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Temperature and pH stimuli-responsive polymers and their applications in controlled and self-regulated drug delivery

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ABSTRACT

Controlled drug delivery is useful because it allows to obtain better drug product effectiveness, reliability and safety. Interest in stimuli-responsive polymers is steadily gaining increasing momentum especially in the fields of controlled and self-regulated drug delivery. Stimuli-responsive or “smart” polymers are macromolecules that display a significant physicochemical change in response to small changes in their environment such as temperature, pH, light, magnetic field, ionic factors, etc. The changes are reversible, and therefore, the smart polymers are capable of returning to its initial state as soon as the trigger is removed. They have become one important class of polymers and their applications have been increasing significantly in the last three decades. Smart polymers have very promising applications in the biomedical field as delivery systems of therapeutic agents, tissue engineering scaffolds, cell culture supports, bioseparation devices, etc. The versatility and untapped potential of smart polymeric materials makes them one of the most promising interfaces of chemistry and biology.

Keywords: Hydrogels. Smart polymers. Stimuli-responsive polymers. Poly (*N*-isopropylacrilamide). Poloxamers.

INTRODUCTION

The ideal drug delivery system is the one in which the drug delivery profile is able to respond to metabolic states and/or physiological variations (Bawa *et al.*, 2009). This kind of system relies on two premises: the first is the temporal drug modulation according the physiological needs and the second is the drug distribution on a specific target.

Smart polymers, or stimuli-responsive polymers, are in the vanguard of drug administration technology since they have show an active response to small signs and changes in the surrounding environment, which translates into significant changes in their microstructure and in the physiological and chemical proprieties, as desired (Grainger, El-Sayed, 2010; Kuckling, Urban, 2011). In other words, smart polymers are able to respond to a stimulus by showing physical or chemical changes in its behavior as, for example, the delivery of the drug carried by itself (Gupta *et al.*, 2002).

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An important feature of these smart polymers is that the macroscopical changes are reversible, i.e., these systems are able to recover their initial state when the sign or stimuli ends (Stuart *et al.*, 2010).

Smart polymers are biocompatible, non-thrombogenic, strong, resilient, flexible, easy shaping and coloring. They keep the drug's stability and are easy to manufacture, good nutrient carriers to the cells, easily changed using cell adhesion ligands and it is possible to inject them *in vitro* as liquid to create a gel with the body temperature (Mahajan, Aggarwarl, 2011).

The addition of smart polymers with drug molecules presents as main advantages the ability to administrate an efficient concentration of a certain drug on the right time and spot, reducing the adverse systemic reactions and increasing the patient's adherence to the therapeutic, allowing also the reduction of the drug dose and, consequently, the costs (Al-Tahami, Singh, 2007).

The signs or stimuli that trigger the structural changes on smart polymers can be classified in three main groups: physical stimuli (temperature, ultrasounds, light, mechanical stress), chemical stimuli (pH and ionic strength) and biological stimuli (enzymes and bio molecules) (Figure 1) (Jeong, Gutowska, 2002; Kumar *et al.*, 2007).

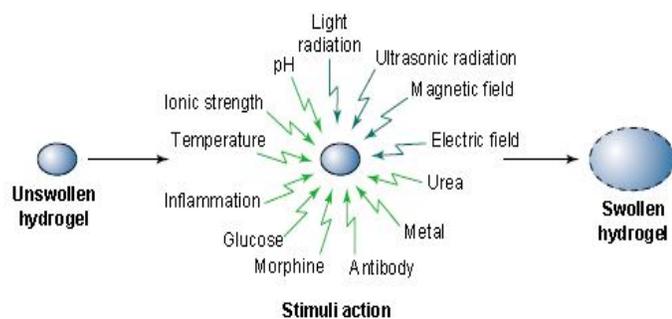


Fig. 1: Response of a smart polymer to different stimuli that triggers the drug delivery (Gupta *et al.*, 2002).

These signs or stimuli can be artificially controlled (with a magnetic or electric field, light, ultrasounds, etc.) or naturally promoted by internal physiological environment through a feedback mechanism, leading to changes in the polymer net that allow the drug delivery without any external intervention (for example: pH changes in certain vital organs or related to a disease; temperature change or presence of enzymes or other antigens) or by the physiological condition (Kopecek, 2007; Kim *et al.*, 2009).

In the presence of a sign or stimuli, changes can happen on the surface and solubility of the polymer as well as on sol-gel transition (Aguilar *et al.*, 2007; Fogueri, Singh, 2009; Shaikh *et al.*, 2010).

Smart polymers can be classified according to the stimuli they're responding or to their physical features. Regarding the physical shape, they can be classified as free linear polymer chain solutions, reversible gels covalently cross-linked and polymer chain grafted to the surface (Jeong, Gutowska, 2002).

Table 1 presents different smart polymers according the stimuli they're responding.

Table 1: Examples Of Smart Polymers and Respective Stimuli They're Responding.

Type of Stimulus	Polymers
pH	Dendrimers Poly(L-lisine) Ester Poly(hydroxyproline) Lactose-PEG grafted poly (L-lysine) nanoparticle Poly(propyl acrylic acid) Poly(ethacrylic acid) Carbopol® Polysilamine Eudragit® S-100 Eudragit® L-100 Chitosan PMAA-PEG copolymer
Organic solvent	Eudragit® S-100
Temperature	PNIPAAm Ploxamers Prolastin PNIPAAm hydrogels containing ferromagnetic material PNIPAAm-co-acrylamide
Magnetic field	
Electrical signals	Chitosan
Ions	Sodium alginate (Ca ²⁺) Chitosan (Mg ²⁺)
Photosensitive	PEG Poly(lactic acid)

Table 2 shows some examples of polymers which respond to different stimuli.

Table. 2: Examples Of Smart Polymers Which Respond To More Than One Stimuli.

