Intelligent Drug Delivery Systems: Polymeric Micelles and Hydrogels

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Abstract: Advanced drug delivery systems try to adjust the site and/or the rate of the release to the physiological conditions of the patient, to the progression of the illness, or to the circadian rhythms. Being different from classical pre-programmed controlled release dosage forms, the new devices aim to provide a drug release profile best for the needs of each patient. Intelligent drug delivery systems are mostly based on stimuli-responsive polymers which sense a change in a specific variable and activate the delivery; this phenomenon being reversible. This review reports on recent advances in the development of open-loop and closed-loop control systems based on stimuli-responsive polymers and their application in the drug delivery field as pulsatile and self-regulated devices. The aim of this review is to describe the most recent advances in the development of intelligent micelles and hydrogels which are sensitive to pH, specific molecules (with a mention to the molecular imprinting), temperature, irradiation or electric field, and the applications of which these mechanisms are intended.

1. INTRODUCTION

In recent years the incorporation into the market of medicines based on new molecules has been limited or even declining. This trend is explained by the small proportion of bioactive substances among the candidates of novel drugs that goes beyond the preclinical phase (one in thousand) and that enters the market (one in five thousand) [1]. A third of all failures are due to biopharmaceutical problems mainly related to the structural complexity of the new chemical entities [1]. To overcome these limitations, innovative formulation technologies are required to develop medicines that can be used at acceptable levels of efficiency and safety [2]. The profound evolution underwent by the science of dosage forms tries to conform to these needs and also to restate drug formulations already in-use in order to extract a therapeutic potential which has not always been completely exploited [3].

The drug delivery systems (DDS) able to release an active molecule at the appropriate site and at a rate that adjusts at every moment to the progression of the disease or to certain functions of the organism have turned out to be particularly attractive [4]. This new approach involves a change of philosophy regarding the design criteria, compared to the first generation of controlled delivery systems which generally only fulfill the function of providing pre-established release profiles for prolonged periods of time. From a therapeutic point of view, a discontinuous release as a function of specific signals can be profitable in many situations. This being the case of the treatments in which: i) the drug is very unstable in the biological medium and a premature release that leads to drug degradation before reaching the site of action should be avoided (as is the case of peptides and therapeutic proteins); ii) the high toxicity of the drug obliges a reconciliation of sufficient concentration at the site of action with a minimal exposure to the other organs or cells (the most typical example is that of the cancer chemotherapeutics); iii) the drug should reach tissues, cells or cellular structures that are not easily accessible from the general circulation (for example, in gene therapy); or iv) the levels of drug should be fitted to biorhythms, as occurs when insulin, estrogens, gastric acid inhibitors, β-blockers, or drugs for heart rhythm disorders or asthma are administered [5].

The DDS that modulate drug release as function of specific stimuli are called "intelligent" or “smart” and can work in an open or closed circuit (Fig. **1**) [6,7]. Open-loop systems, also known as pulsatile, control drug release by sensing a specific external stimulus; the release rate being independent to the conditions of the biological environment. On the other hand, closed-loop or self-regulated systems directly detect certain changes that take place in the biological medium (for example, in the pH, the temperature or the concentration of some substances) activating or modulating the response, i.e. switching on/off drug release or automatically adjusting the release rate. Thereby, in these latter systems, a biological variable directly regulates the delivery process.

The development of intelligent DDS demands materials able to react to the stimuli triggering a response that should be predictable, reproducible, proportional to the intensity of the signal, and reversible. Such materials are rarely commercially available. Therefore, the development process begins with the design or even with the invention of a new material, which generally is a semisynthetic or synthetic polymer [8,9]. Biopolymers are valuable as models for the design of tailored synthetic materials useful to mimic the behavior of the biological systems. Humans possess their own cellular mechanisms for specific recognition, for selective capture and for controlled transfer of substances; macromolecules play a fundamental role in all these functions. Synthetic polymers can overcome some limitations that hinder the direct use of biomacromolecules: i) their use is safer; ii) their structure can be precisely predicted; iii) they can be obtained

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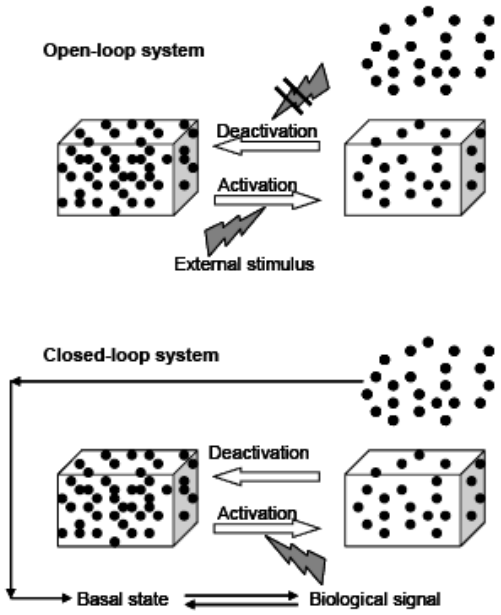

Fig. (1). Schematic view of open-loop or closed-loop intelligent systems.

by versatile procedures that lead to materials with tailored features; and iv) their industrial production is more economic [10].

