC, whereas the opposite was observed for a high molecular weight substance (Bovine Serum Albumin – BSA). The authors concluded that the drug release profile depends on a number of factors, as the temperature, the swelling and degradation characteristics of the hydrogel, as well as the interactions of the drug and the hydrogel macromolecules.

GELLAN GUM

Gellan gum (commercially available) as linear, anionic deacetylated exocellular polysaccharide secreted by the microbe Sphingomonas paucimobilis (formerly known as Pseudomonas elodea) with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucose. It has the characteristic property of temperature dependent and cation-induced gelation involving 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 acetylated form is marketed as Gelrite w or Kelcogel w (Kelco division of Merck and Co, USA), deacetylated gellan gum is approved in the USA and EU as a gelling, stabilizing and suspending agent in food products. The sol-gel transition process is induced by the presence of monovalent or divalent ions such as Na^{+} and Ca^{2+}. Some other parameters influence the phase transition e.g. the concentration of polysaccharide, the temperature of the preparation, and the nature and the concentration of cations.

It was determined that divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gellation of the polysaccharide. Because of its ability to form strong clear gels at physiological ion concentration, deacetylated gellan gum has been widely investigated for use as an in situ gelling agent in ocular formulations. It has been reported to provide a significantly prolonged corneal contact time in comparison with conventional solutions and is currently marketed in the controlled-release timolol formulation Blocadren Depot (Timoptic-XEw). It has also been suggested that gellan gum is a promising polymer for use in nasal formulations.

To our knowledge, however, it has only been included in one study on this subject where it was shown to moderately enhance the local and serum antibody response in mice after nasal administration of viral antigens. Other in situ gelling systems, such as temperature and pH responsive gels, have, on the other hand, appeared more frequently in nasal drug delivery studies and have been shown to increase the residence time and improve drug absorption. Much of the interest in the pharmaceutical application of this material has concentrated on its application in ophthalmic drug delivery; aqueous solutions of gellan dropped into the eye undergo transition to the gel state due to the temperature and ionic conditions in the tear fluid. However the concentration of sodium chloride (2-6 g/l) is quite sufficient to induce the gelation, as the presence of lachrymal fluid is required to induce gel formation, accidental gelation during storage does not occur as with thermo reversible gels.

Efficacy of Gellan gum has been evaluated by measuring pharmacokinetics parameters and pharmacological response. Increased ocular bioavailability of timolol maleate was observed when gelrite formulations were incorporated when compared to commercially available timpotics solution. This result was confirmed by Vogel et al., who observed a twofold decrease of the intraocular pressure of patients after administration of gelrite containing timolol. The feasibility of using gellan formulations for the oral sustained delivery of theophylline is reported. The formulation was a gellan solution containing calcium chloride (as a source of Ca^{2+} ions) and sodium citrate, which complexed the free Ca^{2+} ions and released them only in the highly acidic environment of the stomach. In this way the formulation remained in liquid form until it reached the stomach, when gelation was instantaneous. The in situ gelling properties of deacetylated gellan gum are attributed to its responsiveness to cations. In an ion-free aqueous medium, the polymer chains form double helices, resulting in a fluid that has a viscosity close to that of water. In the presence of gel-promoting cations (Na^+, K^+, Ca^{2+}), a portion of the helices associates and the cation-mediated aggregates cross-link the gel network. A rapid gelling can be expected upon contact with the mucosa since, even at low polymer concentrations, small quantities of ions sufficient for the formation of a strong gel, gellan gum which having the properties like to up take the water and it having good swelling properties and due to this it gives good bioadhesive with the GIT.[9]

SODIUM ALGINATE

Alginic acid is a linear block copolymer polysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,4-glycosidic linkage. 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 the addition of di- and tri-valent metal ions by a co-operative process involving consecutive guluronic residues in the G blocks of the alginate chain. This property has been widely exploited for the fabrication of vehicles for the sustained delivery of bioactive molecules, usually as matrix devices. It consists chiefly of sodium salt of Alginic acid; a polyuronic acid composed of β- D- mannuronic acid carboxyl group of each unit is free while the aldehyde group is shielded by a glycosidic linkage.

There have been only few reports on the use of alginates in liquid sustained release preparations for oral administration. Zatz and Woodford developed a suspension formulation of theophylline which contained sodium alginate and which formed a gel when in contact with simulated gastric fluid. Another author reported a liquid sustained release formulation containing sodium alginate intended for the eradication of Helicobacter pylori in which in situ gelling was achieved by the separate oral administration of a calcium salt solution immediately following that of the sodium alginate solution. An alternative strategy to achieve in situ gelation of sodium alginate solutions, which was similar to that described above for the in situ gelation of gellan, has been reported. In this method

gelation of a solution of sodium alginate containing Ca^{2+} ions is delayed until the preparation reaches the acidic environment of the stomach through complexation of the Ca^{2+} ions with sodium citrate. It should be noted that although the commercial preparations cited above contain sodium alginate, they do not include a source of metal ions. It is not, of course, the intention with these commercial preparations that the alginate should form a gel matrix in the stomach as in the formulations discussed, but rather should form a raft on the surface so reducing acid regurgitation.

Use of alginate in a formulation for the oral delivery of cimetidine is reported and has determined optimum amounts of calcium chloride and sodium citrate for effective gelation. The in situ gelling compound examined, sodium alginate, is widely used in pharmaceutical formulation. Gelation of dilute solutions of sodium alginate occurs on addition of di- and trivalent metal ions by a co-operative process involving consecutive guluronic residues in the α -l-guluronic acid (G) blocks of the alginate chain. When formulated for use in sustained drug delivery, the alginate is usually in the form of a matrix, and there have been only a few studies on the use of alginates in liquid sustained release preparations for oral administration. The formulation described by Zatz and Woodford gelled when in contact with simulated gastric fluid; gelation of that reported by Katayama was achieved by the separate oral administration of a solution of a calcium salt immediately following that of the sodium alginate solution.[9]

POLOXAMER

The poloxamers (Fig. 4) consist of more than 30 different non-ionic surface-active agents.