Type Of Stimulus	Polymers
Ca ²⁺ And Peg	Carboxymethyl Celulose
Ca ²⁺ And Temperature	Eudragit® S-100
Ca ²⁺ And Acetonitrile	Eudragit® S-100
Ph And Temperature	Poly(N-Acryloyl-N-Propyl Piperazine)
	Poly(Vinyl Alcohol)-Graft-Poly-Acrylamide-Triphenylmethane
Light And Temperature	Leucocyanide Derivatives

Smart polymers can be used to different biomedical proposes, from which we can highlight: tissue engineering; extended drug delivery; usage as biosensors of different molecules as glucose or antigens; usage in disposable diapers providing the absorption of the urine and other fluids; in contact lenses; in drain tubes and gloves; in breast implants; in compresses for burns, creating or maintaining an humid environment in the damaged areas; in artificial tendons or cartilage; and replacing vocal cords (Roy, Gupta, 2003; Bajpai *et al.*, 2008).

THERMO-RESPONSIVE POLYMERS

These smart polymers are sensitive to the temperature and change their microstructural features in response to change in temperature. These are the most studied, most used and most safe polymers in drug administration systems and biomaterials.

Thermo-responsive polymers present in their structure a very sensitive balance between the hydrophobic and the hydrophilic groups and a small change in the temperature can create new adjustments (Bajpai *et al.*, 2008).

An important feature of this kind of polymers is the critical solution temperature. If the polymeric solution has a phase

below the critical solution temperature, it will become insoluble after heating, i.e., it has one lower critical solution temperature (LCST). Above the critical solution temperature (LCST), the interaction strengths (hydrogen linkages) between the water molecules and the polymer become unfavorable, it dehydrates and a predominance of the hydrophobic interaction occurs, causing the polymer swelling (MacEwan *et al.*, 2010). The LCST can be defined as the critical temperature in which the polymeric solution shows a phase separation, going from one phase (isotropic state) to two phases (anisotropic state). The hydrophilic or hydrophobic incorporation on a polymeric system of this kind has as consequence the changing of the LCST. Figure 2 shows the transition to the hydrogel phases. We can easily see that from a certain temperature the phase transition occurs, causing an alteration in the polymeric changes and promoting, for example, the drug delivery.

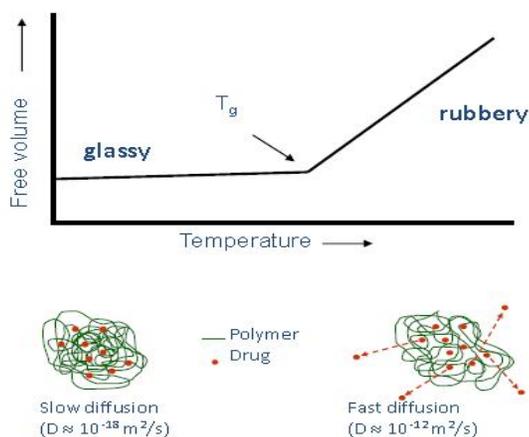


Fig. 2: The Effect of Phase Transition Temperature on the Polymers Volume and Drug Delivery (Hoogenboom, 2011).

The polymers with a lower critical solution temperature (LCST) are the most used on drug delivery systems. The therapeutic agents, as drugs, cells or proteins can be mixed with the polymer when this is on its liquid state (temperature below the transition temperature) being able to be injected in the human body on the subcutaneous layer or in the damaged area and forming a gel deposit on the area where it was injected after increasing the temperature (Jeong, Gutowska, 2002). This kind of pharmaceutical system delivers the drug on a controlled way without being too invasive. Smart polymers that are sensitive to the temperature can be used to increase chemotherapeutic agents in solid tumors. Thus, the local stimulation with heat, ultrasounds and light or tumor stimulation through, for example, a low pH or proteases suppression, leads to an increase of these pharmaceutical systems on the tumor tissue (MacEwan *et al.*, 2010). The accumulation of temperature sensitive polymeric systems in solid tumors are due to the increased impermeability effect to the tumor vascular net retention and to the use of an external impulse (heat source) on the tumor area. This temperature increase promotes the changing of the microstructure of the polymeric system, turning it into gel and releasing the drug, thus increasing the drug in the intra-tumoral

area and the therapeutic efficiency, and reducing the side effects (MacEwan *et al.*, 2010).

As example of this kind of polymers one can highlight the poly(*N*-substituted acrylamide) polymers' family, in which have a special reference the polymers poly(*N*-isopropylacrilamide) (PNIPAAm), poly(*N,N'*-diethyl acrylamide), poly (dimethylamino ethyl methacrylate and poly (*N*-(*L*)-(1-hydroxymethyl) propyl methacrylamide).

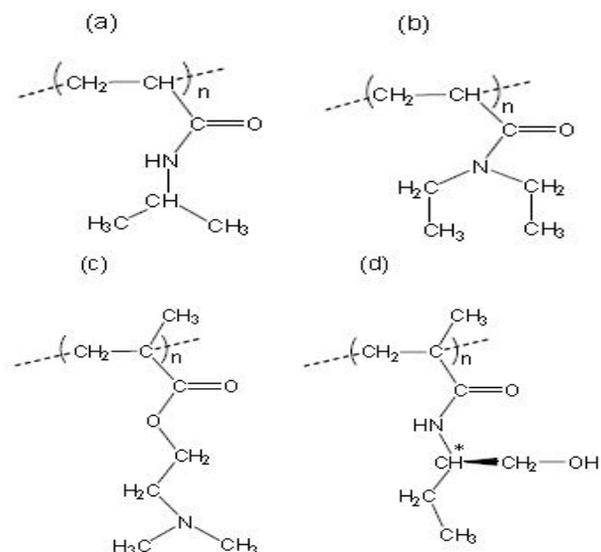


Fig. 3: Molecular structure of some smart polymers and respective LCST. (a) PNIPAAm, LCST 32°C; (b) PDEAAm, LCST 26-35°C; (c) PDMAEMA, LCST 50°C; (d) poly(*N*-(*L*)-(hydroxymethyl)propylmethacrylamide), LCST 30°C (Aguilar *et al.*, 2007).