Until recently the possibilities of advancing the use of polymers in pharmaceutical technology have been hindered by the inability to easily obtain reproducible polymeric materials due to the poor knowledge of their structural properties [11]. In the XIX century, the Chemistry experienced an intense development leading to the synthesis of numerous polymers, but the chemists of the moment did pay attention to these new materials mainly because their amorphous structure. At that time, the new materials were identified by their melting point and those with unclear melting temperature were routinely discarded as impure substances. It was necessary to wait until the beginning of XX century when Hermann Staundinger (1881-1965) discovered that polymers are formed by long molecular chains [12]. The Second World War meant an intense breakthrough in the development of new materials destined for a great variety of sanitary applications. In the second half of XX century, synthetic polymers became popular as components of the sutures, plasma expanders, dialysis tubings, hip replacements, dental fillings, contact lenses, sustained release systems or tissue scaffolds [13]. The elucidation of the structure of numerous biomacromolecules facilitated the understanding of some biological complex mechanisms in which macromolecules play a fundamental role, and led to a quick evolution of the biomedical sciences. The confluence of polymer science with biomedical sciences became unavoidable, as Hermann Mark stated, for doing research into "a multilingual borderland beyond which only a well-established interdisciplinary team can expect to progress" [14]. Thirty years after this statement was made, the joint work in polymer science and in pharmaceutical technology has already given relevant fruits. Biomaterials is greatly impacting the therapeutic field as components of targeting drug delivery systems, stealth particles, polymer-drug conjugates, drug-eluting stents, non-viral gene vectors or biohybrid artificial organs [15-17]. The level of the procedures of synthesis and of the analytical techniques enables a well-characterized variety of polymers with a wide range of structures (multiblock, hyperbranched, cross-linked, hybrid) to be obtained, which can carry out functions that few years ago were difficult to imagine [18,19].

A polymer can be useful as a component of an intelligent DDS if it is endowed with functionality; i.e. a specific macromolecular feature derived from certain chemical and structural characteristics. The functionality of stimuli-sensitive polymers can be shown to have different levels of complexity; for example, as an aptitude for changing the solubility, the shape, the volume or the state of aggregation in response to an external stimulus (e.g., irradiation, heat, electrical or magnetic field, or compression) or to an internal alteration of the microenvironment (the pH or presence of certain ions or molecules). Furthermore, the polymeric system must be capable of transforming the stimulus into a specific function that can be performed in a biological environment; i.e. the system should behave as sensor and actuator [20]. The stimuli-sensitive polymers that respond to the signal reversibly, activated when the stimulus is applied/ appears and deactivated when it stops/ disappears, are accorded with intelligence [21]. Therefore, polymers that undergo irreversible changes or are intended for unidirectional work, such as for example pH-dependent solubility polymers for the coating of solid dosage forms, can not be considered intelligent, strictly speaking. This review will mainly focus on intelligent systems based on polymeric micelles and hydrogels that have been tested for successive switch on and off performance. Examples, particularly relevant, are shown below.

2. INTELLIGENT POLYMERIC MICELLES AND HYDROGELS

2.1. Nature and Structure

Intelligent polymeric micelles and hydrogels are the result of the association of polymeric chains that are sensitive to stimuli. The polymers are kept together through weak interactions in the polymeric micelles and also in the polymersomes, whereas hydrogel formation requires stronger physical or chemical cross-linking among the chains (Fig. **2**).

Amphiphilic copolymers spontaneously aggregate in water forming nanometric aggregates with a hydrophobic core surrounded by a hydrophilic shell (polymeric micelles) or forming vesicles similar to liposomes but with alternating layers of water and amphiphilic copolymers organized as a palisade (polymersomes) [22]. Both polymeric micelles and polymersomes are useful as drug carriers since they can host nonpolar substances in the hydrophobic regions and relatively polar substances in the hydrophilic regions. Polymeric micelles tend to accumulate in tissues with enhanced permeability and retention (EPR effect) of macromolecules when parenterally administered [23]. Their size, similar to virus, lipoproteins and other biological systems of transport, provide polymeric micelles to behave as drug carriers towards the interior of the cells. Intelligent micelles are able to retain the drug until a change in the physiological conditions or an

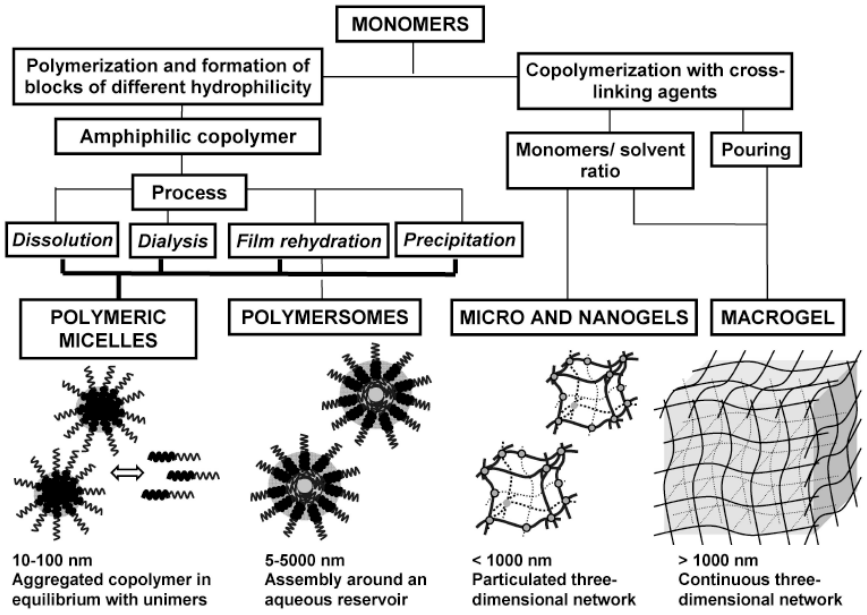

Fig. (2). Preparation routes and structure of polymeric micelles, polymersomes, and hydrogels.

external stimulus alters the hydrophilicity or conformation of the unimers (for comprehensive reviews see [24, 25]). The number of micelles that disintegrate or destabilize and, consequently, the drug release profile depends on the intensity of the stimulus. As soon as the stimulus stops, the micelles are re-formed and the release is interrupted.

