CHAPTER 6

# Smart Polymers and Their Applications as Biomaterials

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## Summary

Living systems respond to external stimuli adapting themselves to changing conditions. Polymer scientists have been trying to mimic this behaviour for the last twenty years creating the so called smart polymers. These are defined as polymers that undergo reversible large, physical or chemical changes in response to small external changes in the environmental conditions, such as temperature, pH, light, magnetic or electric field, ionic factors, biological molecules, etc. Smart polymers have very promising applications in the biomedical field as delivery systems of therapeutic agents, tissue engineering scaffolds, cell culture supports, bioseparation devices, sensors or actuators systems. This chapter is focused on pH and temperature sensitive polymers and their most recent and relevant applications as biomaterials in drug delivery and tissue engineering. Dual-stimuli-responsive materials will also be presented because of their high potential in the biomedical field.

**Keywords:** Stimuli sensitive polymers, drug delivery, tissue engineering

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## *Introduction*

Stimuli-sensitive or "*smart*" polymeric systems are polymers that may overcome dramatic property changes responding to small changes in the environment. The most important systems, also from a biomedical point of view, are those sensitive to pH or temperature (T). Human body presents variations on pH along the gastrointestinal tract, and also in some specific areas like certain tissues (and tumoral areas) or sub-cellular compartments. Thermosensitive polymers with critical T close to the physiological value, i.e. poly(*N*-isopropyl acrylamide) (PNIPAm), offer many possibilities in the biomedical field, as it will be shown in this paper.

The polymer-polymer and the polymer-solvent interactions (solvent that in biomedical applications will be usually water) show an abrupt re-adjustment in small ranges of pH or temperature, and this is translated to a chain transition between extended and compacted coil states. In the case of pH-sensitive polymers, the key element of the system is the presence of ionizable weak acidic or basic moieties attached to a hydrophobic backbone. Upon ionization, the coiled chains extend dramatically responding to the electrostatic repulsions of the generated charges (anions or cations).

Temperature-responding polymers present a fine hydrophobic-hydrophilic balance in their structure, and small temperature changes around the critical T, make the chains to collapse or to expand responding to the new adjustments of the hydrophobic and hydrophilic interactions between the polymeric chains and the aqueous media.

Macroscopic response of the polymer will depend on the physical state of the chains, as it is indicated in Table 1. If the *macromolecular chains* are *linear and solubilized*, the solution will change from mono-phasic to bi-phasic due to polymer precipitation when the transition occurs.

Hoffman *et al*. [1] demonstrated, in a very elegant design, that the action of an enzymatic receptor can be modulated when this kind of polymer is conjugated close to its active place. They were able to switch on-off the receptor using the transition between extended and coiled form of the molecule [2]. Soluble pH and T-responsive polymers that overcome transition at physiological conditions (37ºC and/or physiological pH) have been proposed as minimally invasive injectable systems. The soluble systems may be easily injected, however they precipitate or gel *in situ* forming

an implant or scaffold useful for drug delivery systems (DDS) or tissue engineering applications [3-6] .

**Table 1.** Physical forms of the smart polymer chains, together with the type of response they exhibit and examples of their possible application.

| Physical form of the chains | Type of response | Examples and references |
|---|---|---|
| Uncrosslinked free linear chains (Conjugates) | Solubilization/precipitation | Use of polymer-active compound conjugates. |
| | Sol-gel transition (reversible physical gel formation) | Injectable *in situ* gel forming formulations. BST-Gel® (BioSyntech) and ReGel® of Macromed. |
| Amphiphilic (uncrosslinked) block and graft copolymers | Micellization | Pluronics or Poloxamers (PEO-PPO-PEO) [50] |
| Chemically cross-linked hydrogels | Swelling-deswelling response | Pulsed drug delivery [9;10] |
| Modified surfaces | Responsive interfaces | New substrates for cell culture [11-14] |

*Sol-gel reversible hydrogels* are usually constituted by block or graft copolymers as Pluronics, or more recently proposed, PEO-biodegradable polyester (PLGA, PLLA; PCL). These systems are already in the market as minimally invasive injectable solutions (BST-Gel® of BioSyntech and ReGel® of Macromed). Block or graft copolymers may give other type of transition than soluble polymers, consisting in micellization or micelle aggregation [7,8]. This behaviour is closely related to the reversible sol-gel transition because in some cases micelles are parent shape in gelation.

If the sensitive polymer is forming part of an *infinite crosslinked network*, the chain reorganization will mean a gel transition between a collapsed and an expanded state, that is, between shrunk and swollen state (Figure 1). The difference between these two states could reach several orders of magnitude. This behaviour has been very attractive for the preparation of pulsed DDS [9,10].

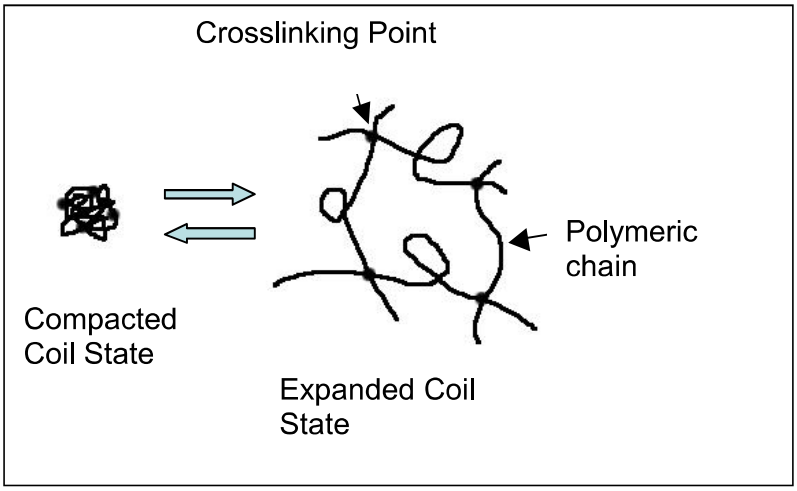


**Figure 1**: Schematic representation of a gel in its collapsed and swollen states. The lines and the points correspond to polymeric chains and crosslinking points respectively.