Other examples of thermo-responsive polymers are: copolymers blocks of poly(ethylene glycol)/poly(lactide-co-glycolide) (PEG/PLGA, Regel[®]), polyoxyethylene-polyoxypropylene (PEO/PPO), triple blocks of copolymers polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO-PPO-PEO) and poly(ethylene glycol)-poly(lactic acid)- poly(ethylene glycol) (PEG-PLA-PEG). There are already in the market some polymers with these properties, from which we highlight BST-Gel[®] from BioSyntech and ReGel[®] from Macromed.

Poly(*N*-isopropylacrilamide) (PNIPAAm)

Poly(*N*-isopropylacrilamide) (PNIPAAm), from the family of poly(*N*-substituted acrylamide) is a synthetic temperature-sensitive polymer and the most studied and most used on drug delivery systems and biomaterials because it presents as main feature the fact that it is soluble in water at room temperature. This happens due to the hydrophilic interactions, i.e. the predominance of hydrogen. Above the lower critical solution temperature (LCST- 32°C) the solutions become opaque and turns into a gel with a transition temperature very similar to the body temperature, with predominance of the hydrophobic interactions (Kulkarni, Aloorkar, 2010).

We can conclude that at temperatures below LCST, the hydrogen bonds predominate between the polymer amide groups

and the water molecules. When the temperature is above LCST the hydrogen bonds get departed and the polymer expels water molecules and dehydrates.

This polymer, incorporated in therapeutic systems, when injected in the human body turns into a gel at the body temperature, forming a small deposit and providing a controlled delivery of the drug.

The transition temperature (LCST) can be adjusted through the co-polymerization of the hydrophobic monomers or through controlling the polymer molecular weight (Reul-Gariépy, Leroux, 2004). For example, an increase of the hydrophobic monomers (as, for example, the butyl methacrylate) or on the molecular weight, results in a LCST decrease (Jeong, Gutowska, 2002). On the other hand, the incorporation of hydrophilic monomers (as, for example, acrylic acid or hydroxyethyl methacrylate) fosters the creation of hydrogen bonds with thermosensitive monomers which increases LCST (Kim *et al.*, 2009). The co-polymers NIPAAm conjugated with hydrophilic unities (as, for example, acrylic acid) promotes the increase of LCST to temperatures around 37°C, i.e., the body temperature. The same way, polymers with 2-hydroxyethyl (methacrylate) (HEMA) promotes the increase of LCST above the body temperature (Aguilar *et al.*, 2007).

The topic administration of levotiroxine (a synthetic hormone) helps to decrease the adipose tissue deposits on the skin. Levotiroxine is usually administrated orally to treat hypothyroidism and gout, since it stimulates the lipid metabolism and induces lipolysis. Azarbayjani *et al.* (2010) have prepared smart polymeric nanofibers from a blend of PVA (poly vinyl alcohol) and PNIPAAm. Different polymeric solutions were prepared with different concentration of both polymers and the drug was dissolved with water or ethanol. These researchers concluded through *in vitro* analysis that the nanofibers only with PNIPAAm had delivered 97% of the drug, while the nanofibers with PVA had delivered only 65% of the drug (lower degradation of this kind of nanofiber). Azarbayjani *et al.* (2010) concluded also that nanofibers are able to promote a sustained delivery of levotiroxine through diffusion and erosion mechanisms, maintaining the appropriated levels of the drug for a long time and promoting the accumulation of active substances in the superficial skin layers, thus decreasing the side effects of systemic absorption.

Thermosensitive monolithic hydrogels can be used to promote drug delivery profiles called "ON-OFF". The hydrogel constituted by poly(*N*-isopropylarilamide) crosslinked to butyl methacrylate (BMA) can, for example, deliver indomethacin in the presence of low temperatures (ON) and stop delivering when the temperature increases (OFF). This happens due the thick low permeable gel that is formed when the temperature changes (Bawa *et al.*, 2009).

These thermosensitive polymers can be used to turn soluble some hydrophobic drugs as paclitaxel (an anticancer drug) and to produce excellent formulations with low solubility drugs (Kim *et al.*, 2009). The anticancer drugs not only have low solubility but have also problems of accessibility due to the

heterogeneity, toxicity and immunogenicity of the tumors, which limit and affect the therapeutic effect.

Other example of a micellar polymeric system can be obtained through the use of polymeric blocks as hydrophobic polymers, such as butyl methacrylate (BMA), polystyrene or polylactic acid prepared with PNIPAAm, incorporating the drug adriamycin. This polymeric block forms a micellar structure in water solution at a lower temperature than the transition temperature of PNIPAAm and the proprieties of hydration/dehydration changes. In its hydrated form the polymer acts like an inert material, however, above 32°C the polymeric chains become hydrophobic due to dehydration, and precipitation occurs (Bawa *et al.*, 2009).

Poloxamers and derivatives

The use of hydrosoluble vehicles of high viscosity, as hydrophilic gels, is one of many strategies to obtain a controlled delivery and represents an area very important in scientific research (Escobar-Chávez *et al.*, 2006). The copolymer blocks based on PEO-PPO sequences constitutes one family of triple blocks of commercialized copolymers with the following names: Pluronic[®], Poloxamers[®] and Tetronics[®].

Poloxamers[®] are non ionic polymers polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO_n-PPO_n-PEO_n), with many pharmaceutical uses (Ricci *et al.*, 2005). These polymers are composed by white granules soluble in water with no odor or taste. They present a sol-gel transition phase below or near the physiologic body temperature and a gel-sol transition around 50° C in relatively high concentrations (Kulkarni, Aloorkar, 2010).