Hydrogels are three-dimensional polymer networks in which water can be imbibed at relatively high proportions [26]. For a hydrogel to behave as sensor and actuator, its degree of cross-linking should be low enough to enable the polymeric network to undergo remarkable conformational changes due to the stimuli, but high enough to provide the network with mechanical stability to be able to maintain its functionality after several cycles. In general, the drug release rate from a chemically cross-linked hydrogel depends on the size of mesh, i.e. on the free space among adjacent chains, which determines the drug diffusion rate. Hydrogels able to modify their degree of swelling in a fast and reversible way in response to external stimuli or specific changes in the biological environment can be obtained by introducing adequate functional groups and tuning the porosity and morphology [19]. This new generation of materials has interesting applications in the development of advanced biomedical devices, scaffolds for cellular cultures and implants, sutures with shape memory, biosensors and DDS (comprehensive reviews have been recently published [20,27-30]). In general, the intelligent hydrogels release the drug when swollen, whereas the release becomes slower or even stops when they shrink. Only in few cases is the opposite behavior observed; i.e. at the swollen state, the drug-hydrogel interactions prevent the diffusion towards the exterior, whereas when the hydrogel shrinks, the drug is squeezed out together with the expelled water (Fig. **3**).

2.2. pH-Responsive Intelligent DDS

The gastrointestinal tract is typical with different pH. The pH gradients can also be found in other regions of the body. For example, the extracellular pH of tumor tissues (6.5-7.0) is slightly lower than that of the blood and the healthy tissues (7.4) [31]. Inside cells, the differences of pH among the cytosol (7.4), endosome (5.5-6.0) and lysosome (5.0) are considerable [32]. Polymers with ionizable groups can provide polymeric micelles and hydrogels with pH-sensitive drug release. The triggering pH depends on the copolymer composition [33]. For example, micelles of amphiphilic copolymers containing amino groups in one block may serve for selective delivery to tumors. This is the case of poly(2-vinylpyridine)-block-poly(ethylene oxide), P2VP-b-PEO; poly(2-(dimethylamine)ethyl methacrylate)-b-poly(ethylene oxide), PEO-b-DMAEMA; or poly(ethylene glycol)-b-poly(L-histidine), PEG-b-PLH [34,35]. At pH above pKa, the blocks are not ionized and behave hydrophobically, being able to form the micellar core. The hydrophilicity increases and the micelle breaks when the pH decreases and the groups protonize. These micelles retain the drug while in the bloodstream (pH 7.4), can accumulate in the tumor tissue by the

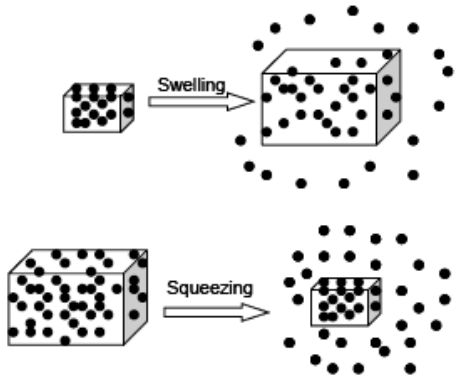

Fig. (3). Control of drug delivery through the volume phase transition of the hydrogel. The swelling promotes the delivery by diffusion; and the shrinking causes the squeezing of the drug with the flow of water.

EPR effect, penetrate in the cells by endocytosis and release the drug in the endosome or lysosome (pH 5-6) of tumor cells, without affecting the healthy ones [25,32,36].

In addition, pH-sensitive polymeric micelles have great potential as synthetic vectors for the systemic gene delivery [37]. DNA interacts with the amino groups of the copolymer, forming a complex that is included inside the micelle (polyplex micelle), which protects it from the enzymatic degradation. Despite the incipient state of this research line, some very promising results have been already published with three-layered polyplex micelles. For example, micelles of poly(ethylene glycol)-b-poly[(3-morpholinopropyl) aspartamide]-b-poly(l-lysine) (PEG-b-PMPA-b-PLL) combine the buffering capacity of PMPA with the excellent aptitude to condense DNA of PLL, resulting in a high transfectional efficiency [37].

Most pH-responsive hydrogels described in literature are designed to act *in vivo* only unidirectionally; i.e. they should stay collapsed until the system reaches a specific region of the body where they have to swell to release the drug and this process is not stopped or reverted. The few exceptions in which reversibility is shown are described in next section.

2.3. Molecule-Responsive Intelligent DDS

Molecule-responsive intelligent systems can enable a continuous control of the drug delivery rate as a function of the concentration of a specific substance. These systems try to imitate the physiological feed-back mechanisms. Among other approaches, this functionality can be achieved using a specific sensor of the triggering molecule (for example, an enzyme), which is attached to a hydrogel that also presents ionizable groups [27,38]. For example, insulin release controlled by glucose levels can be achieved by immobilizing glucose-oxidase in acrylic hydrogels which have amine groups [39]. When glucose concentration reaches a critical value, the glucose-oxidase produces enough glucuronic acid to significantly decrease the pH. The consequent ionization of the amine groups causes the swelling of the hydrogel and the release of insulin. As soon as the glucose recovers its basal level, the production of glucuronic acid is interrupted, the pH inside the hydrogel returns to its initial value, the hydrogel shrinks and the insulin release stops. Similar enzyme-controlled drug release hydrogels have been already successfully tested *in vivo* [40,41].

In other cases, the biomolecule directly activates the system. Hydrogels that contain concanavalin A can regulate insulin release answering quickly to the evolution of the glucose levels [42]. Thus, glycosylated insulin forms a complex with concanavalin A and, in the absence of glucose, no delivery occurs. Glucose competes with insulin for the binding to concanavalin A and, consequently, a quantity of hormone proportional to the glucose level is released (Fig. **4a**). Another approach consists of preparing insulin-loaded hydrogels using concanavalin A to cross-link glycosylated groups of the polymeric network [43]. In absence of glucose, the hydrogel is in the shrinking state. When a certain concentration of glucose is reached, the complexes of concanavalin A with the glycosylated groups break, the hydrogel swells and insulin is released; the process being reversible (Fig. **4b**). The encouraging results obtained *in vivo* [44] have compelled the intense research on new hydrogels capable of staying functional for long periods of time without compromising the stability of insulin [45]. An elegant approach may be the combination of genetically-engineered cells which can act as an insulin source (but are unable to provide glucose-regulation in patients) with a glucose-responsive material able to undergo a gel to sol transition at high glucose concentrations due to the presence of concanavalin A [46] (Fig. **5**).