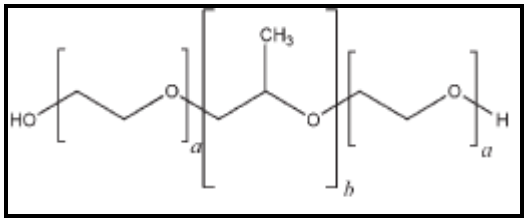


Figure: 4 Chemical Structure of Poloxamer

These polymers are ABA-type triblock copolymers composed of PEO (A) and PPO units (B). The poloxamer series covers a range of liquids, pastes, and solids, with molecular weights and ethylene oxide-propylene oxide weight ratios varying from 1100 to 14,000 and 1:9 to 8:2, respectively. Concentrated aqueous solutions of poloxamer form thermoreversible gels.

The gelation mechanism of poloxamer solutions has been investigated extensively, but is still being debated. Ultrasonic velocity, light-scattering and small-angle neutron scattering measurements of aqueous poloxamer solutions have clearly indicated a micellar mode of association.[34–39] Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration.[36,40] With increasing temperature, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as cubic crystalline phase, has been proposed as the driving force for gel formation[37-39], but this hypothesis has been questioned recently. Thus, packing of micelles and micelle entanglements may be possible mechanisms of poloxamer solution gelation with increased of temperature.[41]

Poloxamer 407 (Pluronics F127) was found to gel at a concentration of 20 %wt at 25°C, which is less than that of the other members of the poloxamer series. At room temperature (25°C), the solution behaves as a mobile viscous liquid, which is transformed into a semi-solid transparent gel at body temperature (37°C). Preliminary toxicity data indicate that this copolymer is well tolerated.[42] Taken together, these results have prompted the use of poloxamer 407 in the design of medical, pharmaceutical, and cosmetic systems. Early studies evaluated poloxamer 407 thermosensitive solutions for the treatment of burns[42], topical administration of anticancer agents[43], and sustained delivery of drugs after extravascular parenteral injection[44]. Poloxamer gels interesting for short-term therapies like infection treatment[45,46], pain management[47], and

fertility control[48]. Besides injectables, other administration routes have been evaluated, such as rectal[49,50], vaginal[51,52], transdermal[52,53] and ophthalmic[54,55]. Poloxamer formulations generally increased drug residence time at application sites, resulting in improved bioavailability and efficacy.

Potential drawbacks of poloxamer gels include their weak mechanical strength, rapid erosion (i.e. dissolution from the surface), and the non-biodegradability of PEO-PPO-PEO, which prevents the use of high molecular weight polymers that cannot be eliminated by renal excretion. To circumvent the biodegradability issue, new polymers were synthesized by linking together a few (usually 3) poloxamer 407 'monomers' via degradable carbonate linkage.[56] As the carbonate linkages were hydrolyzed under physiological conditions, the hydrogel degraded into soluble poloxamer 407 units and carbonate.

PECTIN

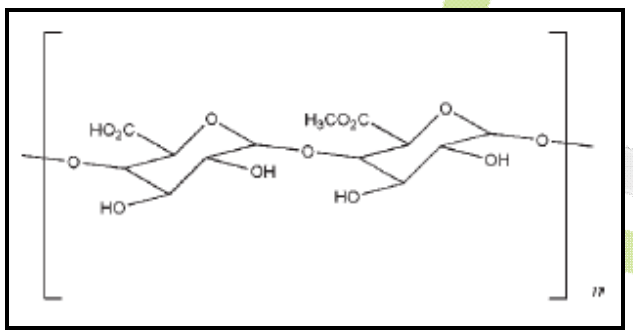


Figure: 5 Chemical Structure of Pectin

Pectin is a biodegradable acidic carbohydrate polymer. Pectin is commonly found in plant cell walls. The cell wall of a plant is divided into three layers consisting of the middle lamella, the primary wall and the secondary cell wall. The middle lamella is richest in pectin. Pectin's 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 %) such as those used in the study readily formed gels in aqueous solution in the presence of free calcium ions, which cross-linked the galacturonic acid chains in a manner described by the 'egg-box' model. In another study, the potential for the sustained delivery of ambroxol of a liquid formulation comprising a dilute aqueous solution of pectin that is designed to form gels in situ in the acidic environment of the stomach was examined. The procedure by which gelation is achieved is similar to that described previously in the design of in situ-gelling formulations of the polysaccharides gellan and sodium alginate, aqueous solutions of which also readily form gels in the presence of Ca^{2+} ions. Reproducible gelation of these polysaccharides is ensured by including a source of Ca^{2+} ions in the formulation, but gelation is delayed until the administered solution reaches the stomach by complexing the calcium with sodium citrate. Here the acidic environment causes breakdown of the complex, releasing free Ca^{2+} ions and causing instantaneous gelation[9].

CHITOSAN

Chitosan is produced with the deacetylation of chitin, which can be found in the outer skeleton of shrimp and insects, among others.

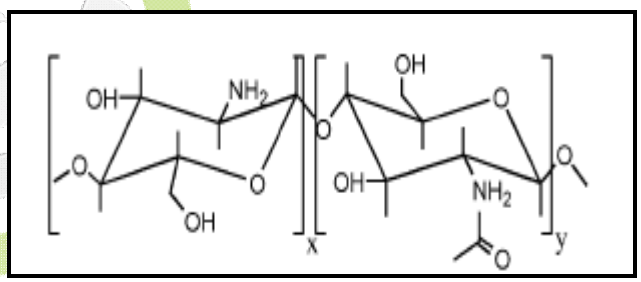


Figure: 6 Chemical Structure of Chitosan

It is a biocompatible, pH-dependent cationic polymer, which is soluble in water up to pH 6.2. Basification of chitosan aqueous solutions above this pH leads to the formation of a hydrated gel-like precipitate. Phase separation occurs from the neutralization of chitosan amine groups and the consequent elimination of repulsive inter chain electrostatic forces, which subsequently allow for extensive hydrogen bonding and hydrophobic interactions between chains. pH - gelling, cationic chitosan solutions have been transformed into thermally sensitive, pH dependent, gel-forming systems by the addition of polyol salts (e.g. β-glycerophosphate, GP). These formulations possess a neutral pH, remain liquid at or below

room temperature, and form monolithic gels at body temperature. The stability of the sol at room temperature and gelation time increase as the chitosan degree of deacetylation decreases. Like other polysaccharide systems, the gels are obtained at low polymer concentrations (~2 %wt).