The triple block of copolymers PEO-PPO-PEO (Pluronic[®] or Poloxamers[®]) get into gel at body temperature in concentrations above 15% (m/m); however this kind of formulas, when injected by intraperitoneal via, presents high toxicity and increases the plasma concentration of cholesterol and triglycerides. The Poloxamers[®] normally used are: 188 (F-68), 237 (F-87), 338 (F-108) and 407 (F-127). "F" refers to the polymer in the form of flakes.

Pluronic[®] and Tetronics[®] are polymers approved by FDA to be used as food additives, pharmaceutical ingredients, drug carriers in parenteral systems, tissue engineering and agricultural products. Pluronic F-127 (Polaxamer 407, PF-127) can also be used as carrier in several routes of administration, including oral, cutaneous, intranasal, vaginal, rectal, ocular and parenteral. In the last years, PF-127 have had a special role on dermal and transdermal drug delivery systems.

Pluronic[®] F127 (PF-127) or Poloxamer 407 (P407) (copolymer polyoxyethylene₁₀₆-polyoxypropylene₇₀-polyoxyethylene₁₀₆) contains about 70% of ethylene oxide which contributes to its hydrophilicity. PF-127 is a copolymer with 12,000 Daltons, a PEO/PPO ratio of 2:1, non toxic, with low viscosity below 4°C and forming a semisolid gel at body temperature.

PF-127 is more soluble in cold water than in hot water due to the hydrogen linkages at low temperatures (Escobar-Chávez *et*

al., 2006). The aqueous solution of PF-127 or P407 at a concentration between 20 and 30% (m/m) turns reversibly into gel at a certain temperature, i.e., it gets liquid at lower temperatures (4-5°C) and turns into gel at the room temperature (this transformation is reversible returning to a liquid state at low temperatures) (Escobar-Chávez *et al.*, 2006). This characteristic, as well as its low toxicity, makes PF-127 a common polymer used on drug delivery systems.

The drug delivery from polymer P407 occurs by diffusion and dissolution through the gel that is formed on the area where it was administrated. Poloxamer 407 presents a complete dissolution in water in 4 hours and this feature can be used to prepare pharmaceutical formulas that act in short time after administration (short term therapy). Ricci *et al.* (2005) showed that the diffusion coefficient of a drug decreases when the concentration of this

polymer is increased. These authors concluded as well that the time of dissolution increases by decreasing the drug diffusion through the gel matrix. The incorporation of organic solvents or salts (sodium chloride or PEG 400 – hydrophilic substances) changes the sol-gel transition temperature of P407, increasing also the drug delivery rate (lidocaine hydrochloride). Thus, Poloxamer 407 can be used to increase the time of action of lidocaine, increasing the drug efficiency and decreasing the side effects.

Some studies have been developed to incorporate Pluronic® F127 in short term therapies as, for example, fertility control, pain management and infections treatment (Ruel-Gariépy, Leroux, 2004). This kind of polymer has been already tested on external uses (local and sustained delivery) of anticancer and anti-inflammatory drugs (Ruel-Gariépy, Leroux, 2004; Aguilar *et al.*, 2007). It has been also tested in ophthalmology using pilocarpine (Escobar-Chávez *et al.*, 2006). There have been reported studies related to parenteral formulations (intramuscular and subcutaneous) with interleukin-2 and antibiotics (Ricci *et al.*, 2005). In the same way, this poloxamer has been successfully tested to carry peptides and proteins (insulin, urease and growing factors), presenting a sustained delivery profile for several hours (Kulkarni, Aloorkar, 2010c).

Table 3: Some examples of the use of Pluronic® F127 (PF-127) in pharmaceutical systems (adapted from Escobar-Chávez *et al.*, 2006).

Pharmaceutical Formulations with PF-127	Results
Indomethacin, anticancer agents (adriamycin, 5-fluorouracil) and mitomycin C in PF-127.	PF-127 is a good vehicle for topical and rectal administration of indomethacin and excellent for sustained-release of mitomycin. <i>In vitro</i> studies show that increasing the polymer concentration caused a decrease in the rate of release of the anticancer drugs (adriamycin and 5-fluorouracil). The rate of drug release increases with increasing temperature from 30 to 40 °C.
<i>In vitro</i> and <i>in vivo</i> skin absorption of capsaicin and nonivamide from hydrogels.	Moderate correlation between <i>in vitro</i> skin permeation and <i>in vivo</i> erythema responses of topically applied capsaicin and nonivamide. The incorporation in PF-127 delays the release of drugs.
Development and <i>in vitro</i> evaluation of sustained release PF-127 gel formulation of ceftiofur.	The overall rate of release of ceftiofur is controlled by the dissolution of PF-127. The ceftiofur release show a zero-order kinetics and correlated with the percentage of PF-127 dissolved, indicating that drug release is related to the dissolution of the polymer. An increased concentration of polymer in the pharmaceutical system decreases the rate of drug release.
PF-127 gel formulations of deslorelin and GnRH reduce drug degradation and sustain drug release.	PF-127 gel formulations can sustain peptide release and reduce peptide degradation.
Development of a thermoreversible gelling formulation in PF-127 solution containing Paclitaxel (Taxol, Bristol-Myers Squibb).	Significant enhancement in the anti-tumor efficacy was noted following intratumoral administration of paclitaxel-PF-127. Initial tumor growth rate was delayed by 67%.
Development of a transdermal gel of PF-127, using ketoprofen as the active substance.	This formulation provided an anti-inflammatory and analgesic sustained effect, greater physical and chemical stability and fewer side effects compared to oral administration of ketoprofen.
Preparation of a gel of Poloxamer 407 with the recombinant human growth hormone	After intramuscular or subcutaneous injection, the gel promoted the controlled release of the hormone.
Preparation of a gel of Poloxamer 407 or Poloxamer 188 with clotrimazole.	Prolongation of the antifungal effect.