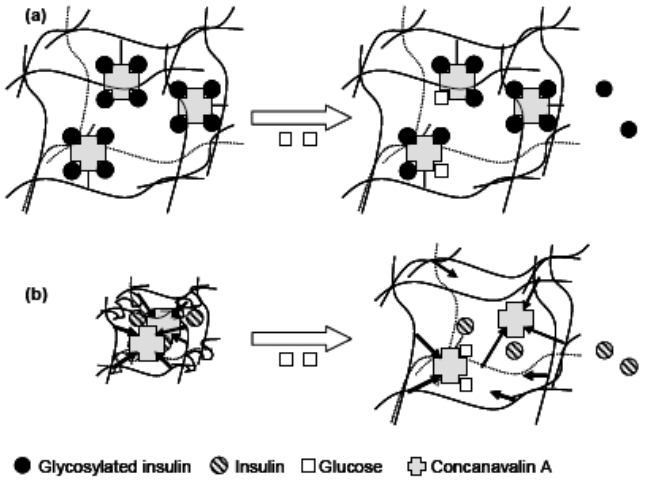

Fig. (4). Delivery of insulin from glucose-responsive hydrogels that (a) contain concanavalin A bound to the network and forming complexes with glycosylated insulin, or (b) contain concanavalin A as transient cross-linker interacting with the glycosylated groups of the network.

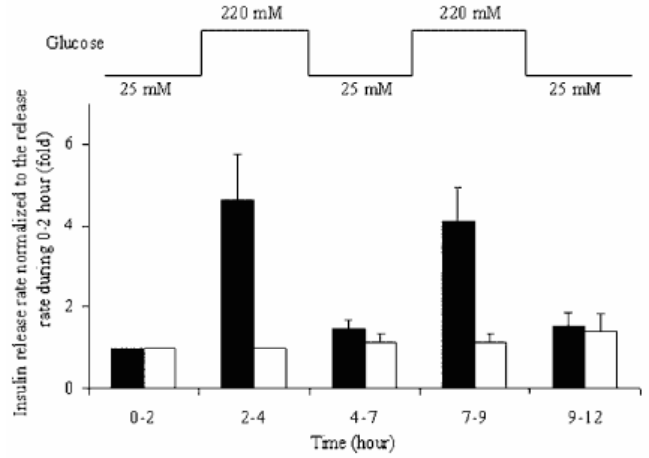

Fig. (5). Insulin release profiles from hybrid construct of alginate encapsulated insulin-secreting C2C12 cells with a concanavalin A-based material (black columns) and with alginate (control, white columns). Reprinted from [46] with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

Antigen-sensitive hydrogels can be prepared by copolymerizing monomers of an antigen and its antibody using the antigen-antibody interactions as cross-linking points [47]. The hydrogel stays shrunk until the same free antigen appears in the medium. The competition for binding to the antibodies of the polymer provokes the hydrogel swelling and the drug release. Molecule-responsive polymeric micelles

have been recently developed using copolymers with diamidopyridina (DAP) and thymine (THY). DAP and THY form complexes that break in presence of substances that contain thymine, causing the separation of the unimers [48].

Molecule-responsive materials represent a starting point for the design of self-regulated DDS able to control the delivery of the drug as a function of the substance concentration that serves as an index of the evolution of a pathological state [49].

2.4. Temperature-Responsive Intelligent DDS

Temperature-sensitive polymers used to prepare intelligent systems are hydrophilic below their critical temperature of dissolution (LCST). When the temperature is above LCST, the polymer becomes hydrophobic and its conformation changes from expanded (soluble) to globular (insoluble) state [50]. Amphiphilic copolymers containing poly(N-isopropyl acrylamide), PNIPA, or one of its derivatives can be used to obtain temperature-sensitive micelles [51]. The PNIPA segment may be at the hydrophilic block or at the hydrophobic one. In the first case, the shell formation takes place below LCST. Above this temperature, the micelle destabilizes and the drug is released [52]. For example, the LCST of block copolymers of poly(lactic acid) and of PNIPA copolymerized with dimethylacrylamide, PLA-b-(PNIPA-co-DMAAm), is close to 40ºC. Once loaded with doxorubicin, the micelles of this copolymer slowly release the drug at 37ºC, but the release becomes faster when the temperature rises to 42ºC [53]. Similarly, polymersomes consisting of poly(ε-caprolactone) and PNIPA have shown reversible disintegration in response to tiny changes of temperature [54]. These micelles and polymersomes could be useful for the development of intelligent systems that release the drug when hyperthermia occurs either at systemic level or at a specific region (such as an inflamed tissue or tumor). The release could also be triggered by an external source of heat applied to a delimited area of the body.

PNIPA-based hydrogels that switch on the delivery below the LCST (when the network is hydrated and swollen) and switch it off at higher temperatures (when the network collapses) have been shown to retain their functionality after several temperature-cycles [55-57]. This behavior could be useful in stopping the delivery by employing an external source of heat. The potential of hydrogels that combine natural polysaccharides and temperature-responsive polymers in the fields of drug delivery, tissue engineering and wound healing has been recently reviewed [58].

External modulation of drug release, without interference of physiological temperature changes, can be achieved using temperature-sensitive polymeric micelles and nanogels that contain, among other components, gold nanoparticles. When irradiated with infrared light, gold absorbs the radiation and the temperature in the surrounding environment rises, with the subsequent destabilization/collapse of the micelles or the nanogels [59]. Thereby it is possible to obtain a pulsatile release of conventional drugs or of proteins.