Solubility at low temperatures is probably due to hydration of the chitosan promoted by GP. Upon heating, the chitosan chains lose their water of hydration, bonding between chains can occur and gelation proceeds. Three types of interactions may be involved in the gelation process: (1) electrostatic attraction between the ammonium groups of chitosan and the phosphate group of GP; (2) hydrogen bonding between polymer chains as a consequence of reduced electrostatic repulsion after neutralization of the chitosan solution with GP; and (3) chitosan–chitosan hydrophobic interactions.[57,58]

Bhattarai et al.[59] incorporated poly(ethylene glycol) (PEG) into chitosan and were able to form a thermoreversible hydrogel with no additional crosslinking agents. Moreover, PEG grafting improved the solubility of chitosan in water, and the gelation was found to be possible in physiological pH values. Chitosan-based hydrogels have been also investigated as potential cell carriers for tissue engineering applications. A copolymer of NiPAAm and water-soluble chitosan was tested for chondrogenic differentiation of human mesenchymal stem cells (hMSC). The hydrogel showed a stable gelation at 37°C and differentiation of hMSC into chondrocytes was observed, both *in vitro* and *in vivo*. This hydrogel could be used for a minimally invasive treatment of vesicouretral reflux with an endoscopic procedure through a single injection.[60]

A chitosan-glycerophosphate salt (GP) hydrogel was recently tested for its potential in neural tissue engineering. This thermally responsive hydrogel, as developed initially by Chenite et al.[61], has been shown to have good biocompatibility *in vitro*, but it had not been yet tested with nerve cells. Crompton et al.[62] observed that polylysine-chitosan-GP may be a good candidate for neural tissue engineering.

GELATIN

Gelatin is another biopolymer with thermoreversible properties. At temperatures below 25°C, an aqueous gelatin solution solidifies due to the formation of triple helices and a rigid three-dimensional network. When the temperature is raised above approximately 30°C, conformation changes from a helix to the more flexible coil, rendering the gel liquid again.[63] As the opposite thermal behavior is desired for biomedical applications, researchers have combined gelatin with other polymers, which show thermal gelation close to body temperature. Gelatin has the advantage of allowing for easy modification on the amino acid level; moreover, it is biodegradable and biocompatible.[64]

Another protein-based hydrogel was proposed by Gil et al.[65]. Gelatin was blended with silk fibroin to yield a thermoresponsive gel, which was stabilized at 37°C by the presence of b crystals of silk fibroin. The swelling profile at temperatures below and above the helix-to-coil transformation of gelatin was evaluated, as well as the protein release from the matrices. The gel showed a higher swelling at physiological temperatures as compared to 20°C, but also higher mass loss due to dissolution and release of gelatin.

SYNTHETIC POLYMERS

N-ISOPROPYLACRYLAMIDE COPOLYMERS

Poly (N-isopropylacrylamide) (pNiPAAm) (Fig. 7) is a non-biodegradable polymer with a LCST, 32ºC in water[66], and cross-linked gels of this material collapse around this temperature.[67,68] Hydrogels based on poly(N-isopropylacrylamide) (pNiPAAm) and its copolymers belong to the most intensively investigated thermoreversible systems. Recent developments on pNiPAAm based hydrogels include their use for drug delivery[69-72] cell encapsulation and delivery[73,74]

and cell culture surfaces.[75] The pNiPAAm LCST can be controlled by copolymerization with other monomers. The addition of hydrophilic monomers typically increases the LCST whereas the incorporation of more hydrophobic units has the opposite effect.[76] Below the LCST, pNiPAAm assumes a flexible, extended coil conformation in aqueous solutions. At the LCST, it becomes hydrophobic and the polymer chains seem to collapse prior to aggregation in globular structures.[77,78] Copolymerization of NiPAAm with a more hydrophilic monomer increases the overall hydrophilicity of the polymer, and the stronger polymer-water interactions lead to an increase in the LCST. Likewise, copolymerization with a more hydrophobic monomer results in a lower LCST than pNiPAAm.[79] Moreover, the phase transition temperature is influenced by the presence of salts[80] and pH to a certain extent.[81] It was found that an aqueous solution of high molecular weight NiPAAM/acrylic acid (2-5 %mol) copolymer synthesized in benzene showed reversible gelation above a critical concentration (4 %wt), without noticeable hysteresis around 32ºC, rather than polymer precipitation.[82] The polymers were characterized as having a distribution of polymer composition. A new family of polymers that self-assemble to form gels in a thermoreversible fashion has been proposed recently by Lin and Cheng.[83] It consists of block linear and star copolymers with a central hydrophilic PEO segment and temperature-responsive PNIPAM terminal segments. Linear and star copolymers of PEO and PNIPAM form liquid aqueous solutions at room temperature that transform to relatively strong elastic gels upon heating. Multiple-arm copolymers yield gels via physical cross-links between aggregates of PNIPAM segments, whereas diblock copolymers gel by a micellar aggregation mechanism. More recently, diblock and star-shaped block copolymers AB, A(B)2, A(B)4, and A(B)8, where A is the central hydrophilic star-shaped PEG block (molecular weight (MW) per arm 2000-2460) and B is the temperature-responsive NiPAAM oligomer block (MW 1900-2400), have been synthesized. These were reported to form a somewhat viscoelastic gel upon heating (gelation temperature 26-33ºC) when the typical polymer concentration was 20 %wt, and the resulting gels showed no syneresis[83]. This process was reversible without hysteresis. Based on differential scanning calorimetry (DSC) and dynamic mechanical analysis, the gelation mechanism was observed to be micellar aggregation for the AB diblock copolymer. It was found to be a strong associative network formation for the other polymer architectures via hydrophobic interaction of collapsed NiPAAM oligomer blocks. The polymer architecture influenced the resulting gel strengths and A(B)4 showed the highest gel strength of 860 Pa yield stress.