PH-RESPONSIVE POLYMERS AND DRUG DELIVERY SYSTEMS

In the human body we can see remarkable changes of pH that can be used to direct therapeutic agents to a specific body area, tissue or cell compartment (Table 4). These conditions make the pH sensitive polymers the ideal pharmaceutical systems to the specific delivery of therapeutic agents.

The ionic pH sensitive polymers have as main feature the fact that they are able to accept or release protons in response to pH changes (Urban, 2010; Grainger, El-Sayed, 2010). These polymers contains in their structure acid groups (carboxylic or sulphonic) or basic groups (ammonium salts) (You *et al.*, 2010). In other words, pH sensitive polymers are polyelectrolytes that have in their structure acid or basic groups that can accept or release protons in response to pH changes in the surrounding environment.

Table 4: pH values from several tissues and cells compartments (adapted from Bawa *et al.*, 2009).

Tissue / Cell compartment	pH
Blood	7.4-7.5
Stomach	1.0-3.0
Duodenum	4.8-8.2
Colon	7.0-7.5
Lysosome	4.5-5.0
Golgi complex	6.4
Tumor – Extracellular médium	6.2-7.2

This group of smart polymers changes its solubility by changing the electrical charge of the polymer molecule (Shaikh *et al.*, 2010). Thus, the transition from a soluble state to an insoluble state is caused by the decrease of the electrical charge in the polymeric molecules. The polymer's electric charge can be decreased by decreasing its pH, neutralizing the electric charge and reducing the hydrophilicity (increasing hydrophobicity) of the

polymeric macromolecules (Kumar, 2011).

As examples of this kind of polymers we can highlight: polyacrylamide (PAAm), poly(acrylic acid) (PAA) (Carbopol[®]) and derivatives, poly(methacrylic acid) (PMAA), poly(2-diethylaminoethyl methacrylate) (PDEAEMA), poly(ethylene imine), poly(L-lysine) and poly(*N,N*-dimethylaminoethylmethacrylate) (PDMAEMA).

Polymers with functional acid groups

pH sensitive polymers named as polyacids or polyanions, such as, poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMAA) are polyanions that have in their structure a great number of ionizable acid groups, like carboxylic acid or sulfonic acid (Grainger, El-Sayed, 2010). The carboxylic groups accept protons at low pH values and release protons at high pH values (Gil, Hudson, 2004). Thus, when the pH increases the polymer swells due to the electrostatic repulsion of the negatively charged groups. The pH in which acids become ionized depends on the polymer's pKa (depends on the polymer's composition and molecular weight).

Thus, in an oral drug delivery system, the poly(acrylic acid) polymer retains the drug on the presence of acid pH (stomach), delivering it in alkaline pH (small intestine). The drug delivery occurs due to the ionization of pendant groups of carboxylic acids, forcing the polymer to swell.

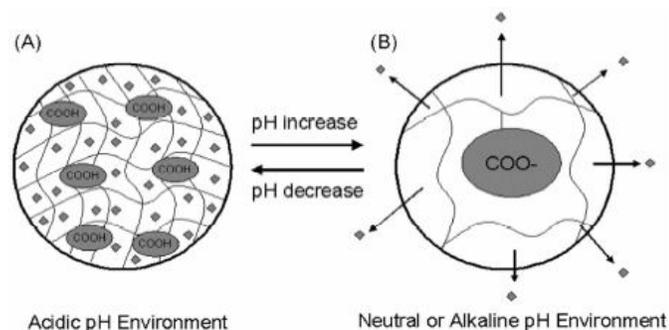


Fig. 4: Illustration of the poly(acrylic acid) polymer carrying a specific drug. (A) The pendent carboxylic acid groups in the stomach are not ionized yet and retain the drug in their interior. (B) However, in the presence of the alkaline or neutral environment of small intestine the pendent carboxylic acid groups become ionized and the polymer swells due to the ionized groups' electrostatic repulsions, delivering the drug molecules to the environment (Grainger, El-Sayed, 2010).

Polymers with functional basic groups

The polymers named as polybases, or also polycations, such as poly(4-vinylpyridine), poly(2-vinylpyridine) (PVP) and poly(vinylamine) (PVAm), are protonated at high pH values and positively ionized at neutral or low pH values, i.e., they go through a phase transition at pH 5 due to the deprotonation of the pyridine groups (Gil, Hudson, 2004).

Other polybases are poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) and poly(2-diethylaminoethyl methacrylate) (PDEAEMA), with amino groups in their structure which in acid environments gain protons, and in basic environments release the protons. Examples of polycationic

polyelectrolyte polymers are poly(*N,N*-diakyl aminoethyl methacrylate), poly(lysine) (PL), poly(ethyleneimine) (PEI) and chitosan (Aguilar *et al.*, 2007).

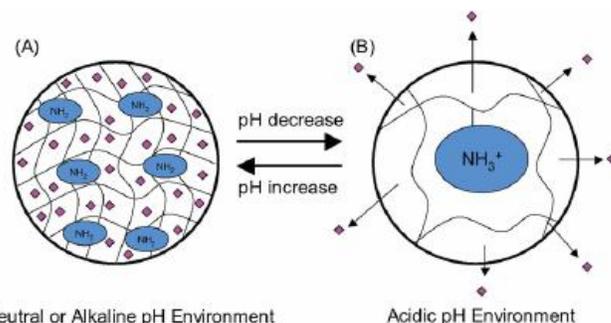


Fig. 5: Illustration of the polymer poly(vinyl amine) with a specific drug. (A) In a neutral or alkaline environment, the pendent amino groups are not ionized and retain the drug molecules in their interior. (B) However, when the pH decreases to values under the pKa they become protonated and the polymeric net swells due to electrostatic repulsions between the positive groups, delivering the drug molecules in the environment (Grainger, El-Sayed, 2010).

