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TOPICAL REVIEW

Stimuli-responsive polymers and their applications in drug delivery

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Abstract

Interest in stimuli-responsive polymers is steadily gaining increasing momentum especially in the fields of controlled and self-regulated drug delivery. Delivery systems based on these polymers are developed to closely resemble the normal physiological process of the diseased state ensuring optimum drug release according to the physiological need. Also termed 'environmental-sensitive' or 'smart', these polymers experience rapid changes in their microstructure from a hydrophilic to a hydrophobic state triggered by small changes in the environment. The changes are reversible; therefore, the polymer is capable of returning to its initial state as soon as the trigger is removed. Stimuli may occur internally (e.g. a change in pH in certain organs or diseased states, a change in temperature or the presence of specific enzymes or antigens). External stimuli include magnetic or electric fields, light, ultrasound, etc. This review will delve into the various internally and externally stimuli-responsive polymers and the drug delivery systems that exploit them.

1. Introduction

With the emergence of more novel and effective drug therapies, increased importance is being placed upon the methods by which these drugs are being delivered to the body. Conventional drug delivery methods result in a peak in plasma drug concentrations, followed by a plateau and finally a decline. As a result, these methods of drug delivery may lead to toxic plasma drug concentrations or ineffective plasma levels. Commercially available controlled release devices offer several advantages, e.g. they maintain the drug within the desired therapeutic range with just a single dose, they allow localized drug delivery which ultimately may reduce the need for follow-up care, they preserve drugs that are rapidly destroyed by the body and ultimately improve patient compliance.

Even though these systems are therapeutically advantageous over the conventional systems, they remain insensitive to the changing metabolic states of the body. Mechanisms capable of responding to these physiological variations must be provided in order to synchronize drug-release profiles with changing physiological conditions. Ideally, a drug delivery system should respond to physiological requirements, sense the changes and alter the drug-release profile accordingly [1]. This encompasses the two concepts of (1) temporal modulation and (2) site-specific drug targeting [2]. The symptoms of most disease states follow a rhythmic pattern, e.g. angina pectoris, diabetes mellitus, etc, and require drug delivery as per the rhythms. More importantly, if the drug possesses side effects, drug release when not required poses an extra burden on the body's metabolic system [1]. A more appropriate and effective approach of managing some of these conditions is by chronotherapy. This approach allows for pulsed or self-regulated drug delivery which is adjusted to the staging of biological rhythms, since the onset of certain diseases exhibit strong circadian temporal dependence.

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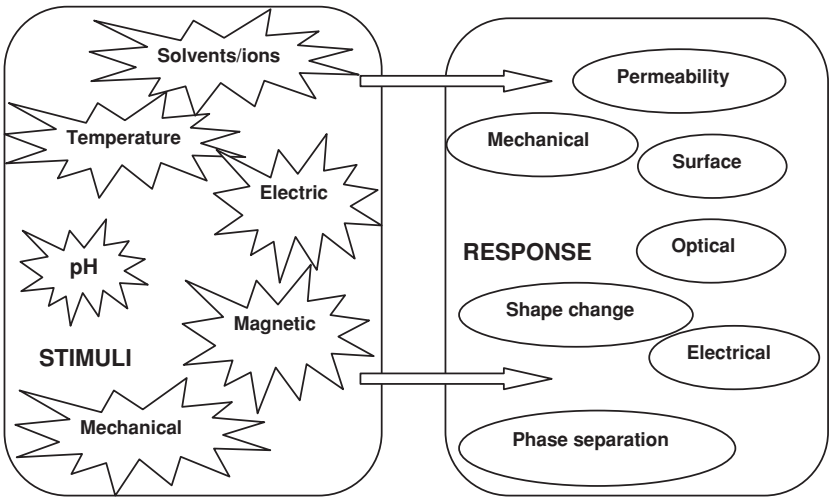

Figure 1. Potential stimuli and responses of synthetic stimuli-responsive polymers (adapted from Schmaljohann [6]).