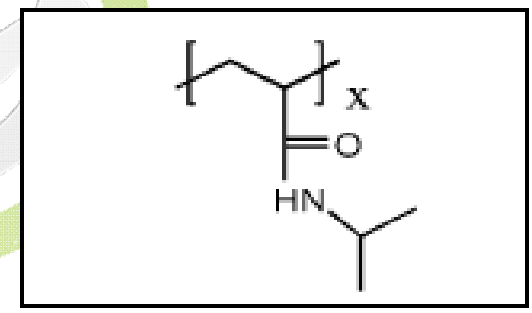


Figure: 7 Chemical Structure of Poly (N-isopropylacrylamide)

PEG/PLGA BLOCK COPOLYMERS

A novel concept, which combines thermogelation, biodegradability, and no toxicity, has been proposed for an injectable gel system with better safety and longer gel duration.[84] Poly(ethylene glycol-b-lactic acid-b-ethylene glycol) (PEG-PLLA-PEG) was synthesized by ring-opening polymerization of L-lactide onto monomethoxy poly(ethylene glycol) (MW 5000), which produced PEG-PLLA diblock copolymers, followed by coupling of the resulting diblock copolymers with hexamethylene diisocyanate to produce

triblock copolymers with a PLLA central block (MW 2000-5000). The copolymers only exhibited a single sol-to-gel transition with decreasing temperature in water, like a gelatin solution. In the gelation phase diagram of the triblock copolymer, the gelation concentration (10-30 %wt) and temperature (20-60ºC) were influenced by the length of the middle block when the terminal PEG block was kept constant. When FITC-labelled dextran (MW 20,000) was mixed with an aqueous solution of the 5000-2040-5000 triblock copolymer above the critical gelation temperature at a given polymer concentration, and the mixture was gelled by cooling to body temperature.

The use of high molecular weight-PLGA combined with low molecular weight-PEG resulted in a hydrogel with quick gelation at physiological temperature. The combination of hydrophobic/hydrophilic units created a surfactant behavior of the polymers in water, thus facilitating also the solubilization of hydrophobic drugs. *In vivo* studies showed sufficient mechanical properties and integrity for longer than a month.[85] Another recent approach towards a thermoresponsive system involved the synthesis of a multiblock copolymer with biodegradable polyester. Alternating multiblock poly(ethylene glycol)/poly(L-lactic acid) (PEG/PLLA) copolymers were produced. It was shown that sol-to-gel transition was depending on both the total molecular weight (MW) and the MW of each building block. *In vitro* and *in vivo* gelation studies determined that a copolymer with a total MW of 6700 Da and 600/1300 (MW of PEG/PLLA blocks, respectively) holds potential as an injectable carrier for biomedical applications in terms of transition temperature and modulus at 37ºC (Fig. 8).[86]

Changing the polymer composition further, particularly the middle block composition, the block length, and the block ratio, produced the next generation of poly(ethylene glycol-b-L-lactic acid-co- glycolide-b-ethylene glycol) (PEG-PLGA-PEG) tri-block copolymers (Fig. 8).

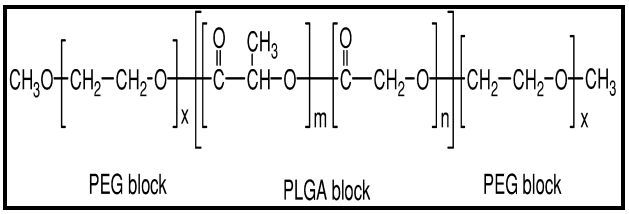


Figure: 8 Chemical structure of a PEG-PLGA-PEG triblock copolymer.

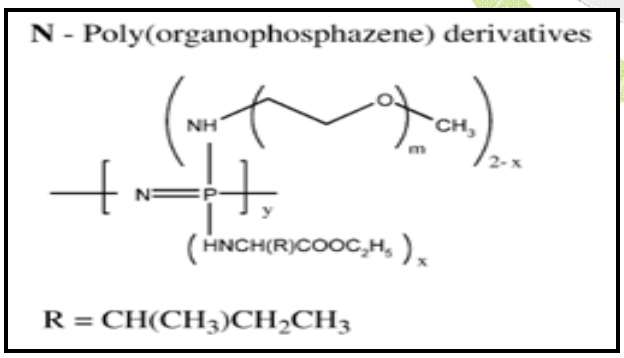
The aqueous polymer solution is a free-flowing sol at room temperature and becomes a gel at body temperature.[87] The mechanism of the sol-to-gel transition (lower transition) of an aqueous solution of a PEG-PLGA-PEG triblock copolymer is believed to be a micellar expansion accompanying an increase in aggregation number driven by hydrophobic forces.[88,89] From SLS and DLS studies on PEG-PLGA-PEG triblock copolymer aqueous solutions, two important facts were noted.[90] First, water becomes less favorable for the PEG-PLGA-PEG triblock copolymers, as reflected in the second viral coefficients, and such a trend is rather abrupt at about 30ºC, which is the sol-to-gel transition temperature at high concentration. A decrease in the second viral co-efficient indicates that the polymer-polymer attraction increases relative to polymer-solvent interactions. Second, the micelles grow by an increase in aggregation number as well as the diameter of the micelle. The growth of the micelles also occurred abruptly around 30ºC. Therefore, micellar growth and an increase in polymer-polymer attraction may drive the sol-to-gel transition at high concentrations (above CGC) with increasing temperature.

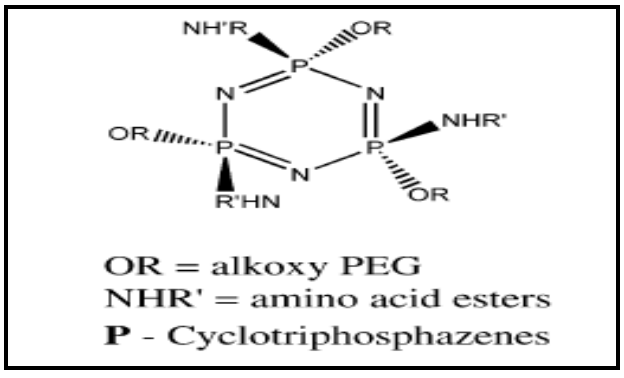
POLY (ORGANOPHOSPHAZENE) DERIVATIVES

Current advances on poly(organophosphazenes) (PPZ) include their use as drug[91,92] and cell[93] delivery systems. Poly(organophosphazenes) grafted with mPEG and amino acid esters were reported as a new class of biodegradable and thermosensitive polymers in 1999.[94] PPZ derivatives were shown to exhibit sol-gel phase transitions as a function of temperature. Sohn and colleagues[95] developed a correlation for the