2.5. Photo-Responsive Intelligent DDS

The use of light as a triggering agent enables localized drug release in very well delimited regions of the body, reducing the affection of adjacent tissues to a minimum. Ultraviolet light or blue light can be used for treatments applied on the skin or the mucous, as is normally used in photodynamic therapy. Radiation of greater wavelengths (infrared), which have a high capacity of tissue penetration, although still innocuous, are particularly useful at modulating the release in areas of more difficult access [60,61].

Copolymers with photoactive groups that, when exposed to the light, undergo reversible alterations of the hydrophilic-lipophilic balance (HLB) have been assayed as components of light-sensitive micelles [24]. Photosensitive polymers with azobenzene groups are attracting the most interest. A trans to cis isomerization occurs without secondary chemical reactions when irradiated at 365 nm. This isomerization alters the hydrophilicity of the copolymer, being hydrophobic at trans (forming micelles) and hydrophilic at cis (staying as unimers). The wavelength that triggers the isomerization depends on the nature of the substituent groups and, thus, can easily be tuned [62]. The cis form is unstable at the corporal temperature so that, in darkness or if exposed to higher wavelength radiation, it reverts to the trans form. Therefore, cycles of micellar destabilization/ reconstitution can be obtained applying light pulses (Fig. **6**; [63]).

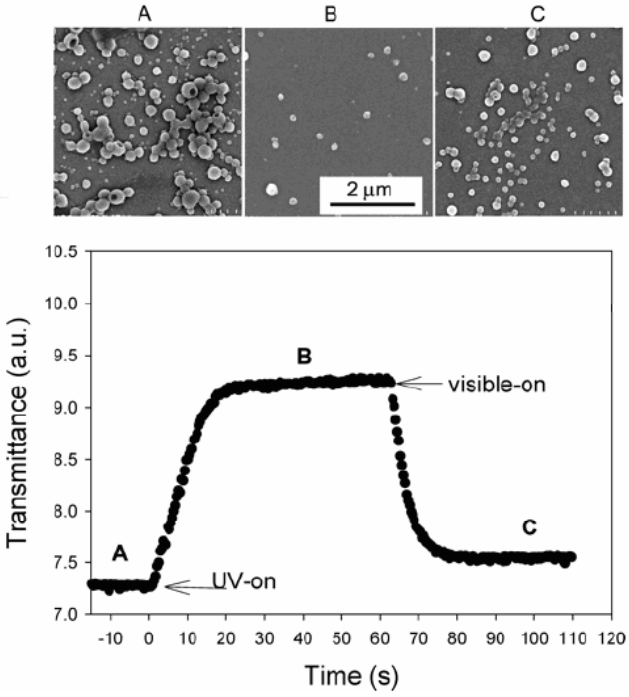

Fig. (6). Changes in transmittance for a vesicle solution of $PAzo_{74}$-*b*-($t$$BA_{46}$-$AA_{22}$) exposed to UV (360 nm, 18 mW cm^{-2}) and visible (440 nm, 24 mW cm^{-2}) light irradiation, vesicles being formed by adding 16%, in volume, of water in a dioxane solution with initial polymer concentration of 1 mg mL^{-1}. Typical SEM images (with the same scale bar) for samples cast from the solution at different times indicated in the figure show the vesicles before irradiation, their dissociation under UV irradiation, and the reformation after visible light exposure. Reprinted with permission from [63]. Copyright (2005) American Chemical Society.

In addition, the conformation of the azobenzene groups determines the interactions of the copolymers with hydro-

phobic regions of other macromolecules. As shown in Fig. **7**, the dispersions of seroalbumin and micelles of poly(acrylic acid)-1,2-aminoundecilamido-4-phenylazobenzene present a high viscosity at dark due to intra and intermicellar interactions, and this prevents the release of the protein. The azo groups adopt the cis conformation and the micelles break when irradiated with ultraviolet light. Consequently, the viscosity drops and the protein can be released. If the system is kept in the dark or exposed to visible light (436 nm), the azo groups recover the trans conformation, the viscosity rises again and the release stops [64]. These copolymers may be particularly useful in developing protein pulsatile delivery systems.

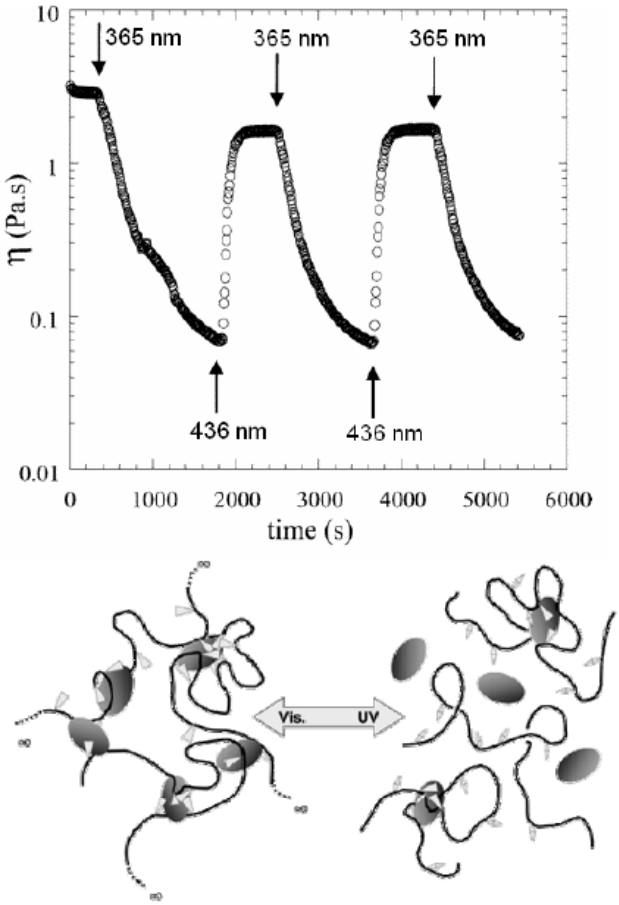

Fig. (7). Variation of viscosity of 1 wt % azobenzene copolymer solution (225-2C12Azo) in the presence of 0.7% bovine serum albumin, under exposure to light with alternating the wavelength between UV (365 nm) and visible (436 nm). Initially, the sample was equilibrated for 24 h in the dark (all-trans form) and first exposed to UV at time 400 s. The sketch of light-triggered binding of proteins on the azobenzene copolymer shown below indicates that proteins are mostly bound under exposure to visible light and that are released under UV exposure. The trans-azobenzene side groups are drawn as triangles, and their cis form appears as mostly unbound lozenges. Reprinted in part with permission from [64]. Copyright (2006) American Chemical Society.