Stimuli-responsive polymeric drug delivery is another field of controlled drug delivery. These systems closely follow the normal physiological process of the disease state where the amount of drug released is affected according to the physiological need [3]. Biopolymers such as proteins, carbohydrates and nucleic acid are all basic components of living organic systems that are responsible for the construction and operation of the cells' complicated machinery [4, 5]. These 'natural' polymers have led to the development of numerous synthetic polymers that have been designed to mimic these biopolymers. Based on their chemical and physical properties, these synthetic polymers have been coined with different names, e.g. 'stimuli-responsive' polymers, 'smart' polymers, 'environmental-sensitive' polymers or 'intelligent' polymers. The distinguishing characteristic of these stimuli-responsive polymers is their ability to undergo rapid changes in their microstructure from a hydrophilic to a hydrophobic state which is triggered by small changes in the environment. The macroscopic changes that occur are reversible; therefore the system is capable of returning to its initial state when the trigger is removed [4, 5].

Common stimuli that drive these changes are represented in figure 1 [1, 3–6]. These stimuli are then further categorized as either external or internal stimuli. Externally controlled systems rely on externally applied stimuli that are produced with the help of different stimuli-generating devices, which ultimately results in pulsed drug delivery [1, 3]. Internally regulated systems are also known as self-regulated devices, where the release rate is controlled by a feedback mechanism that is produced within the body to control the structural changes in the polymer network and to exhibit the desired drug release, without any external intervention [1, 3]. Responses to these stimuli may be manifested as changes in shape, surface characteristics, solubility, formation of an intricate molecular assembly or a sol-to-gel transition [5]. This review will be focused on the characteristic behavior, advances and limitations of various stimuli-responsive polymers and the drug delivery systems that utilize them.

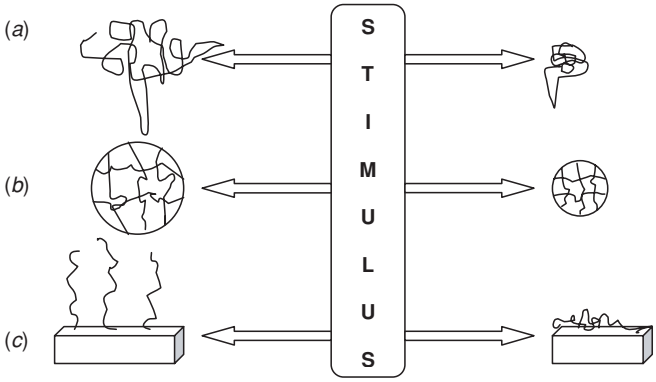

Figure 2. Classes of stimuli-responsive polymers based on their physical forms. (*a*) Linear free chains in solution, (*b*) covalently cross-linked reversible and physical gels and (*c*) chain adsorbed or surface-grafted forms (adapted from Kumar *et al* [5]).

2. Categorization of stimuli-responsive polymers

Synthetic biopolymers can be classified into different categories based on their chemical and physical properties; however, when categorized based on their physical properties, they fall under three categories as represented in figure 2.

2.1. Linear free chains in solution

Polymers under this category undergo a reversible collapse after the application of an external stimulus [5]. An appropriate hydrophilic and hydrophobic proportion is required in the molecular structure of the polymer in order for phase transition to occur; therefore, in solution a delicate balance between these two has to be maintained in order for phase transition to occur. With increasing hydrophobicity, the soluble polymer is precipitated out of solution and forms a totally different insoluble phase. This conversion is achieved either due to a reduction in the number of hydrogen bonds that the polymer forms with water or because of the neutralization of the electric charges that are present on the polymeric network [5]. These

Table 1. Effects of various external stimuli on drug release from various stimuli-responsive hydrogels (adapted from Soppimath *et al* [46]).

Stimulus	Hydrogel type	Release mechanism
pH	Acidic or basic	A change in pH causes swelling of the hydrogel.
Ionic strength	Ionic	Change in ionic strength causes a change in the concentration of ions inside the gel. This causes a change in swelling.
Chemical species	Electron-accepting groups	Electron-donating compounds cause charge transfer. This causes a change in swelling.
Enzyme substrate	Immobilized enzymes	When a substrate is present enzymatic conversion occurs. The product causes swelling.
Magnetic	Magnetic particles in microspheres	An applied magnetic field causes a change in pores in gel. This results in change in swelling.
Thermal	Thermo-responsive	Change in temperature causes a change in polymer–polymer and water–polymer interactions. This causes a change in swelling.
Electrical	Polyelectrolyte	Applied electric field causes membrane charging. Electrophoresis of charged drug changes swelling.
Ultrasound	Ethylene-vinyl alcohol	Ultrasound irradiation causes temperature increase.

polymers in solution have various applications such as for the bio-separation of proteins, cells and other bioparticles.