Examples of pH-responsive polymers

Some of this kind of polymers are already on the market: Eudragit L[®] and Eudragit S[®] from RÖhm Pharma GmbH (with methacrylic acid and methylmethacrylate in their composition), CMEC (a cellulose derivative) from Freund Sangyo Co., CAP by Wako Pure Chemicals Ltd., HP-50 and ASM by Shin-Etsu Chemical Co., Ltd. Other pH-responsive polymers in marketed formulations are: SQZ Gel[™] (chitosane and PEG – diltiazem hydrochloride tablets) and Cervidil[®] (polyoxyethylene and urethane – vaginal gel with dinoprostone (natural prostaglandine E2)) (Gupta *et al.*, 2002).

Natural pH-responsive polymers

There are several natural polymers (for example, albumine, gelatin and chitosan) that present pH sensibility. Chitosan is a cationic amino polysaccharide, derivative from chitin, that is biocompatible and resorbable (cationic polymer, soluble in water at pH 6.2, becoming a hydrated gel above this value) that can be used in oral or mucosal administration due to mucoadhesive properties. This polymer can also carry DNA as it presents a negative charge that can be easily incorporated on the chitosan's amino groups due to its positive charge (Gil, Hudson, 2004).

Chitosan/glycerophosphate pharmaceutical system is a good alternative to pharmaceutical implants due to its porosity and greater ability to promote the controlled delivery of macromolecules and drugs with low solubility in water. The incorporation of paclitaxel (a hydrophobic anticancer drug) in the chitosan/glycerophosphate system promotes the sustained delivery of this drug, inhibiting the growth of the cancer cells EMT-6 (Reul-Gariépy, Leroux, 2004).

Conjugation of pH-responsive polymers with anticancer drugs

There are some features in the tumor tissues that is necessary to take into account, as the hydrostatic pressure on the tumor is high (difficult the diffusion of the drug on the interstitial

matrix), the metabolic profile is very different (low oxygen concentration, high lactic acid concentration and a consequent pH decrease to values between 6.0-7.4). Thus, to direct the drug to the tumor tissue it is necessary to take advantage of both features: the extra cell pH on the tumor tissue that can be between 6.5 and 7.2 (lower than the body pH that is usually 7.4) and the hydrolytic enzymes on the lysosomes (interior pH between 4.5 and 5.0) that after the cell caption can be used to separate the drug from the carrying polymer. Based on this kind of polymers, other pharmaceutical systems were designed to promote a specific delivery of anticancer drugs (as, for example, paclitaxel) on tumor cells. Thus, a biodegradable pH sensitive polymer was used, poly(β -amino ester), to produce a paclitaxel nanoparticles system (Shenoy *et al.*, 2005). This system showed to be more effective in reducing the tumor cells than the drug on its original state.

Other possibility is to prepare paclitaxel nanoparticles with the polymers poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) and 2-hydroxyethyl (methacrylate) (HEMA) (You *et al.*, 2010). PDMAEMA has on its structure third amino groups with a pKa of 7.5. *In vitro* studies have shown that paclitaxel is only delivered when in the presence of low pH values (for example: tumor tissue). Chawla *et al.* (2002) based on the same principles, developed nanoparticles using the polymer poly(ϵ -caprolactone) (PCL) to increase the local tamoxifen concentration in estrogen receptors (ER)-positive in breast cancer.

In the work of MacEwan *et al.* (2010) the polymeric micelles prepared with the pH-responsive polymers poly(histidine)- β -PEG and poly(L-lactic acid)- β -PEG delivered the drug (doxorubicine) responding to the pH values that in the tumor environment is slightly acid. These researchers concluded that doxorubicine on the pH sensitive micelles has shown to be efficient in the treatment of tumors MCF-7 and A2780 on rats.

pH-responsive polymers and the endocytosis mechanisms

Endocytosis can lead to further accumulation of the pharmaceutical systems inside the endosomes and intracellular lysosome compartments. The environment of these organs, i.e., the redox potential and the acid pH, different from the extracellular environment, promotes an excellent opportunity to explore the specific drug delivery (Ghandehari *et al.*, 1997). The polyelectrolytes have high potential as biomaterials to carry opposite charged molecules. Therefore, one of the most promising applications of the pH-responsive polymers is their use as non viral gene carriers. The nucleic acid molecules show a great potential in therapeutic activity against cancer, inflammation and neurodegenerative diseases. The therapeutic efficiency of this kind of molecules depends on their ability to reach their intracellular target (Grainger, El-Sayed, 2010). The systemic administration of nucleic acids results on its degradation by the enzymes named nucleases, in the non-specific delivery through all the body, in the fast elimination by the urine and in a very limited cellular uptake. All of this features condition the therapeutic efficiency of this kind of therapeutic agents.

DNA molecules are very difficult to incorporate inside the cells due to their negative charge and large size (Jeong, Gutowska, 2002). Thus, a DNA vector can be conjugated and the encapsulation with a cationic polymer is a reliable and efficient strategy to the specific delivery of this kind of molecules. In these pharmaceutical systems, the negatively charged DNA molecules are encapsulated in positively charged polymers.

The encapsulation protects the DNA molecules in the systemic circulation and allows the interaction with the negative cells' surface, promoting the endocytosis. During the endocytosis the endosome's pH is around 5 and 6. Then, the endosome merges with the pre-lysosome segregated by Golgi's complex, forming a lysosome that has in itself several digestive enzymes including those named as nucleases (able to destroy the DNA molecules). Having as main goal to delivery DNA to the nucleus and not to destroy the DNA molecule, it is necessary to avoid the endosome before the lisosomal fusion (Grainger, El-Sayed, 2010).