*Surface modification* with this type of polymers leads to the preparation of responsive interfaces which may show a very different behaviour in response to one of these small changes in the environmental parameters. Surface may change from hydrophobic to hydrophilic [11-14] or if a membrane is chemically modified, it may vary the pore size itself [15-17]

From this general scheme, different issues will be faced in this paper, as the modulation of the response (T or pH transition range, rate of the transition, etc.) by controlling the structure of the polymeric chains. Some comments about the utility of polycations to carry and deliver genes will also be addressed. Special attention will be placed on dual-stimuli smart polymers, or polymers that can respond to two parameters, i.e. pH and T, simultaneously.

## *pH-sensitive polymers: General considerations*

pH-sensitive polymers are polyelectrolytes that bear in their structure weak acidic or basic groups that either accept or release protons in response to changes in environmental pH. The pendant acidic or basic groups on polyelectrolytes undergo ionization just like acidic or basic groups of monoacids or monobases. However, complete ionization on polyelectrolytes is more difficult due to electrostatic effects

exerted by other adjacent ionized groups. This makes the apparent dissociation constant ($K_a$) different from that of the corresponding monoacid or monobase.

By generating the charge along the polymer backbone, the electrostatic repulsion results in an increase in the hydrodynamic volume of the polymer [10]. This transition between tightly coiled and expanded state is influenced by any condition that modify electrostatic repulsion, such as pH, ionic strength, and type of counterions. The transition from collapsed state to expanded state has been explained by changes in the osmotic pressure exerted by mobile counterions neutralizing the network charges [18]. The pH range that a reversible phase transition occurs can be generally modulated by two strategies:

1. Selecting the ionizable moiety with a $pK_a$ matching the desired pH range. Therefore, the proper selection between polyacid or polybase should be considered for the desired application.
2. Incorporating hydrophobic moieties into the polymer backbone and controlling their nature, amount and distribution. When ionizable groups become neutral – non-ionized- and electrostatic repulsion forces disappear within the polymer network, hydrophobic interactions dominate. The introduction of a more hydrophobic moiety can offer a more compact conformation in the uncharged state and a more accused phase transition. The hydrophobicity of these polymers can be controlled by the copolymerization of hydrophilic ionisable monomers with more hydrophobic monomers with or without pH-sensitive moieties, such as 2-hydroxyethyl methacrylate, methyl methacrylate and maleic anhydride.

Polyacidic polymers will be unswollen at low pH, since the acidic groups will be protonated and unionized. When increasing the pH, a negatively charged polymer will swell. The opposite behaviour is found in polybasic polymers, since the ionization of the basic groups will increase when decreasing the pH. Typical examples of pH-sensitive polymers with anionic groups are poly(carboxylic acids) as poly(acrylic acid) (PAA) or poly(methacrylic acid) (Figure 2). Another kind of polyacidic polymer are the polysulfonamides (derivatives of *p*-aminobenzenesulfonamide) (Figure 3). These weak polyacids present a $pK_a$ that narrowly varies from 3 to 11, depending on the electro-withdrawing nature of the substituent on the nitrogen [19]. A few examples of cationic polyelectrolytes are poly(*N,N*-diakyl aminoethyl methacrylates) (Figure 4), poly(lysine) (PL), poly(ethylenimine) (PEI), and chitosan.

### *Polyacids*

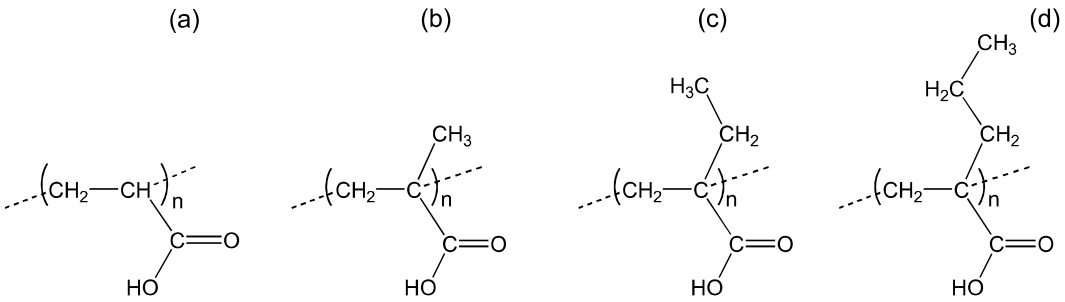


**Figure 2:** Chemical structure of pH-sensitive polyacids (a) poly(acrylic acid); (b) poly(methacrylic acid); (c) poly(2-ethylacrylic acid); (d) poly(2-propylacrylic acid)

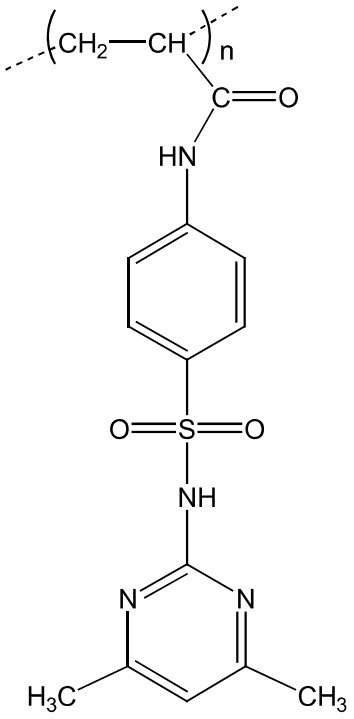


**Figure 3:** Chemical structure of 4-amino-*N*-[4,6-dimethyl-2-pyrimidinyl]-benzenesulfonamine (sulfomethazine) containing polymer [78]