2.2. Covalently cross-linked reversible and physical gels

Investigations into stimuli-responsive polymers have often been focused to a large extent on hydrogels that swell in aqueous media. These hydrogels may respond rapidly to small changes in pH and temperature and recently have been shown to also respond to a change in light intensity, ionic strength, magnetism, inflammation, ultrasound, electric fields and even certain biochemicals (e.g. glucose, urea) [2, 7–9]. These 'smart' hydrogels may be synthesized in the conventional manner to result in hydrogels with small pore sizes or they may be synthesized by various other approaches to result in macroporous hydrogels for different applications [10, 11]. Some of these approaches are by co-polymerization, genetic engineering and irradiation. Another novel hydrogel, termed cryogel, was synthesized under moderately frozen conditions to form pores of large sizes [12]. These hydrogels are candidates for numerous applications. Table 1 describes the phase transitions that occur due to various changes in the external environment. These changes may be gradual and smooth, or they may be abrupt and discontinuous. This all depends on the nature of the system [13].

2.3. Chain adsorbed or surface grafted form ('smart' surfaces or membranes)

Phase separation of stimuli-responsive polymers occurs only when a sharp conformational change of a macromolecule occurs. This change must be accompanied with a drastic increase in hydrophobicity triggered by a small change in the environment [5]. When a polymer reversibly swells or collapses on a surface it converts the interface from hydrophilic to hydrophobic and vice versa. This occurs when the 'collapsed' hydrophobic macromolecules aggregate and form a separate phase. The 'intelligent' polymers do not aggregate but the conformational transition from a hydrophilic to a hydrophobic state renders the surface hydrophobic. The surface is hydrophilic when the polymer is in its expanded soluble state and hydrophobic when in its collapsed insoluble state. This occurs only once a specific external parameter is modified [5]. This change in surface hydrophobicity may be in response to changing temperature and can easily be exploited to allow separation of substances that interact differently with the hydrophobic matrix.

3. Externally regulated drug delivery systems

There is increasing evidence that constant drug delivery is not always effective from a pharmacological point of view. Almost all functions of the body, including those influencing pharmacokinetic parameters, display significant daily variations or patterns [14]. It has generally been assumed that a constant drug infusion leads to a constant drug concentration and this in turn leads to constant drug effects. However, there are a wide variety of drugs e.g. antiasthmatics, psychotropics, calcium channel blockers, diuretics and anticancer drugs that have shown daily variations in their effect in clinical studies. These findings have provided the basis for an increased interest in the field of chronopharmacology which is focused on coordinating biological rhythms with medical treatment [14]. In these situations, externally regulated drug delivery systems are recommended. This allows for pulsatile drug delivery which may be defined as the rapid and transient release of a certain amount of drug within a short time period immediately after a predetermined off-release period [15]. Also, in the last decade, a lot of attention has been given to the development of targeted drug delivery systems that allow for drug delivery to specific sites, organs, tissues or cells in the body where drug therapy is required e.g. the specific targeting of drugs to cancer cells [16]. A very effective way of achieving site-specific drug targeting is by employing stimuli-responsive polymers. Stimuli that are specific at target sites may include low pH and high levels of certain enzymes. Among internal and external stimuli, an elevated temperature is one of the best signals in terms of easy and safe medical applications and may be used for both site-specific drug therapy or for pulsatile drug release [16].

3.1. Thermo-responsive polymers

Temperature may act as both an external and internal stimulus. Physiologically, thermal stimuli are very important e.g. during a fever there is an elevation of body temperature due to the presence of pyrogens. This elevation in temperature is mediated by an elevated concentration of prostaglandin E2 within certain areas of the brain thus altering the firing rate of neurons that control thermo-regulation [17, 18]. Changes in temperature that can trigger drug delivery can be either due to increased body temperature in a disease state or due to modulated external temperature (in the form of heat-triggered subdermal implants, etc).