2.6. Electro-Responsive Intelligent DDS

The effects of the electrical stimuli on drug penetration across the skin are well-known and constitute the base of procedures, as the iontophoresis and the electroporation, of transdermal administration. Cross-linked polyelectrolytes with a high density in ionizable groups are particularly adequate in obtaining electro-sensitive hydrogels for pulsatile drug release (for a comprehensive review see [65]). These materials can be prepared as sheets, microparticles or *in situ* gelling injectable systems for subcutaneous implantation. When an electrical field is applied, the cross-linked polyelectrolyte may shrink and expel water, thus releasing the drug by squeezing. The degree of shrinking depends on the amount of electricity passing across the hydrogel; when the electrical field is switched off, the hydrogel swells again. Therefore, alternative shrinking and swelling can easily be achieved by applying pulses of electricity, and this can serve to control the drug release rate. This approach has been tested for the pulsatile delivery of insulin using subcutaneously implanted poly(dimethylaminopropyl acrylamide) microgels [66] and for the transdermal delivery of diclofenac from hydrogels of sodium alginate, carbopol, and their blends [67]. The electrical stimuli can be generated using a commercially available apparatus for transdermal delivery, which enables a precise control of the intensity, the amount of current, the duration of the pulses and the intervals between successive pulses.

2.7. Ultrasound-Responsive Intelligent DDS

Polymeric micelles can be reversibly destabilized by applying ultrasounds for a pulsatile drug delivery in tumors [68]. Once iv injected, polymeric micelles accumulate in tumor tissues *via* the EPR effect. The ultrasounds should be applied when maximum micelle accumulation in the tumor is reached; the waiting period depending on the kinetics of the distribution of the micellar system. Rapoport´s group found that the optimum time of ultrasound application after injection of doxorubicin-loaded Pluronic P-105 polymeric micelles (or PEO-diacylphospholipid mixed micelles) is between 4 and 8 hours. The amount of drug released can be modulated through the control of the frequency, power density, pulse length and inter-pulse intervals [69]. Drug release from the micelles is reversible; during inter-pulse intervals exceeding 0.5 s, the drug can be completely re-encapsulated into the restored micelles (Fig. **8**). The *in vivo* anti-tumor effectiveness of this approach is also promoted by the cell membrane perturbation caused by ultrasounds (sonoporation) which enhances the intracellular uptake of micelles, drugs and genes [70,71].

2.8. Intelligent DDS Capable of Molecular Recognition

The biomedical interest of the stimuli-sensitive materials could be considerably increased if they were able to recognize specific molecules in a similar way as the biological receptors, enzymes or antibodies work. In the case of these biomacromolecules, the evolution is determined by the sequence of the structural unities (which condition the shape and charge distribution) which are responsible for such functionality. Proteins find their desired conformation out of a nearly infinite number, thanks to the unique details of their native state. Similarly, synthetic polymers also require a properly selected or designed sequence of monomers and cross-linkers to be able to memorize a specific conformation and to always revert back into the same conformation after

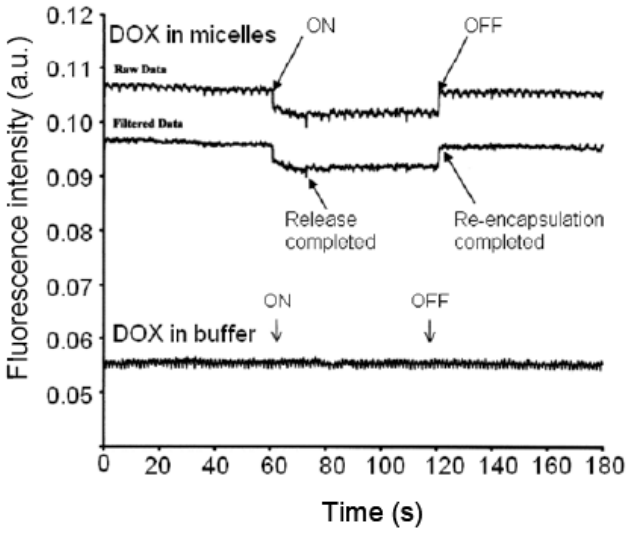

Fig. (8). Example of release profiles of doxorubicine (DOX) from a 10% Pluronic solution and from phosphate buffer. DOX concentration 6.7 μg/ml. Raw and Fourier-filtered data are presented for the 10% Pluronic solution. For the PBS solution, ultrasound was turned on at 60 s and off at 120 s; there was a negligible change of DOX fluorescence under sonication. Reprinted from [69] with permission from Elsevier.

being stretched and unfolded. Otherwise, a polymer with a randomly-made sequence would not fold in just one way [72-74].

Recently, the molecular imprinting technology has been adapted to the synthesis of hydrogels that can adopt specific conformations in order to form active centers capable of selectively taking up molecules. Hydrogels can recognize a substance if they are synthesized in the presence of such a substance (which acts as template or mould) in a conformation which corresponds to the global minimum energy. The monomers arrange themselves around the mould molecules as a function of their affinity (through covalent or non-covalent bonds) and this conformation is fixed during polymerization. A polymeric network with cavities (receptors) with the shape, size and functional groups most suitable for hosting the target molecule, is obtained when the mould molecules are washed out (Fig. **9**). The "memorization" of the receptors conformation, after the swelling of the network and the washing of the template, will only be possible if the network always folds into the conformation adopted upon synthesis [75]. As a consequence, the affinity of the imprinted polymer for the template drug can be significantly larger than that shown by conventionally-prepared polymers of the same composition. Reviews on the basics of molecular imprinting technology and its applications in the biomedical field [76-80] and particularly on the synthesis of intelligent hydrogels [81] have been recently published.