***Polybases***

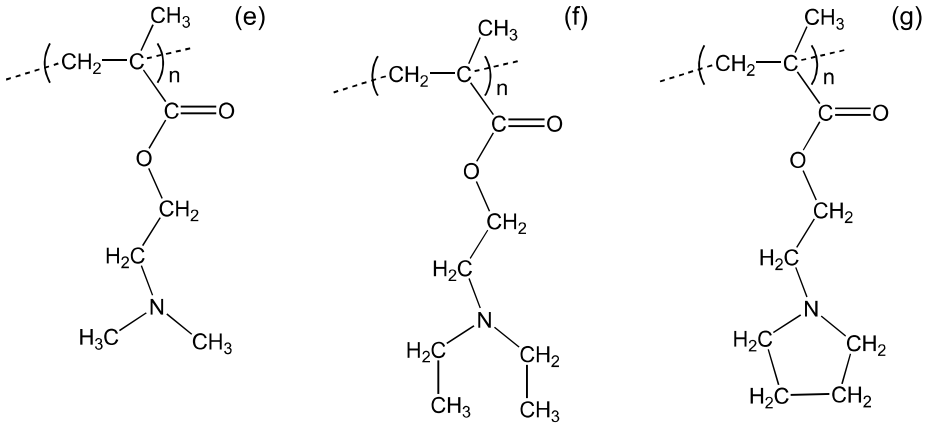


**Figure 4:** Chemical structure of pH-sensitive polybases. (a) PDMAEMA; (b) PDEAEMA; (c) PEPyM

When pH-sensitive polymeric chains are crosslinked forming *hydrogels*, their behaviour is not only influenced by the nature of the ionizable groups, the polymer composition, and the hydrophobicity of the polymer backbone, but also by the crosslinking density. This affects the solute permeability in terms of bioactive compounds release in several applications; the higher the crosslinking density, the lower the permeability, especially significant in the case of high molecular weight solutes.

Most of the materials described in the literature that respond to external stimuli are acrylic hydrogels. In a swollen state, each polymer chain is isolated by solvent molecules and is therefore, exposed as a single molecular unit to tension and shear forces produced during a gel deformation process. Most polyelectrolyte gels exhibit a decrease in modulus with increasing swelling degree. However, poly(silamine) (Figure 5) hardened on swelling by formation of the rigid molecular locks through ionic interactions [20]. The system described by Lou *et al*. consists on a silicon-based polymer, comprising alternating units of *N,N*-diethylethylenediamine and 3,3-dimethyl-3-silapentamethylene. They developed hydrogel microspheres based on this chemistry which formed a very stable skin layer when swollen in acidic media [21]. The externally stable skin layer was explained in terms of the high activation energy for the rod/globule transition along with a lowered mass transport through the skin layer.

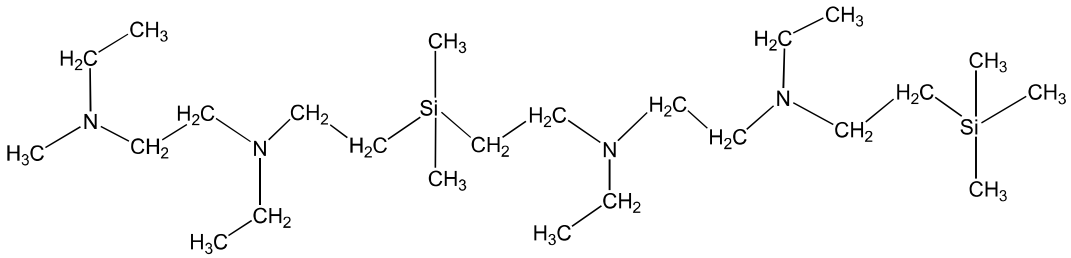

**Figure 5:** Non-protonated polysilamine [21]

## *Applications*

pH-sensitive polymers have been used in several biomedical applications, the most important being their use as drug and gene delivery systems, and glucose sensors. Between all the systems described in the literature, we report in this section the most attractive examples reported in the last years.

### *Drug delivery systems*

pH varies along the gastrointestinal tract (GIT) between 2 (stomach) and 10 (colon). This condition makes pH-sensitive polymers ideal for colon specific drug delivery. The most common approach utilizes enteric polymers that resist degradation in acidic environment and release drug in alkaline media due to the formation of salt. There are several examples of these kind of polymers already commercialized, i.e. Eudragit L, Eudragit S from Röhm Pharma GmBH (based on methacrylic acic and methyl methacrylate) or CMEC from Freund Sangyo Co., Ltd; CAP from Wako Pure Chemicals Ltd.; HP-50 and ASM from Shin-Etsu Chemical Co., Ltd. (derived from cellulose). A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrin, chondroitin sulphate, dextran and locust beam gum, have been also investigated for colon specific drug release [22,23].

Several groups have developed polymeric prodrugs (polymers in which the drug is covalently attached to the macromolecular chain) susceptible to hydrolytic cleavage dependent upon the pH and hence suitable for colon drug delivery. This is the case of poly(*N*-methacryloylaminoethyl 5-aminosalycilamide) or poly(methacryloylethoxyethyl 5-aminosalycilic acid) [24] or the copolymeric system developed in our group based on 2-acrylamido-2-methylpropane sulfonic acid (AMPS) and a methacrylic derivative of an antiaggregant drug called Triflusal [25] (Figure 6).

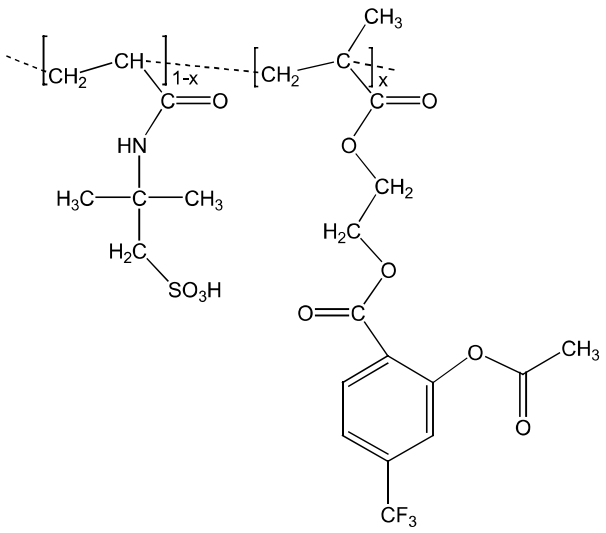


**Figure 6:** Schematic structure of the copolymeric system based on AMPS and the methacrylic derivative of Triflusal [29]

However, pH in GIT varies depending on diet, pathological conditions or even inter- and intra-subject, making the pH-sensitive delivery profile along the GIT not very predictable. Therefore, the strategy that depends on colonic microflora for liberation of entrapped drug seems most suitable, i.e. glycosidase activity of the colonic microflora is responsible for liberation of drugs from glycosidic prodrugs and the presence of azo-reductase from the anaerobic bacteria of the colon plays a main role in the release of drug from azo bound prodrugs [26,27]

Researchers have designed more sophisticated pH-sensitive polymers in order to take advantage of the pH changes that occur in nature. These materials are inspired by living organisms trying to mimic their response mechanisms. Sauer *et al*. [28] have reported the synthesis of pH-sensitive hollow nanocontainers inspired in virion particles. The poly(acrylic acid) vehicles were synthesised by vesicular polymerization and emulsion polymerization (using core-shell latex particles). These nanocapsules combined the protective ability of the nanocontainers in combination with controlled permeability and therefore can be used to trigger the release of encapsulated materials from the inner core.