LCST of these polymers as a function of their molecular structure, which comprises hydrophilic (PEG) and hydrophobic (amino acid esters) side groups. The polymers showed a sustained release profile for both hydrophobic[91] as well as hydrophilic[92] drugs for over 3 and 2 weeks, respectively. PPZ bearing methoxy-PEO and amino acid esters as substituents were synthesized by Song et al. (Fig. 9).[96,97] The polymers were hydrolytically degrade-able and displayed a LSCT in the 25.2-98.5°C range. The same group demonstrated that oligomeric cyclophosphazenes with proper orientation of substituents were also thermosensitive (Fig. 9).[98] PPZ bearing α-amino-ω-methoxy-PEO and hydrophobic L-isoleucine ethyl ester as side groups exhibited reversible sol-gel transition between 29 and 61°C depending on the structure (Fig. 9).[99]

Gelation was attributed to hydrophobic interactions between the side-chain fragments (-CH(CH3)-CH2CH3) of L-isoleucine ethyl ester. Also their use as cell carriers holds promise, as shown recently. Hepatocytes cultured in PPZ hydrogels were able to maintain good viability and liver-specific activity for a period of 4 weeks.[93]





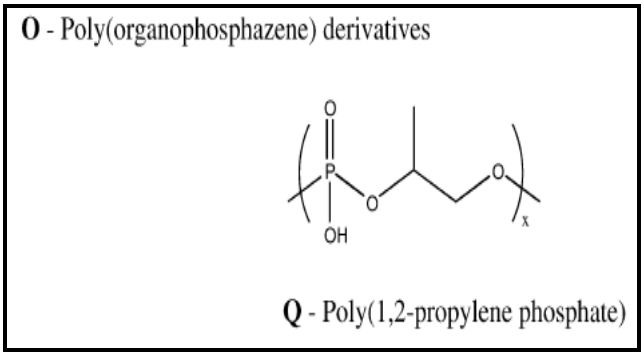


Figure: 9 Poly(Organophosphazene) derivatives

CONCLUSION

The choice of a particular hydrogel depends on its intrinsic properties and envisaged therapeutic use. For instance, the formation of a transparent gel is particularly important when ophthalmic applications are considered. Non-biodegradable gels could prove useful for administration routes other than parenteral. Poloxamer hydrogels perhaps represent the most extensively studied systems. However, despite the clinical acceptance of poloxamers as solubilizer and thickening agents, these polymers have not met initial expectations as biomedical implants, mainly due to their non-biodegradability and inability to provide sustained drug delivery over more than just a few days. Polysaccharides usually demonstrate good biocompatibility and/or biodegradability, and their solutions are thermosensitive at low polymeric concentrations. However, these systems may not be adapted for the sustained release of hydrophilic, low molecular weight drugs because their large, water-filled pores permit rapid diffusion. On the other hand, they provide adequate scaffolds for cell growth and tissue repair. Poly(N-isopropylacrylamide) and its copolymers with other synthetic or natural polymers is one of the most investigated thermoresponsive systems. By the appropriate copolymerization, intrinsic drawbacks of pNiPAAm like its non-biodegradability and mechanical properties may be improved. PEO/PLGA hydrogels are particularly attractive systems for pharmaceutical applications. They are biodegradable and generally have a good safety profile. Their composition can be tailored

to provide drug delivery over weeks or months after parenteral extravascular administration. More recently, in-situ gels exhibiting a thermosensitive sol–gel behavior have been reported as cell carriers for tissue regeneration.

REFERENCES

1. Francis MF, Cristea M, Winnik FM, "Polymeric micelles for oral drug delivery: Why and how", Pure Applied Chemistry, 2004, 76(7-8), 1321– 1335.
2. Amidon GL, Lennernas H, Shah VP, Crison JR, "A theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability", Pharmaceutical Research, 1995, 12(3), 413–420.
3. Lipinski C, "Poor aqueous solubility – an industry wide problem in drug delivery", American Pharma Review, 2002, 5, 82–85.
4. Hasirci V, Vrana E, Zorlutuna P, Ndreu A, Yilgor P, Basmanay FB, Aydin E, "Nanobiomaterials: a review of the existing science and technology, and new approaches", Journal of Biomaterial Science and Polymer, 2006, 17(11), 1241–1268.
5. Hu J, Johnston KP, Williams III RO, "Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs, Drug Delivery". Indian Pharmacy, 2004, 30(3), 233–245.
6. Overhoffa KA, Engstromb JD, Chenc B, Scherzerd BD, Milnerc TE, Johnston KP, Williams III RO, "Novel ultra-rapid freezing particle engineering process for enhancement of dissolution rates of poorly water-soluble drugs", European Journal of Pharmacy and Biopharmaceutics, 2007, 65(1), 57–67.
7. Muller RH, Jacobs C, Kayser O, "Nanosuspensions as particulate drug formulations in therapy rational for drug development and what we can expect for the future", Advanced Drug Delivery Review, 2001, 47(1), 3–19.
8. Kipp JE, "The role of nanoparticle technology in the parenteral delivery of poorly water-soluble drugs", International Journal of Pharmacy, 2004, 284(1-2), 109–122.
9. Saraswat R, Bhan CS, Gaur A, "A review on polymers used in in-situ gel drug delivery systems", International Journal of Pharmaceutical Innovations, 2011, 1(2), 111-118.
10. Guenet JM, "Thermoreversible Gelation of Polymers and Biopolymers", Academic Press, London, 1992, 287.
11. Finch CA, "Chemistry and technology of water-soluble polymers", Plenum Press, New York, 1983, 118.
12. Harrington WF, Von Hippel PH, "The structure of collagen and gelatin", Advances in Protein Chemistry, 1961, 16, 1–138.
13. Anderson NS, Campbell JW, Harding MM, Rees DA, Samuel JW, "X-ray diffraction studies of polysaccharide sulphates: double helix models for k- and i-carrageenans", Journal of Molecular Biology, 1969, 45(1), 85–99.
14. Heymann E, "Studies on sol–gel transformations, I: The inverse sol– gel transformation of methylcellulose in water", Transaction of Faraday Society, 1935, 31, 846–864.
15. Sarkar N, "Thermal gelation properties of methyl and hydroxypropyl methylcellulose", Journal of Applied Polymer Science, 1979, 24(4), 1073–1087.
16. Tate MC, Shear DA, Hoffman SW, Stein DG, LaPlaca MC, "Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury", Biomaterials, 2001, 22(10), 1113–1123.
17. Carlsson A, Karlstrom G, Lindman B, Stenberg O, "Interaction between ethyl (hydroxyethyl) cellulose and sodium dodecyl sulphate in aqueous solution", Colloid Polymer Science, 1988, 266(11), 1031–1036.
18. Carlsson A, Karlstrom G, Lindman B, "Thermal gelation of nonionic cellulose

ethers and ionic surfactants in water", Colloids and Surfaces, 1990, 47, 147–165.