When the centers of molecular recognition are located in a stimuli-sensitive hydrogel, the conformation of the receptors can be deformed and re-constituted as a function of an external or a physiological signal. These materials can serve to develop advanced intelligent systems, which i) selectively and effectively load a certain drug; ii) release the drug at a rate modulated by a stimulus; and iii) uptake again the released drug from the environment if the drug remains around the hydrogel when the stimulus stops or diminishes its intensity and the cavities are reformed (Fig. **9**). The characteristics i) and iii) above differentiate the imprinted hydrogels from the conventional ones.

Stimuli-sensitive imprinted hydrogels can be obtained combining responsive monomers with monomers able to interact with the drug molecules. The synthesis should be carried out under conditions to ensure that the network will grow in the collapsed state. After polymerization, if the hydrogel swells due to a stimulus, the structure of the receptors is altered and the drug is released. If the stimulus disappears or its intensity decreases, the receptors can be reconstituted and the release slows down or stops. The optimization of imprinted stimuli-responsive hydrogels is particularly challenging and requires an even balance between cross-linking density, flexibility, nature of the functional monomers and ratio of drug to functional monomer [79].

Fig. **10** shows the release profile of 5-nitroisophthalic sodium salt from a temperature-sensitive imprinted hydrogel based on PNIPA and different proportions of functional monomer [82]. The drug-loaded hydrogel swells when the temperature is below the LCST and releases drug to the surrounding medium until an equilibrium concentration is reached. If the temperature rises, the hydrogel shrinks and the cavities recover their capacity of recognition; consequently the drug can be resorbed. The response is fast and shows an excellent reproducibility after several swelling/shrinking cycles. Similarly, temperature-sensitive imprinted gels for 4-aminopyridine and l-pyroglutamic acid, which had significantly larger saturation and affinity constants than the non-imprinted ones and were also highly selective, have been obtained [83,84].

pH- and temperature-sensitive gels imprinted for serum albumin (BSA) have also been prepared [85]. The ionic

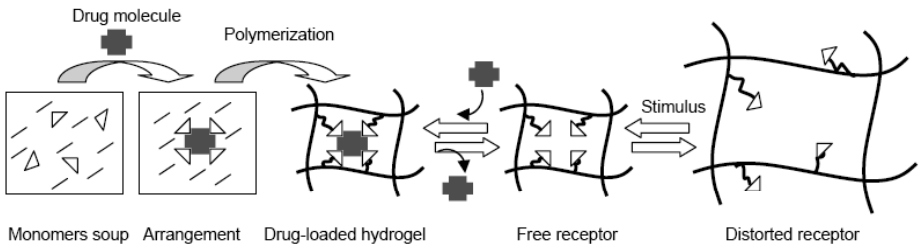

Fig. (9). Diagram of the synthesis of an imprinted hydrogel and the washing out/release and re-loading processes. The effect of stimuli on the conformation of the drug-receptors is also depicted.

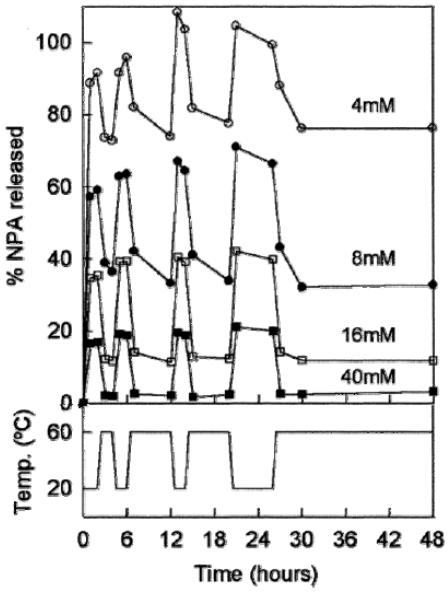

Fig. (10). Influence of temperature on the release and readsorption process of NPA by imprinted gels prepared with different concentrations of functional monomer. Cross-linker concentration was 40 mM. Degrees of swelling at 20 and 60 ºC were 6.0-6.5 and 0.9, respectively. Reprinted with permission from [82]. Copyright (2001) American Chemical Society.

poly(N-tertbutylacrylamide-co-acrylamide/maleic acid) hydrogels synthesized in the presence of BSA showed a remarkably greater affinity for the protein compared to the non-imprinted ones, the adsorption being dependent on both pH and temperature. The hydrogels were synthesized at the swollen state (22.8 ºC) and at this temperature the adsorption is maximal. In contrast, when the gel collapses it is difficult for the protein to diffuse into the gel, the imprinted cavities are distorted, and also the nature of the interactions is altered. At a low temperature, the interactions between BSA and the hydrogel are based on the hydrogen bonds. As the temperature rises, hydrogen bonds become weaker while hydrophobic interactions get stronger. These results clearly highlight the relevance of the memorization of the conformation achieved during polymerization to provide the gel with the recognition ability for a given template. The abrupt change in affinity during the gel volume phase transition allows drug release to be switch on and off.

2.9. Multi-Responsive Intelligent DDS

To obtain a very precise adjustment of the drug release or an amplification of the response to the stimuli, new materials that are sensitive to two (dually-responsive) or to more stimuli (multi-responsive) are being developed. These materials can serve also in controlling the simultaneous release of several drugs answering to different signals of the biological environment.