Bellomo and co-workers [29] have prepared a new type of synthetic vesicle with a high degree of architectural control made of amphiphilic block copolypeptides. The hydrophilic block was made of lysine, decorated with a few water soluble ethylene glycol units and the hydrophobic block was constituted by leucine peptide. The synthetic polymer forms a supramolecular structure highly sensitive to environmental signals and able to respond precisely to pH changes and actuate as drug delivery carriers.

Most of the drug release intelligent systems are driven by the phase-volume transition. However, Liu and co-workers [30] reported the preparation of novel β-cyclodextrin microgels for drug delivery applications driven by inclusion effects.

pH-sensitive polymers have also been incorporated into organic-inorganic composites obtaining materials that presented both the advantages of inorganic materials (high mechanical stability) and conventional polyeletrolyte capsules (the controlled release/uptake properties of the capsule shell resulting from changes in, among others, pH values and ionic strength). Shchukin *et al*. presented in 2003 [31] the synthesis and characterization of new inorganic/organic composites capsules where the inorganic particles acted as building blocks glued together by a pH-sensitive polyelectrolite. Besides the common applications of polyelectrolyte capsules in controlled release, these composite inorganic/organic capsules can be also applied as mechanically stable microreactors for enzymatic reactions and synthesis employing gas-phase reagents, in the form of hollow catalytically microcontainers. The inorganic part can also find medical application that, together with encapsulated drug material, can provide synergistic curing effects, i.e. the application of hydroxyapatite-containing capsules in bone repair. These capsules can also be used as protective solid microcontainers due to their ability to preserve the initial spherical shape and controlled release properties upon drying.

*Gene carriers*

Polyelectrolytes have high potential as biomaterials in delivering oppositely charged molecules. One of the most promising applications of pH-sensitive polymers is as non-viral gene carriers. Naked DNA is very difficult to incorporate into the cells because it is negatively charged and it has a very large size at physiological conditions. Liposomes and polycations are the two major classes of chemical (non-viral) gene delivery methods to condense DNA in charge balanced nanoparticles that can be carried into cell

compartments. Godbey and Mikos reviewed some of the advances in non-viral gene delivery research [32] describing the use of poly(ethylenimine) (PEI) and poly(L-lysine) (PLL) as two of the most successful candidates for this application. PEI is a highly polycationic synthetic polymer that condense DNA in solution, forming complexes that are readily endocytosed by many cell types. Chitosan, a biocompatible and resorbable cationic aminopolysaccharide, has also extensively been used as DNA carrier [33-37].

Lim *et al*. [38] prepared a self-destroying, biodegradable, polycationic polyester, poly(*trans*-4-hydroxy-L-proline ester) (PHP ester), with hydroxyproline, a major constituent of collagen, gelatine, and other proteins, as a repeating unit. PHP ester formed soluble polymer/DNA complexes with average diameters of less than 200 nm. These complexes could transfect the mammalian cells, being comparable to the transfection obtained with PLL, the most common polymer for gene delivery.

Lim *et al*. also presented [39] a degradable non-toxic PLL analogue employing poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA) for its application as gene carrier. This polymer condensed DNA into a spherical shaped polymer and showed accelerated degradation when free, and decreased degradation during the formation of complexes with DNA. The transfection efficiency of PAGA/DNA complexes was about twice that of PLL/DNA complexes. The most important characteristics of this polymer against cationic liposomes and polyamidoamine (PANAM) dendrimers are its high solubility, non-toxicity and degradability when used as systemic gene carrier.

Kataoka [40] recently communicated the development of polymeric micelles as nanocarriers for gene and drug delivery based on doxorubicin-conjugated block copolymer poly(ethylene glycol)-poly(aspartame hydrazinedoxorubicin) [(PEG-p(Asp-Hid-dox)]. The polymer retained drugs and genes at physiological pH and released the drugs as pH decrease below 6.0.

Anionic polyelectrolytes have been used in the development of new intracellular delivery systems by membrane destabilizing mechanisms. These polymers can be tailored to interact actively with phospholipid membranes upon external stimulation, such as acidification of the surrounding medium. This strategy has been exploited to improve the cytoplasmic delivery of biomolecules (DNA, proteins) that enter cells by endocytosis and end up in acidic organelles [41,42].

Hoffman's group has dedicated great efforts to obtain new delivery systems to introduce efficiently biomolecules to intracellular targets [43-45]. They mimicked the molecular machinery of some viruses and pathogens that are able to sense the lowered

pH gradient of the endosomal compartment and become activated to destabilize the endosomal membrane. This mechanism enhances protein or DNA transport to the cytoplasm from intracellular compartments such as endosome. They demonstrated the utility of poly(2-propylacrylic acid) (PPAA) (Figure 2d) to enhance protein and DNA intracellular delivery. They also constructed more versatile carrier systems, designing a new functionalized monomer (pyridyl disulfide acrylate, PDSA), that allows efficient conjugation through disulfide linkages that can be reduced in the cytoplasm after endosomal translocation of the therapeutics. PDSA was copolymerized with alkylacrylic acid monomers and alkylacrylate monomers. The membrane destabilizing activity of the polymers depended on the lengths of the alkyl segment and their ratio in the final polymer chains [46].