19. Nystrom B, Walderhaug H, Hansen FK, "Rheological behavior during thermoreversible gelation of aqueous mixtures of ethyl(hydroxyethyl) cellulose and surfactants", Langmuir, 1995, 11(3), 750–757.
20. Carlsson A, Lindman B, Watanabe T, Shirahama K, "Polymersurfactant interactions. Binding of N-tetradecylpyridinium bromide to ethyl (hydroxyethyl) cellulose)", Langmuir, 1989, 5, 1250–1252.
21. Lindman B, Carlsson A, Karlstrom G, Malmsten M, "Nonionic polymers and surfactants—some anomalies in temperature dependence and in interactions with ionic surfactants", Advances in Colloid and Interface Science, 1990, 32(2-3), 183–203.
22. Shirakawa M, Yamotoya K, Nishinari K, "Tailoring of xyloglucan properties using an enzyme", Food Hydrocolloid, 1998, 12(1), 25–28.
23. Ruel-Gariepy E, Leroux JC, "In situ forming hydrogels-review of temperature-sensitive systems", European Journal of Pharmacy and Biopharmaceutics, 2004, 58(2), 409–426.
24. Nisbet DR, Crompton KE, Hamilton SD, Shirakawa S, Prankerd RJ, Finkelstein DI, Horne MK, Forsythe JS, "Morphology and gelation of thermosensitive xyloglucan hydrogels", Biophysics Chemistry, 2006,121(1), 14–20.
25. Miyazaki S, Suisha F, Kawasaki N, Shirakawa M, Yamatoya K, Attwood D, "Thermally reversible xyloglucan gels as vehicles for rectal drug delivery", Journal of Controlled Release, 1998, 56(1-3), 75–83.
26. Suisha F, Kawasaki N, Miyazaki S, Shirakawa M, Yamatoya K, Sasaki M, Attwood D, "Xyloglucan gels as sustained release vehicles for the intraperitoneal administration of mitomycin C", International Journal of Pharmacy, 1998, 172(1-2), 27–32.
27. Kawasaki N, Ohkura R, Miyazaki S, Uno Y, Sugimoto S, Attwood D, "Thermally reversible xyloglucan gels as vehicles for oral drug delivery", International Journal of Pharmacy, 1999, 181(2), 227–234.
28. Miyazaki S, Kawasaki N, Endo K, Attwood D, "Oral sustained delivery of theophylline from thermally reversible xyloglucan gels in rabbits", Journal of Pharmacy and Pharmacology. 2001, 53(9), 1185–1191.
29. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D, "In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride", International Journal of Pharmacy, 2001 , 229(1-2), 29–36.
30. Bilensoy E, Rouf MA, Imran V, Murat S, Hincal AA. "Mucoadhesive thermosensitive prolonged release vaginal gel for clotrimazole: β-cyclodextrin complex". AAPS Pharma Science Technology, 2006, 7(2), 38.
31. Miyazaki S, Suisha F, Kawasaki N. "Thermally reversible xyloglucan gels as vehicles for rectal drug delivery". Journal of Controlled Relase, 1998, 56(1-3), 75-83.
32. Zhang X, Wu D, Chu CC, "Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex-MA/ pNIPAAm hydrogels", Biomaterials, 2004, 25(19), 4719–4730.
33. Huang X, Lowe TL, "Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs", Biomacromolecules, 2005, 6(4), 2131–2139.
34. Rassing J, Attwood D, "Ultrasonic velocity and light-scattering studies on the polyoxyethylene-polyoxypropylene copolymer Pluronic F127 in aqueous solution", International Journal of Pharmacy, 1982, 13(1), 47–55.

35. Zhou Z, Chu, B "Anomalous association behavior of an ethylene oxide/propylene oxide ABA block copolymer in water", Macromolecules, 1987, 20(12), 3089–3091.

36. Zhou Z, Chu B, "Light-scattering study on the association behavior of triblock polymers of ethylene oxide and propylene oxide in aqueous solution", Journal of Colloid Interface Science, 1988, 126(1), 171–180.

37. Wanka G, Hoffman H, Ulbricht W, "The aggregation behavior of poly-(oxyethylene)-poly-(oxypropylene)-poly-(oxyethylene)-block-copolymers in aqueous solution", Colloid Polymer Science, 1990, 268, 101–117.

38. Mortensen K, "Block copolymer in aqueous solution: Micelle formation and hard-sphere crystallization", Progr. Colloid Polymer Science, 1993, 93, 72–75.

39. Schille´n K, Glatter O, Brown W, "Characterization of a PEO-PPOPEO block copolymer system", Progress in Colloid Polymer Science, 1993, 93, 66–71.

40. Bohorquez M, Koch C, Trygstad T, Pandit N, "A study of the temperature-dependent micellization of pluronic F127", Journal of Colloid Interface Science, 1999, 216(1), 34–40.

41. Cabana A, Ait-Kadi A, Juhasz J, "Study of the gelation process of polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer (Poloxamer 407) aqueous solutions", Journal of Colloid Interface Science, 1997, 190(2), 307–312.

42. Schmolka IR, "Artificial skin: Preparation and properties of Pluronic F-127 gels for treatment of burns", Journal of Biomedical Material Research, 1972, 6(6), 571–582.

43. Miyazaki S, Takeuchi S, Yokouchi C, Takada M, "Pluronic F-127 gels as a vehicle for topical administration of anticancer agents", Chemical and Pharmaceutical Bulletin, 1984, 32(10), 4205–4208.