Polymeric micelles with a dual response to temperature and radiation have been prepared from copolymers of NIPA and azobenzene monomers [86]. In the absence of UV-irradiation, the system shows the characteristic behavior of a temperature-sensitive material. Under UV light, the hydrophilicity of the copolymer rises as a consequence of the trans to cis photoisomerization of the azobenzene groups, and the LCST increases above body temperature. Thus, under these conditions the micelles disintegrate. With the same purpose in mind, mixed micelles that combine copolymers with azobenzene groups and triblock copolymers which are temperature-sensitive (Pluronic®) were prepared [87]. The azobenzene groups can modify the gel temperature of Pluronic's micelle, therefore it is possible to obtain fluid dispersions of low viscosity in the dark but undergo a sol to gel transition when irradiated at 365 nm. These changes in the viscosity have great potential in modulating drug release rate. Aqueous solutions of the pentablock copolymer, consisting of poly(2-diethylaminoethyl-methyl methacrylate)-poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)-poly(2-diethylaminoethyl-methyl methacrylate) ($PDEAEM_{25}$-PEO_{100}-PPO_{65}-PEO_{100}-$PDEAEM_{25}$) exhibit temperature and pH dependent micellization due to the LCST of the PPO blocks and the polyelectrolyte character of the PDEAEM blocks, respectively [88]. Such responsiveness enables the preparation of *in situ* gelling solutions that, once at body temperature, can regulate drug release precisely as a function of small changes in pH. Micellization/demicellization cycles due to changes in temperature and pH have also been reported for copolymers of NIPA, N,N-dimethylacrylamide and N-acryloylvaline [89].

Hydrogels sensitive to both pH and temperature offer interesting possibilities to formulate thrombolytic agents such as heparin or streptokinase [90]. IPNs of methacrylic acid and NIPA have been shown to be useful for adjusting drug release to the small changes of pH and temperature that accompany the formation of the thrombi. Chitosan-NIPA IPNs provide pH and temperature-dependent release of diclofenac [91]. Similarly, hydrogels of NIPA, butylmethacrylate and acrylic acid has been designed to coat the vaginal tissue and to release an anti-HIV microbicide in response to semen-induced pH change [92].

Of late, a DDS capable of detecting and analyzing the subtle multiple signals generated in the biological medium and which can finely adjust the release has been developed [93]. The device combines the electro-responsiveness of a hydrogel made of sodium acrylate and tetraethylenglycol dimethacrylate with the power of a computer analysis system. The biological signals (concentration of glucose, light, temperature, pH, electrical field or their binary combinations) are detected by sensors and transmitted to a microcomputer to be processed. The microcomputer emits the necessary orders to the hydrogel, as specific electric stimuli through a number of electrodes, to control drug/s release. A simultaneous and independent control of the release of several drugs registering different stimuli can be achieved using such a device; for example, the release of a certain drug can be externally regulated by applying light pulses, whereas the release of another drug can be regulated by voltage pulses (Fig. **11**).

CONCLUSIONS

The intelligent systems arise as a consequence of a profound change in the mentality in designing DDS and can be brought to fruition thanks to the remarkable progress of polymer science. The central idea of this new approach is to

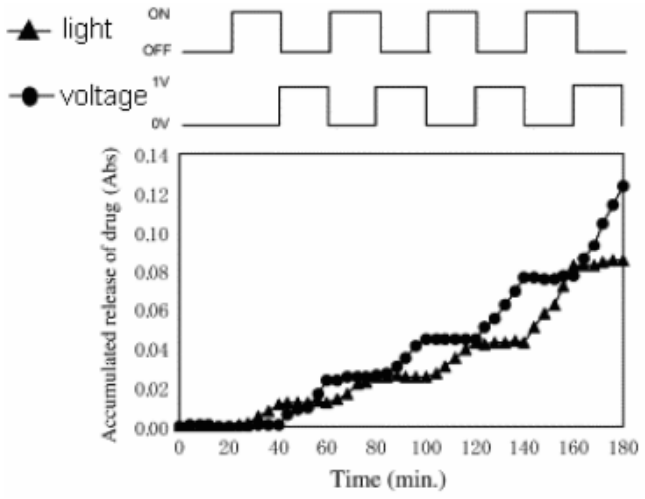

Fig. (11). Accumulated released amounts of two different drugs from binary inputs–binary outputs intelligent DDS. Reprinted from [93] with permission from Elsevier.

reproduce the recognition and delivery behavior of biological systems in order to extract the maximum therapeutic advantages of the drugs that must be delivered according to rhythms which are hardly predictable or require a very rigorous control of their concentration in specific areas of the body. The incorporation of stimuli-sensitive polymers to polymeric micelles or to hydrogels constitutes a very attractive way in the development of systems with such functionality and is currently under intensive investigation. The clinical use of new intelligent systems, based on these polymeric structures, still requires considerable additional efforts, especially regarding the following aspects:

i) Design and synthesis of new biocompatible materials in order to increase the range of stimuli-sensitive polymers that fulfill the requirements of generally recognized as safe (GRAS) products.

ii) Optimization of the synthesis procedures to guarantee the homogeneity, the reproducibility and the purity of the new polymeric materials. It is essential to develop manufacture procedures that lead to "formats", in the case of hydrogels (for example, nanogels, microgels or sheets), which will facilitate the administration of the DDS and, if needed, the application of external stimuli.

iii) Development of truly reversible systems. The correct operation of an intelligent system needs the response to the stimuli to be reproducible during hundreds or even thousands of cycles, without dysfunction during the period of use.

iv) *In vivo* evaluation of the performance of new delivery systems. Up until now most information comes from *in vitro* studies. This must be supplemented by carrying out tests with biological substratum.

The progress already accomplished in the intelligent drug delivery field is undoubtedly remarkable. However, the enormous complexity of the living beings makes foreseeable a long journey until the use of devices able to detect biomarkers and, as a function of their levels, to automatically adjust the release of the adequate drug becomes a common practice. In the near future, it will be necessary to confront the challenge of developing new materials which offer increasingly precise and sophisticated features. Therefore, the basic and translational research in the field of the stimuli-responsive polymers has an undoubtedly brilliant future [94].

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