*Glucose sensors*

One of the most popular applications of pH-sensitive polymers is the fabrication of insulin delivery systems for the treatment of diabetic patients. Delivering insulin is different from delivering other drugs, since insulin has to be delivered in an exact amount at the exact time of need. Many devices have been developed for this purpose and all of them have a glucose sensor built into the system. In a glucose-rich environment, such as the bloodstream after a meal, the oxidation of glucose to gluconic acid catalysed by glucose oxidase (GluOx) can lower the pH to approximately 5.8. This enzyme is probably the most widely used in glucose sensing, and makes possible to apply different types of pH-sensitive hydrogels for modulated insulin delivery [47].

## *Temperature responsive polymers*

### *General considerations*

Polymers sensitive to temperature changes are the most studied class of environmentally sensitive polymers as they have potential applications in the biomedical field [18]. This type of systems exhibit a critical solution temperature (typically in water) at which the phase of polymer and solution is changed in accordance with their composition. Those systems exhibiting one phase above certain temperature and phase separation below it, possess an upper critical solution temperature (UCST). On the other hand, polymer solutions that appear as monophasic below a specific temperature and biphasic above it, generally exhibit the so-called lower critical solution temperature (LCST). These represent the type of polymers with most number of applications [48]. The typical

example is poly(*N*-isopropylacrylamide) (PNIPAAm) that presents a LCST at 32ºC in water solution [49]. Below that temperature the polymer is soluble as the hydrophilic interactions, due to hydrogen bonding, are predominant, whereas a phase separation occurs above the LCST (cloud point) due to predomination of hydrophobic interactions. Other type of temperature sensitivity is based on the intermolecular association as in the case of Pluronics or Poloxamers (PEO-PPO-PEO) [50] where hydrophobic associations of PPO blocks lead to the formation of micelle structures above critical micelle temperature (CMT).

*Polymers with LCST*

The LCST can be defined as the critical temperature at which polymer solution undergo phase separation from one phase (isotropic state) to two phases (anysotropic state) rich and poor in polymer [18]. Below the LCST the enthalpy term, related to the hydrogen bonding between the polymer and the water molecules, is responsible for the polymer dissolution. When raising the temperature above the LCST, the entropy term (hydrophobic interactions) dominates leading to polymer precipitation. The LCST of polymers in water solutions can be modulated by incorporating hydrophilic or hydrophobic moieties. For example, when NIPAAm is copolymerized with hydrophilic monomers such as AAm, the LCST increases up to about 45ºC when 18% of AAm is incorporated to the polymer, whereas LCST decreases to about 10ºC when 40% of hydrophobic *N*-tert-butylacrylamide (N-tBAAm) is added to the polymer [51].

When hydrogels are prepared by crosslinking T-sensitive polymers the temperature sensitivity in water results in changes in the polymer hydration degree. Below the transition temperature the polymer swells up to equilibrium hydration degree being in an expanded state. By increasing the temperature above the transition hydrogel deswells to a collapsed state. This process is generally reversible and can be applied in a pulsatile manner making the polymer to behave as an on-off system when the stimulus is applied or removed.

The most representative group of polymers showing LCST is the poly(*N*-substituted acrylamide) family (Figure 7). PNIPAAm (Figure 7a) is the most investigated temperature sensitive polymer exhibiting a LCST close to body temperature. Related polymers such as poly(*N,N'*-diethyl acrylamide) (Figure 7b) exhibit a LCST in the range 26-35 ºC [10], poly(dimethylaminoethyl methacrylate)

(Figure 7c) close to 50ºC [52] and poly(N-(L)-(1-hydroxymethyl) propylmethacrylamide) (Figure 7d) close to 30ºC [53].

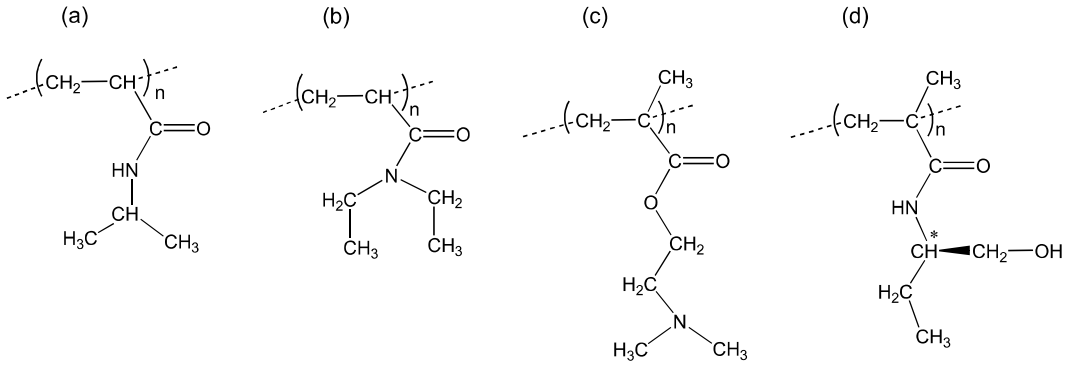


**Figure 7:** Schematic structure of polymers showing a LCST. (a) PNIPAAm, LCST 32ºC; (b) PDEAAm, LCST 26-35ºC ; (c) PDMAEMA, LCST 50ºC; (d) poly(N-(L)-(hydroxymethyl) propylmethacrylamide) LCST 30ºC

PNIPAAm has been deeply studied in terms of transition mechanism and also in specific applications. When PNIPAAm is crosslinked for example with *N,N'*-methylene-*bis*-acrylamide (MBAAm), temperature sensitive hydrogels are obtained showing hydration degrees from about 60% below the transition temperature associated to the LCST, to approximately 0% above it [49]. This swelling behaviour can be modulated in terms of kinetics and swelling degree by controlling different aspects of the molecular design. A rapid response to deswelling can be increased by incorporating hydrophilic units by copolymerization of NIPAAm with acrylic acid (AA) [54], or by obtaining comb-like PNIPAAm molecular architectures [55], or by grafting hydrophilic chains onto PNIPAAM hydrogel networks [56]. LCST modulation can be obtained by copolymerization with other monomers as described above, in order to have LCST close to physiological temperature (37ºC) for applications for drug delivery. When NIPAAm was randomly copolymerized with ionisable monomers such as AA the LCST of the prepared copolymers is modified or disappeared at the ionisable groups $pK_a$ whereas when AA is grafted onto PNIPAAm the value of the LCST is not altered [57]. In the case of random copolymers based on NIPAAm, 2-hydroxyethyl methacrylate (HEMA) lactate, LCST is increased above the body temperature [58]. Then, using the poly(NIPAAM-*co*-HEMA lactate) system to prepare block copolymers with poly

(ethylene glycol) (PEG), a micelle structure is formed at physiological temperature that can be destabilized when hydrolyzing the lactate groups [58]. When considering NIPAAm terminal modification by incorporating hydrophilic units the LCST increase is more pronounced in comparison with the random copolymers, the rate of the transition being slowed down when linking AA or DMA as end groups [54].