44. Johnston TP, Miller SC, "Inulin disposition following intramuscular administration of an inulin/poloxamer gel matrix", Journal of Parenteral Science Technology, 1989, 43(6), 279–286.

45. Veyries ML, Couarraze G, Geiger S, Agnely F, Massias L, Kunzli B, Faurisson F, Rouveix B, "Controlled release of vancomycin from poloxamer gels", International Journal of Pharmacy, 1999, 192(2), 183–193.

46. Zhang L, Parsons DL, Navarre C, Kompella UB, "Development and in-vitro evaluation of sustained release poloxamer 407 (P407) gel formulations of ceftiofur", Journal of Controlled Release, 2002, 85(1-3), 73–81.

47. Paavola A, Kilpelainen I, Yliruusi J, Rosenberg P, "Controlled release injectable liposomal gel of ibuprofen for epidural analgesia", International Journal of Pharmacy, 2000, 199(1), 85–93.

48. Wenzel JGW, Balaji KSS, Koushik K, Navarre C, Duran SH Rahe, CH, Kompella UB, "Pluronic F127 gel formulations of Deslorelin and GnRH reduce drug degradation and sustain drug release and effect in cattle", Journal of Controlled Release, 2002, 85(1-3), 51–59.

49. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK, "Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository", International Journal of Pharmacy, 1998, 165(1), 33–44.

50. Ryu JM, Chung SJ, Lee MH, Kim CK, Shim CK, "Increased bioavailability of propranolol in rats by retaining thermally gelling liquid suppositories in the rectum", Journal of Controlled Release, 1999, 59(2), 163–172.

51. Chang JY, Oh YK, Choi HG, Kim YB, Kim CK, "Rheological evaluation of thermosensitive and mucoadhesive vaginals gels in physiological conditions", International Journal of Pharmacy, 2002, 241(1), 155–163.

52. Chang JY, Oh YK, Kong HS, Kim EJ, Jang DD, Nam KT, Kim CK, "Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis", Journal of Controlled Release, 2002, 82(1), 39–50.

53. Shin SC, Cho CW, Oh IJ, "Enhanced efficacy by percutaneous absorption of piroxicam from the poloxamer gel in rats", International Journal of Pharmacy, 2000, 193(2), 213–218.

54. El-Kamel AH, '*In vitro* and *in vivo* evaluation of Pluronic F127- based ocular delivery system for timolol maleate", International Journal of Pharmacy, 2002, 241(1), 47–55.

55. Wei G, Xu H, Ding PT, Li SM, Zheng JM, "Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies", Journal of Controlled Release, 2002, 83(1), 65–74.

56. Zhao X, Yan J, Battle W, Allums S, Bentley M, "Oligomers of poloxamer 407 as degradable thermal sensitive depot materials for sustained drug release", Proc. Int. Symp. Control. Rel. Bioact. Mater., 2003, 30, 167.

57. Chenite A, Buschmann M, Wang D, Chaput C, Kandani N, 'Rheological characterization of thermogelling chitosan/glycerol phosphate solutions", Carbohydrate Polymer, 2001, 46(1), 39–47.

58. Ruel-Garie´py E, Chenite A, Chaput C, Guirguis S, Leroux JC, "Characterization of thermosensitive chitosan gels for the sustained delivery of drugs", International Journal of Pharmacy, 2000, 203(1-2), 89–98.

59. Bhattarai N, Matsen FA, Zhang M, "PEG-grafted chitosan as an injectable thermoreversible hydrogel", Macromole Bioscience, 2005, 5(2), 107–111.

60. Cho JH, Kim SH, Park KD, Jung MC, Yang WI, Han SW, Noh JY, Lee JW, "Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer", Biomaterials, 2004, 25(26), 5743–5751.

61. Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, Leroux JC, Atkinson BL, Binette F, Selmani A, "Novel injectable neutral solutions of chitosan form biodegradable gels in situ", Biomaterials, 2000, 21(21), 2155–2161.

62. Crompton KE, Goud JD, Bellamkonda RV, Gengenbach TR, Finkelstein DI, Horne MK, Forsythe JS, "Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering", Biomaterials, 2007, 28(3), 441-449.

63. Joly-Duhamel C, Hellio D, Djabourov M, "All gelatin networks: Biodiversity and physical chemistry", Langmuir, 2002, 18, 7208–7217.

64. Yang H, Kao WJ, "Thermoresponsive gelatin/monomethoxy poly (ethylene glycol)-poly (D, L-lactide) hydrogels: formulation, characterization and antibacterial drug delivery", Pharmacy Research, 2006, 23(1), 205–214.

65. Gil ES, Frankowski DJ, Spontak RJ, Hudson SM, "Swelling behavior and morphological evolution of mixed gelatin/silk fibroin hydrogels", Biomacromolecules, 2005, 6(6), 3079–3087.

66. Heskins M, Guillet JE, "Solution properties of poly(N-isopropylacrylamide)", Journal of Macromolecular Science and Chemistry, 1968, A2, 1441–1455.

67. Hoffman AS, Afrassiabi A, Dong LC, "Thermally reversible hydrogels: II. Delivery and selective removal of substances from aqueous solutions", Journal of Controlled Release, 1986, 4, 213–222.

68. Hirotsu S, Hirokawa Y, Tanaka T, "Volume-phase transitions of ionized N-isopropylacrylamide gels", Journal of Chemical Physics, 1987, 87, 1392–1395.

69. Feil H, Bae YH, Feijen J, Kim SW, "Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of N-isopropylacrylamide copolymers", Macromolecules, 1993, 26, 2496–2500.

70. Liu YY, Shao YH, Lu J, "Preparation, properties and controlled release behaviors of pH-induced thermosensitive amphiphilic gels", Biomaterials, 2006, 27, 4016–4024.

71. Yin X, Hoffman AS, Stayton PS, "Poly(N-isopropylacrylamide-copropylacrylic acid) copolymers that respond sharply to temperature and pH", Biomacromolecules, 2006, 7, 1381–1385.

72. Schild HG, "Poly(N-isopropylacrylamide): experiment, theory and application", Progress in Polymer Science, 1992, 17, 163–249.

73. Nakayama M, Okano T, Miyazaki T, Kohori F, Sakai K, Yokoyama M, "Molecular design of biodegradable polymeric micelles for temperature-responsive drug release", Journal of Controlled Release, 2006, 115(1), 46-56.