Temperature-sensitive or thermo-responsive hydrogels and polymers are the most commonly studied class of stimuli-responsive polymeric systems [16, 19, 20]. A major characteristic feature of these polymers is the presence of hydrophobic groups such as methyl, ethyl and propyl groups [2]. A unique property of temperature-responsive polymers is the presence of a critical solution temperature, which is the temperature at which the phase of polymer and solution is discontinuously changed according to their composition [20]. If the polymer solution has one phase below a specific temperature, in other words they become insoluble upon heating, they have a lower critical solution temperature (LCST). Otherwise, it is called a higher critical solution temperature (HCST) or an upper critical solution temperature (UCST) [6, 20]. Generally, the solubility of most polymers increases with an increase in temperature. However, in the case of polymers with a LCST, the solubility decreases as temperature increases and therefore hydrogels made of these polymers shrink as the temperature increases above the LCST. This type of swelling behavior is known as inverse temperature dependence and occurs due to predominating hydrophobic interactions [2, 17]. These hydrogels are made of polymer chains that possess either moderately hydrophobic groups or a mixture of hydrophilic and hydrophobic segments. At lower temperatures, hydrogen bonding between the hydrophilic segments and water leads to enhanced dissolution; however, as the temperature increases, the hydrophobic segments are strengthened, thus resulting in shrinking of the hydrogels due to inter-polymer chain associations [2].

In the field of drug delivery it is the LCST of polymers and systems that are generally more relevant. In terms of thermodynamics, the phenomenon of polymer aggregation at the LCST is owed to the entropy (S) of the two-phase polymer and water system [19]. A positive ΔS allows the increase in temperature to contribute to the system aggregation. This is also encouraged by the positive enthalpy ΔH (which is smaller than the entropy term). Under these circumstances, association is favorable as the free energy of association ($\Delta G = \Delta H - T\Delta S$) is negative [19, 21]. Theoretically, a negative excess entropy of mixing is required for an LCST; however, the nature of the polymer–water interactions may also be responsible [22–27].

N-substituted polyacrylamide has recently been of interest in the field of controlled drug delivery. In particular, poly(*N*-isopropylacrylamide) (PNIPAAm) is the most widely researched and used synthetic temperature-responsive polymer in drug delivery, biomaterial and intelligent material studies [2]. The main reason for its frequent use is because its phase-transition occurs at approximately body temperature. It also exhibits phase transition at 32 °C in water, and for the purposes of drug targeting, its phase-transition temperature can easily be adjusted to an appropriate temperature (around 40 °C) by the introduction of a hydrophilic co-monomer such as *N*, *N*-dimethylacrylamide (PDMAAm) [28, 29].

Another popular temperature-responsive polymer is poly(*N,N′*-diethylacrylamide) (PDEAAm) which has a LCST in the range of 25–35 °C [2]. However, the transition temperature of PDEAAm is dependent on the tacticity of the polymer which is in contrast to PNIPAAm [6, 16]. Poly(2-carboxyisopropylacrylamide) (PCIPAAm) offers two benefits: the similar temperature-responsive behavior as PNIPAAm and an additional functionality due to its pendent groups [30]. Poly(*N*-(l)-1-hydroxymethyl-propylmethacrylamide) (poly(l-HMPMAAm)) is a novel thermo-responsive polymer that was designed to have optical activity and was shown to have quite a different thermo-sensitive phase transition from that of its optically inactive counterpart [31]. Another novel thermo-responsive polymer that in addition possesses pH-responsive characteristics is poly(*N*-acryloyl-*N′*-alkylpiperazine) [28, 29].

3.2. Thermo-responsive hydrogels

Thermo-responsive hydrogels can be classified into two groups based on the origin of thermo-sensitivity in aqueous swelling [32, 33]. The first group is based on polymer–water interactions, specifically hydrophobic–hydrophilic balancing effects and the configuration of side groups. The second group is based on polymer–polymer interactions in addition to polymer–water interactions [34]. The sensitivity of thermo-responsive hydrogels makes them extremely useful e.g. upon injection to the body, when temperature is increased from ambient to physiological, gelation occurs with no other requirement for chemical or environmental treatment as depicted in figure 3 [35].

3.3. Applications of thermo-responsive hydrogels

3.3.1. On–off drug delivery systems. Thermo-responsive monolithic hydrogels are being used to obtain an ‘on–off’ drug release profile in response to a stepwise temperature change [37–39]. Poly(*N*-isopropylacrylamide) cross-linked butyl methacrylate (BMA) hydrogels were loaded with indomethacin and analyzed for their on-off release profile. ‘On’ was achieved at low temperatures and ‘off’ at high temperatures. This occurred due to the formation of a skin-type barrier that formed a dense, less permeable gel surface layer when the temperature was suddenly changed. The barrier was formed due to the faster collapse of the gel surface than the interior and was regulated via the length of the methacrylate alkyl side-chain [40].