Polymers with LCST have been tested in controlled drug delivery matrices and in on-off release profiles in response to a stepwise temperature change. In this sense, polyNIPAAm hydrogels form a thick skin on their surface above the LCST in the collapsed state, which reduces the transport of bioactive molecules out of the hydrogels. NIPAAm has also been copolymerized with alkyl methacrylates in order to increase the hydrogels mechanical properties, maintaining the temperature sensitivity. Poly(NIPAAm-*co*-butyl methacrylate) (poly(NIPAAm-*co*-BM)) was studied for the delivery of insulin in a temperature on-off profile, below and above the LCST that in this system was lowered to about 25ºC [59]. The terpolymer NIPAAm, based on AAm and BM, was tested for the delivery of indomethacin being the LCST in this case close to physiological temperatures [60].

Clinical applications of NIPAAm-based T-sensitive hydrogels have limitations since they are not biodegradable but have been evaluated as drug release carriers consisting in semi-IPN of PNIPAAm and poly(tetramethylene glycol) crosslinked with MBAAm, analysing the release of indomethacin as a model drug, showing also an on-off pulsatile release behaviour [61]. Other combinations with biodegradable systems have been prepared by dispersing PNIPAAm hydrogel microparticles into a crosslinked gelatine matrix [62] or by encapsulating the drug core with ethylcellulose containing nano-sized PNIPAAm hydrogel particles [63]. Novel hydrogels showing both T-sensitivity and biodegradability have been prepared using PNIPAAm crosslinked with degradable poly(amino acid) [64]. Kumashiro *et al*. [65] have designed a new semi-interpenetrating network (SIPN) based on dextran grafted with T-sensitive polymer chains, that is degraded by specific enzymes at a definite temperature range.

Several approaches have been performed in the tissue engineering field as temperature sensitive scaffolds or surface modifications for the manipulation of cell sheets. Poly(NIPAAm-*co*-acrylic acid) (poly(NIPAAm-*co*-AA)) gels have been applied as extracellular matrix for pancreatic islets in biohydrid pancreas [66]. Composite membranes have also been prepared maintaining and exploiting the LCST. For example, NIPAAm has been crosslinked on the surface of glass disks forming hydrogels

inside the glass pores. For making stable thermally controlled on-off devices, PNIPAAm hydrogel had been grafted onto the entire surface of a rigid porous polymer membrane [67]. Disks were placed between donor and receptor cells by a permeation chamber and the transport of salicylic acid and bovine serum albumin were tested as a function of temperature. The delivery rate of both drugs across the composite membrane was found to be temperature dependent as the rate was increased when the temperature raised from 20 to 40 ºC, going from an expanded state to a collapsed state where pores are opened [17].

Surface modifications incorporating temperature responsive polymers have been carried out in order to immobilize specific molecules or to manipulate cell sheets in tissue engineering processes. In this sense, the application of temperature responsive polymers to modified surfaces exploits the fact that most proteins show significantly greater adsorption on hydrophobic surfaces than in hydrophilic ones. Above the LCST the T-sensitive polymer will adsorb peptides and proteins from a solution and these biomolecules can be desorbed by decreasing the temperature as has been done in chromatographic supports incorporating PNIPAAm and using water as eluent [68]. Another approach has shown that the surface of tissue culture polystyrene grafted with PNIPAAm allows cells to adhere and proliferate above the LCST of the polymer whereas a cell detachment was detected at temperatures below LCST [14]. Moreover, this type of application has been tested on biodegradable polymers such as poly(L-lactic acid) [69] and chitosan [11] exhibiting a similar behaviour than that performed on polystyrene culture plates. This type of grafting has also been performed on reversible 3D-matrix for the culture of articular chondrocytes, cells that proliferate adequately on the matrix that are then removed by temperature lowering [70].

### *Polymers with amphiphilic balance*

Block copolymers based on PEO–PPO sequences are a family of commercially available triblock copolymers which have the following trade names: Pluronics® or Poloxamer, Tetronics® (Figure 8). These systems exhibit a sol-gel transition below or close to the physiological temperature, a gel-sol transition around 50ºC and a LCST. They are considered separated temperatures as the gelation responds by a 3-dimensional packing of micelles due to the hydrophilic-hydrophobic balance, increasing the micelle volume and provoking micelle packaging when sol-gel transition takes place [71]. This *balance* can be modulated by incorporating different side chains with hydrophilic or

hydrophobic segments. In this sense, one important aspect when applying these polymers is their high concentration required to form a gel at 37ºC [72]. By grafting PAA onto the polaxomer backbone in one step reaction by radical polymerization of AA in the presence of polaxomer [73], the sol-gel transition occurs at lower concentration than the polaxomer because PAA forms physical crosslinking points at low concentrations.