The endocytosis of this kind of cationic polymers results on a endosome pH blockage, promoting the entrance of hydrogen ions inside the endosome's and the exit of counterions as, for example, chloride ions, increasing the osmotic pressure of the endosome. The osmotic gradient that is formed causes the water entrance on the endosome, the disruption of the endosome membrane and the dissociation of the therapeutic genes.

Other available strategy is the association of a pH-responsive polymer (cationic) with a lipidic double layered system (liposome) which can carry in its interior structure the DNA molecules. Therefore, the positive polymers are used to balance the negative charges and to condense the DNA in nanoparticles with around 100 nm diameter. The anionic polymers can also be used to increase the delivery efficiency of DNA molecules overcoming the endosome membrane through a different mechanism of cationic polymers. These polymers has as a main feature their ability to change the hydrophilic state to a hydrophobic state by destabilizing the endosome membrane and causing its disruption (Grainger, El-Sayed, 2010). These polymers are physical or chemically mixed with the DNA molecule forming a particle in which can be incorporated an additional cationic polymer to promote a greater condensation of the nucleic acid molecules. These particles are thus internalized through the endocytosis mechanism on the targeted cells and will change to a hydrophobic state, promoting the disruption of the endosome membrane and the consequent delivery of its content. Nanoparticles composed by the anionic polymer PEAA (polyethacrylic acid) or by PPAA (polypropyl acrylic acid) promotes the stability of the formulation, increasing the efficiency of the DNA transfection.

Polypropylacrylic acid (PPAA) and polyethacrylic acid (PEAA) are examples of pH-responsive polymers that can be used to carry genes. The hemolytic activity of PPAA and PEAA polymers' increases rapidly when the pH decreases to values between 5 and 6 and doesn't present any hemolytic activity at a value of 7.4 (Jeong, Gutowska, 2002). Aguilar *et al.* (2007) used a biodegradable polycationic polyester polymer named poly(trans-4-

hydroxy-L-proline ester) (PHP ester) mixed with hydroxyproline (collagen, gelatin and other peptides). A complex between the soluble polymer, the PHP ester and the DNA was formed and allowed the gene transfection into the mammal's cells (Aguilar *et al.*, 2007). There were also developed polymeric micelles using the copolymer poly(ethylene glycol)-poly(aspartame hydrazine doxorubicin) [(PEG-p(Asp-Hid-dox) mixed with doxorubicin (Aguilar *et al.*, 2007). This copolymer retained the drug and the genes at a physiological pH and released the drug when the pH was below 6.

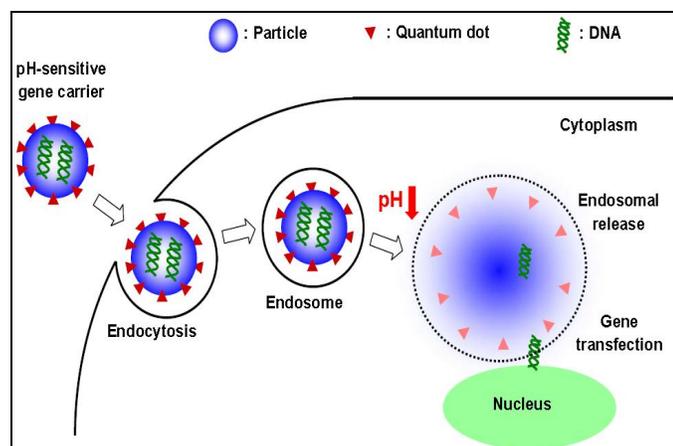


Fig. 6: Illustrative scheme of the genes' delivery through the pH-responsive polymers, as, for example, the DMAEMA/HEMA nanoparticles (You *et al.*, 2010).

Glucose-responsive polymers for insulin delivery

Pharmaceutical systems for insulin delivery constituted by pH-responsive polymers covalently bound to the enzyme glucose oxidase were also developed (Kulkarni, Aloorka, 2010). The glucose oxidase oxidizes the glucose in the blood, forming glucuronic acid which causes changes on the pH environment. As the hydrogel is pH sensitive, it changes its volume, swelling and releasing insulin. One example of this kind of system is the use of polycationic polymers, as, for example, the polymer poly(2-diethylaminoethyl methacrylate) (PDEAEMA), in which the pH decrease causes an increase of the membrane's permeability, delivering the insulin (Fogucri, Singh, 2009). This permeability increase is caused by the ionization of the polymer in an acid environment.

The modified insulin delivery with pH-responsive polymers was also described by Morishita *et al.* (2002). These authors have prepared microparticles with poly(methacrylic acid-g-poly(ethylene glycol)) P(MAA-g-EG) that in the presence of an acid environment promote the delay of insulin delivery through the gel, while in neutral or alkaline pH the delivery is faster. Other pH-responsive polymer used on the modified delivery of insulin is acrylic acid (AA). Foss *et al.* (2004) prepared nanoparticles of poly(AA)-g-PEG to delivery insulin orally and reported that AA with pH above the pKa value of 4.5 become hydrophilic due to the ionization (Foss *et al.*, 2004).

With the same goal, a pH-sensitive hydrogel was prepared from the monomer *N,N*-dimethylaminoethyl methacrylate (DMAEMA) (low weight molecule) obtaining a copolymer by copolymerization of the 2-hydroxyethyl (methacrylate) and the monomer DMAEMA (Gil, Hudson, 2004). This hydrogel was prepared with the following molecules: glucose oxidase, catalase and insulin. The glucose excess diffuses to the hydrogel's interior and the glucose oxidase converts glucose in glucuronic acid, decreasing the pH within the polymeric net, and causing the DMAEMA protonation of the tertiary amino groups. This causes the swelling of the hydrogel, increasing the electrostatic repulsion strength. The swelling increases the pores and causes the increase of the insulin delivery from the polymeric matrix. The catalase incorporation promotes the hydrogen peroxide conversion into oxygen, which is essential to oxidize the glucose. This pharmaceutical system was studied *in vivo* in rats and the decrease of the glucose levels on the blood was shown.