74. Coughlan DC, Quilty FP, Corrigan OI, "Effect of drug physichochemical properties on swelling/deswelling kinetics and pulsatile drug release from thermoresponsive poly(N-isopropylacrylamide) hydrogels", Journal of Controlled Release, 2006, 98, 97–114.

75. Na K, Park JH, Kim SW, Sun BK, Woo DG, Chung HM, Park KH, "Delivery of dexamethasone, ascorbate, and growth factor (TGF b-3) in thermo-reversible hydrogel constructs embedded with rabbit chondrocytes", Biomaterials, 2006, 27, 5951–5957.

76. Hacker MC, Ma BB, Kretlow JD, Mikos AG, "Novel macromers for the fabrication of injectable, calcium-binding hydrogels, Transactions of the 31st Annual Meeting of the Society for Biomaterials", Pittsburgh, USA, 2006, April 26–29.

77. Hatakeyama H, Kikuchi A, Yamato M, Okano T, "Bio-functionalized thermoresponsive interfaces facilitating cell adhesion and proliferation", Biomaterials, 2006, 27, 5069–5078.

78. Pei Y, Chen J, Yang L, Shi L, Tao Q, Hui B, Li J, "The effect of pH on the LCST of poly(N-isopropylacrylamide) and poly (N-isopropylacrylamide-co-acrylic acid)", Journal of Biomaterials Science, Polymer Edition, 2004, 15, 585–594.

79. Zhang XZ, Yang YY, Chung TS, Ma KX, "Preparation and characterization of fast response macroporous poly(N isopropylacrylamide) hydrogels", Langmuir, 2001, 17, 6094–6099.

80. Feil H, Bae YH, Feijen J, Kim SW, "Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of N-isopropylacrylamide copolymers", Macromolecules, 1993, 26, 2496–2500.

81. Liu XM, Wang LS, Wang L, Huang J, He C, "The effect of salt and pH on the phase-transition behaviors of temperature-sensitive copolymers based on N-isopropylacrylamide", Biomaterials, 2004, 25, 5659–5666.

82. Han CK, Bae YH, "Inverse thermally reversible gelation of aqueous *N*-isopropylacrylamide copolymer solution", Polymer, 1998, 39, 2809–2814.

83. Lin HH, Cheng YL, "In-situ thermoreversible gelation of block and star copolymers of poly(ethylene glycol) and poly(*N*-isopropylacrylamide) of varying architectures", Macromolecules, 2001, 34, 3710–3715.

84. Jeong B, Bae YH, Lee DS, Kim SW, "Biodegradable block copolymers as injectable drug-delivery systems", Nature, 1997, 388, 860–862.

85. Jeong BM, Lee DS, Bae YH, Kim SW, "In situ gelation of PEGPLGAPEG triblock copolymer aqueous solutions and degradation thereof", Journal of Biomedical Materials Research, 2000, 50, 171–177.

86. Lee J, Bae YH, Sohn YS, Jeong BM, "Thermogelling aqueous solutions of alternating multiblock copolymers of poly(L-lactic acid) and poly(ethylene) glycol", Biomacromolecules, 2006, 7, 1729–1734.

87. Jeong B, Choi YK, Bae YH, Zentner G, Kim SW, "New biodegradable polymers for injectable drug delivery systems", Journal of Controlled Release, 1999, 62, 109–114.

88. Jeong B, Bae YH, Kim SW, "Micellization of PEG–PLGA–PEG triblock copolymer aqueous solutions", Colloids Surfaces B: Biointerfaces, 1999, 16, 185–193.

89. Kim YJ, Choi S, Koh JJ, Lee M, Ko KS, Kim SW, "Controlled release of insulin from biodegradable triblock copolymer", Pharmaceutical Research, 2001, 18, 548–550.

90. Jeong B, Kibbey MR, Birnbaum JC, Won YY, Gutowska A, "Thermogelling biodegradable polymers with hydrophilic backbones: PEG-g-PLGA", Macromolecules, 2000, 33, 8317–8322.

91. Kang GD, Cheon SH, Song SC, "Controlled release of doxorubicin from thermosensitive poly(organophosphazene) hydrogels", International Journal of Pharmaceutics, 2006, 319, 29–36.

92. Kang GD, Cheon SH, Khang G, Song SC, "Thermosensitive poly(organophosphazene) hydrogels for a controlled drug delivery", European Journal of Pharmacy and Biopharmaceutics, 2006, 63, 340–346.

93. Park KH, Song SC, "Morphology of spheroidal hepatocytes within injectable, biodegradable and thermosensitive poly - (organophosphazene) hydrogel as cell delivery vehicle", Journal of Bioscience and Bioengineering, 2006, 101, 238–242.

94. Song SC, Lee SB, Jin JI, Sohn YS, "A new class of biodegradable thermosensitive polymers. I. Synthesis and characterization of poly(organophosphazenes) with methoxy-poly(ethylene glycol) and amino acid esters as side groups", Macromolecules, 1999, 32, 2188–2193.

95. Sohn YS, Kim JK, Song R, Jeong B, "The relationship of thermosensitive properties with structure of organophosphazenes", Polymer, 2004, 45, 3081–3084.

96. Song SC, Lee SB, Jin JI, Sohn YS, "A new class of biodegradable thermosensitive polymers I. Synthesis and characterization of poly(organophosphazenes) with methoxy-poly(ethylene glycol) and amino acid esters as side groups", Macromolecules, 1999, 32, 2188–2193.

97. Lee SB, Song SC, Jin JI, Sohn YS, "A new class of biodegradable thermosensitive polymers 2 Hydrolytic properties and salt effect on the lower critical solution temperature of poly(organophosphazenes) with methoxypoly(ethylene glycol) and amino acid esters as side groups", Macromolecules, 1999, 32, 7820–7827.

98. Lee SB, Song SC, Jin JI, Sohn YS, "Thermosensitive cyclotriphosphazenes", Journal of American Chemical Society, 2000, 122, 8315–8316.

99. Lee BH, Lee YM, Sohn YS, Song SC, "A thermosensitive poly(organophosphazene) gel", Macromolecules, 2002, 35, 3876–3879.