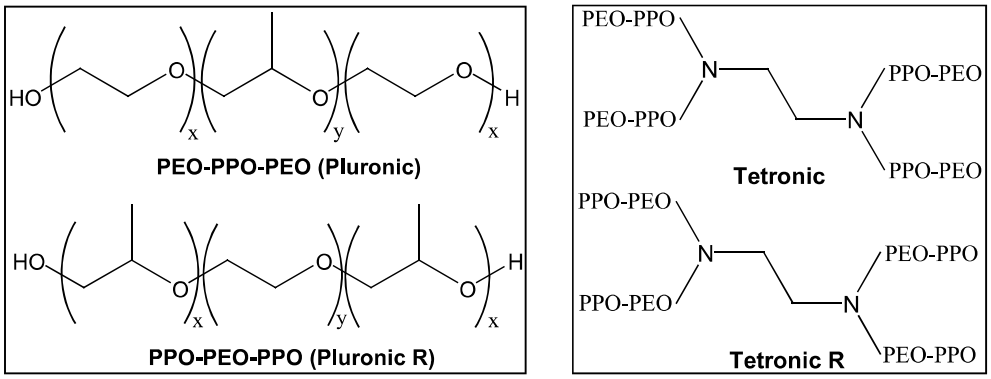


**Figure 8:** Schematic structure of polymers with amphiphilic balance

Pluronics® and Tetronics® are used as thermoreversible gels and some of them have been approved by the FDA and EPA for applications as food additives, pharmaceutical ingredients and agricultural products, as drug delivery carriers and as injectable systems for tissue engineering processes [10]. Their gelation temperature depends on polymer composition and solution concentration. For example Pluronic F127 gels at 37ºC in solutions that contain about 20%-wt of polymer. These systems have been used in treatment of burns and other wound healing applications [74]. More recent PEO/PPO/PEO triblock copolymers, Poloxamer 407, were mixed with isolated chondrocytes and applied with a brush on an osseous surface forming a sticky gel in a short period of time (minutes). A new cartilage was formed on the osseous substrate in the bone cartilage interface [75]. Poloxamer 407 in solution with isolated chondrocytes was also applied as an injectable cartilage formulation testing the formation of tissue after subcutaneous injections in mice. Histological examination of all samples

demonstrated the presence of new cartilage formation indicating that the polymer/cell suspension is very promising for orthopaedic and reconstructive surgery [75].

The Pluronics® sol-gel transition has made them very attractive systems as injectable drug delivery carriers forming an *in situ* drug depot. Poloxamer is again the most commonly used in this kind of application and it has been tested in the delivery of protein and peptides (insulin, urease, bone morphogenic protein and growth factors) showing in most of the cases sustained release profiles over several hours [76]. Due to their rapid dissolution in water (for example Poloxamer 407 is completely dissolved in about 4 hours), Poloxamer formulations are functional during short periods of time after administration.

In this sense poly(ethylene glycol)-poly(L-lactic acid)-poly(ethylene glycol), PEG-PLLA-PEG, triblock copolymers and PEG-PLLA block copolymers exhibit the sol-gel transition when decreasing the temperature in water like a gelatine solution, which is influenced by the length of PLLA block when PEG is constant. When preparing the Poly(lactic-*co*-glycolic acid)-PEG (PLGA-PEG) diblock and triblock copolymers the aqueous solution is *sol* at room temperature and *gel* at physiological one, *sol-gel* transition can be modified by changing the blocks length. These systems have been evaluated for the release of either hydrophilic or hydrophobic drugs, the release of hydrophilic one lasting about two weeks whereas the hydrophobic one over two months. Degradation of the polymer matrix was slowed down by the incorporation of the PLGA blocks [77].

Similar systems incorporating biodegradable segments and adjustment of the sol-gel transition are those based on poly(ethylene oxide)-PLGA (PEO-PLGA) triblock copolymers. They present sol-gel transitions in aqueous solutions at about 30ºC resulting in the formation of an *in situ* transparent gel with maintained structural integrity and mechanical strength [78]. Other systems based on aqueous solutions of PEO-g-PLGA and PLGA-g-PEO form soft gels at 37ºC that are biodegradable and can be applied in tissue engineering.

Synthetic block copolypeptides also with hydrophobic and hydrophilic segments, present similar temperature sensitivity to Pluronics, but in this case hydrogels are also formed at low concentrations based on the formation of α-helix conformations [79;80]. Other approaches to prepare synthetic polypeptides are those using recombinant DNA leading to triblocks that can be sensitive to both temperature and pH

changes [81]. Elastin-like polypeptides have also been reported to exhibit reversible sol-gel thermal transitions when incorporating silk like segments [82].

## *Polymers of natural origin*

Gellan (composed of glucose and β-D-glucuronic acid and α-L-rhamnose), gelatine (protein obtained from the collagen hydrolysis), amylopectin, amylose and agarose are some biopolymers that also exhibit temperature sensitivity by different gelation mechanisms that lead to the formation of helix conformations by physical crosslinks. These polymers are *sol* at high temperatures and become *gel* at lower by formation of aggregation of double helices that act as crosslinking knots [83]. The polysaccharide gellan and derivatives as gellan benzyl ester, attain these conformations by hydrogen bonding in aqueous media [61]. In the case of gelatine, gels are formed in aqueous solution when lowering the temperature that promotes the formation of gel networks due to the change from random to triple helix conformation [84]. The low stability of gelatines under physiological conditions has promoted their conjugation with other polymers such as chitosan being stable at temperatures of up to 50ºC [85].

## *Polymer-protein bioconjugates*

Hoffman *et al*. have been also involved in the synthesis and development of polymer-protein bioconjugates [86-88] useful for affinity separations, biosensors, diagnostics, enzyme processes, and targeted delivery of drugs or chemical agents, labels and other signals. Two different kinds of bioconjugates including stimuli-responsive polymers have been prepared by:

1. Random polymer conjugation to lysine amino groups of a protein.
2. Site-specific conjugation of the polymer to genetically engineered specific amino acid sites. The placement of stimuli-sensitive polymers near the active place of a recognition protein can provide a highly environmental-sensitive system.

PNIPAAm was taken as the stimuli-sensitive synthetic polymer of choice. Conjugation was carried out using dual-stimuli responsive macromolecules such as the copolymer of *N,N*-dimethylacrylamide (DMA) and 4-phenylazophenyl acrylate (AZAA) or *N*-4-phenylazophenyl acrylamide (AZAAm) [88].The copolymer based on

NIPAAm and AAc provided pH control of biotin binding and triggered release from a genetically modified protein [89].

## *Polymers with dual stimuli-responsiveness*

It is possible to obtain polymeric structures sensitive to both temperature and pH, by the simple combination of ionisable and hydrophobic (inverse thermosensitive) functional groups. It has mainly been achieved by the copolymerization of monomers bearing these functional groups [89-92], combining thermosensitive polymers with polyelectrolytes (SIPN, IPN) [93] or by the development of new monomers that respond simultaneously to both stimuli [94].