POLYMERS WITH DUAL STIMULI-RESPONSIVENESS

To obtain a temperature and pH sensitive polymer it is only necessary to combine temperature sensitive monomers (as, for example, poly(*N*-isopropylacrylamide-co-methacrylic acid and PNIPAm) with pH sensitive monomers (as, for example, AA and MAA) (You *et al.*, 2010). Combining PNIPAm and MAA with different ratios, nanoparticles containing vitamin B₁₂ were prepared. The increase of the temperature from 37 to 43 °C and the pH decreasing from 6 to 4 increased the permeability, causing the release of vitamin B₁₂ (You *et al.*, 2010).

On the other hand, nanoparticles constituted by a polymeric net of poly(*N*-isopropylacrylamide-co-methacrylic acid) [p(NIPAAm-co-MAA)] were prepared. The permeability through the membrane increases for some solutes (as, for example, peptides, leuprolide, vitamin B₁₂ and insulin) when the temperature is higher and decreases when the pH increases (Shaikh *et al.*, 2010).

The copolymer constituted by 2-(dimethylaminoethyl) methacrylate (DMAEM) and the acrylic acid or the itaconic acid obtained by ultraviolet radiation can also respond to both impulses (pH and temperature) (Aguilar *et al.*, 2007).

With the same goal it was prepared an injectable hydrogel by mixing the monomers NIPAm, acrylamide (Aam) and vinylpyrrolidone (VP) containing naltrexone (an opioid receptor antagonist) (You *et al.*, 2010). When the pH decreases from 8.5 to 7.4 the hydrogel swells, decreasing its size when the temperature increases from 25 to 37 °C. *In vitro* studies have shown that an extended drug delivery occurs during 28 days.

The polymer formed by the monomer *N*-ethylpyrrolidine methacrylate (EPyM) presents a temperature of phase separation in water at 15 °C and at acid pH it swells. This polymer has an "ON-OFF" or pulsate behavior (swelled – not swelled) when in presence or absence of the impulse. Its LCST can be modulated through its co-polymerization with the monomer *N,N*-dimethylacrylamide (DMA) (Aguilar *et al.*, 2007).

The polymer poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) presents a temperature sensibility between 38° and 40°C and it is sensitive to a pH of 2.5, presenting also sensibility to electrical fields with a voltage near 3.0 volts (Aguilar *et al.*, 2007).

The membrane systems with microporous grafted with smart polymers, as, for example, PPA and PNIPAAm, are able to respond to temperature and pH impulses (Shaikh *et al.*, 2010). One other example of a membrane system able to respond to more than one impulse is constituted by a membrane film mixed with chitosan, PEG and PNIPAAm which has a LCST around 32°C due to the presence of the polymer PNIPAAm, responding also to pH changes due to the amino groups of chitosan (Shaikh *et al.*, 2010).

CONCLUSIONS

The stimuli-responsive polymers or “smart-polymers” developed in the last decades constitute a very interesting subject for scientific study, with several applications, such as modified release of drugs, tissue engineering, bioseparation devices, active membranes, biosensors, among others. All these applications show very promising results, opening every day a new window of development.

The use of stimuli-responsive polymers in modified release pharmaceutical dosage forms has many advantages, including the release and distribution of the drug in a specific target, thus reducing the side effects or adverse systemic reactions. The selectivity shown by this type of pharmaceutical systems provides a reduction in therapeutic doses, and a consequent patient compliance. In addition to the advantages mentioned above, such systems are relatively easy to produce which is an incentive for pharmaceutical industry.

There are several types of sensitive-polymers; however, temperature-sensitive polymers and pH-sensitive polymers are those that have shown promising results, having already pharmaceutical systems made of such polymers in the market. The path of the research in this area indicates the combination of polymers that respond to different stimulation (physical, chemical and biochemical) in order to optimize the formulation and increase the effectiveness of therapeutic agents. Future trends indicates the creation of polymeric networks capable to detect or change biochemical parameters in response to the presence of certain biomolecules, releasing the drug according to physiological needs from a given disease. The versatility and unexplored potential of stimuli-responsive polymers make this type of pharmaceutical excipients a group of materials with a great potential for exploring in the fields of chemistry, biology, pharmacy and medicine, having as its main objective the creation of innovative solutions and therapeutic strategies for optimizing the effectiveness and adherence to therapy.

LIST OF ABBREVIATIONS

AA- Acrylic acid
 MAA - Methacrylic acid
 PAA – Poly(acrylic acid)
 BMA – Butyl methacrylate

DNA – Deoxyribonucleic Acid
 FDA – Food and Drug Administration
 HEMA – 2-hydroxyethyl (methacrylate)
 LCST – Lower critical solution temperature
 PMAA – Poly(methacrylic acid)
 P407 – Poloxamer 407
 PDEAAm – Poly(*N,N*-diethylacrylamide)
 PDEAEMA - Poly(2- diethylaminoethyl methacrylate)
 PDMAEMA – Poly(*N,N*-dimethylaminoethyl methacrylate)
 PEG – Poly(ethylene glycol)
 PEO – Polyoxyethylene
 PF-127 – Pluronic® F127
 PHP ester - Poly(trans-4-hydroxy-L-proline ester)
 PLA – Poly(lactic acid)
 PLGA – Poly(lactide-co-glicolide)
 PNIPAAm – Poly(*N*-isopropylacrilamide)
 Poly(AA)-g-PEG – Poly(acrylic acid)-graft-Poly(ethylene glycol)
 PPO – Polyoxypropylene
 PVA – Poly vinyl alcohol
 PVAm – Poly(vinylamine)
 PVP – Poly(*N*-vinylpyridine)
 USCT – Upper critical solution temperature

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