Several authors have recently presented their advances in this field, as Leung *et al*. [95] that have prepared smart core-shell microgels based on PNIPAAm, MBAAm and chitosan or poly(ethyleneimine) in the absence of surfactants. The materials were obtained by graft copolymerization and presented a well defined core-shell structure consisting of temperature-sensitive cores (based on PNIPAAm) with pH-sensitive shells (based on cationic water-soluble polymers).

Rodríguez-Cabello´s group [96] has extensively worked in the development of elastin-like polymers (ELPs) by genetic engineering showing their extraordinary potential. Well defined and tailored polymers were obtained covering a wide range of properties. They developed different materials by fermentation, which showed clear environmental advantages. The ELPs presented a modulated pH- and T-sensitivity covering the most interesting range of biomedical applications. ELPs have also been modified with photoresponsive molecules as azobenzenes [96] and spiropyranes [97] getting photosensitive macromolecules. Kurata and Dobashi [98] published the preparation of potential intelligent drug carriers based on *N*-acryloyl-*N´*-alkylamide derivatives of both L-glutamic acid and L-aspartic acid.

New copolymeric systems derived from *N,N*-dimethylaminoethylmethacrylate (DMAEM) and acrylic acid (AAc) or itaconic acid (IAc) were obtained by UV-irradiation. They responded to both pH and temperature as a polyampholyte according to the monomeric compositions and combination of temperature and pH conditions [98].

Kuckling *et al*. presented a systematic study of how LCST varies depending on hydrophilic/hydrophobic balance. With this purpose they prepared copolymers of

NIPAAm with acrylamide derivatives bearing carboxylic groups attached to spacers with different chain length [91] and deeply studied the influence of both temperature and pH on their properties.

Other authors combined NIPAAm with butylmethacrylate and acrylic acid in order to obtain pH-/temperature-sensitive vehicles for peptide delivery. They confirmed that loading efficiency increased with increasing ionic strength, which is predominantly governed by hydrophobic interactions and/or specific interactions between the polymer molecules [99].

Alginate has been modified using PNIPAAm forming dual stimuli responsive SIPNs that could be useful in biomedical fields for stimuli-responsive drug delivery systems [100]. Moreover, Benrebouh *et al.* [101] synthesised and characterised copolymers based on NIPAAm and methacrylate monomers derived from cholic acid (Figure 9) in order to improve the biocompatibility of the polymer.

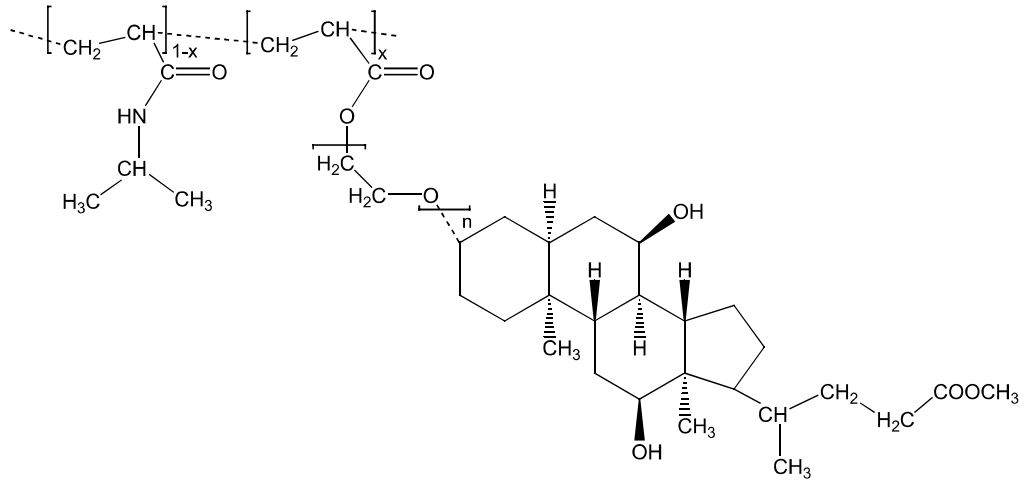


**Figure 9:** Chemical structure of the copolymer of NIPAM and methacrylate monomers derived from cholic acid [101]

Ning *et al.* presented in 2001 [102] the synthesis by γ-irradiation and characterization of poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA), a temperature-sensitive material in a temperature range of 38-40ºC and pH-sensitive at pH=2.5. Electricity-responsive behaviour at a field voltage of approximately 3.0 V was shown to take place. As the synthesised materials were transparent, elastic, and had a

good swelling capacity their utilization as drug delivery systems was proposed. Poly(*N,N*-diethylaminoethyl methacrylate) PDEAEMA (Figure 4b) has also been copolymerized with PEO in order to obtain pH- and T-sensitive copolymers for injectable delivery applications [103]

Gan *et al*. [104] prepared and studied a new water soluble pH- and T-sensitive polymer based on poly(acryloyl-*N*-propylpiperazine) (PAcrNPP) (Figure 10) that exhibited a lower critical solution temperature (LCST) in water at 37ºC. Our group has also been working in this field, developing new polymers based on *N*-ethylpyrrolidine methacrylate (EPyM) that present pH- and T-sensitivity [94,105]. The homopolymer (PEPyM) (Figure 3c), exhibited a phase separation transition temperature in water at 15ºC being also sensitive to pH changes (the polymeric structure was collapsed at basic pH and highly swollen at acidic pH). PEPyM presented a pulsatile swelling-deswelling behaviour when the stimuli were removed or reversed (on-off). LCST of the polymer could be modulated by copolymerization reactions with *N,N*-dimethyl acrylamide (DMA).

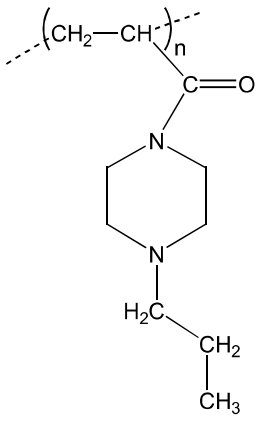


**Figure 10:** Scheme of poly(*N*-acryloyl-*N*-propylpiperazine) structure [104]

## *Conclusions*

This chapter has attempted the compilation of the most recent advances performed in the field of smart polymers and their application in the biomaterials area as drug delivery carriers and in the tissue regeneration processes. pH-sensitive polymer systems characteristics and applications as drug delivery systems, in particular as gene carriers,