3.3.2. Cancer chemotherapeutics. The limited therapeutic activity and insolubility of anti-cancer drugs, problems

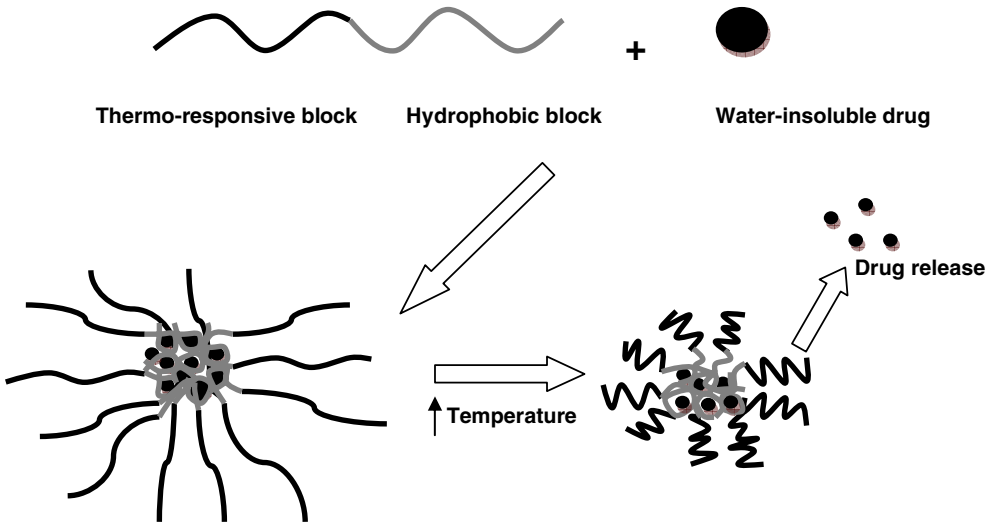

Figure 3. Structure and drug release from a thermo-responsive polymeric micelle (adapted from Alred [36] and Klouda and Mikos [35]).

of accessibility and heterogeneity of tumors, and toxicity/immunogenicity of the delivery agent all intensify the need for newer drug delivery modalities that are capable of eradicating these challenges. Despite the development of many different systems such as low molecular weight prodrugs, liposomes and micro- and nanoparticles, there is still limited therapeutic efficacy in this regard [41, 42]. Soluble polymeric drug carriers bear attractiveness due to their improved drug pharmacokinetic profiles, and they lead to increased tumor accumulation over free drug due to passive targeting. This is termed the enhanced permeability and retention (EPR) effect [43]. However, a major drawback of these carriers is their inability to intrinsically target a certain physiological site. In combination with chemo and radiotherapy, hyperthermia establishes synergistic activity that enhances solid tumor cytotoxicity [44]. Since hyperthermia increases the permeability of tumor vasculature compared to normal vasculature, drug delivery to tumors can be further enhanced [45, 46]. Hyperthermia treatment of cancer patients is usually performed at 42 °C; thus, temperature-responsive delivery systems should have their LCST above that of body temperature [17, 47]. Polymeric micelles have been reviewed as good carriers for drug targeting due to their stealth against the body's defense system (reticulo-endothelial system). Therefore, passive targeting is achievable by these micelles through an enhanced permeation and retention effect of tumor sites [43]. Block copolymers with hydrophobic polymers, such as poly(butyl methacrylate) (PBMA), polystyrene or poly(lactic acid), were prepared with end-functionalized PNIPAAm [48–50]. These block copolymers formed a micellar structure in aqueous solution below the transition temperature of PNIPAAm and showed a change in its hydration/dehydration properties. In the hydrated form, it acted as an inert material toward all biological entities; however, upon a temperature rise above 32 °C, these chains became hydrophobic. This occurred due to the dehydration of the polymer chains, thus resulting in aggregation and precipitation. The cores of both the PBMA and polystyrene micelles then acted as a reservoir for the hydrophobic anticancer drug, adriamycin. Thermo-responsive polymer micelles can be effectively utilized for the thermo-responsive drug delivery to tumor sites in conjunction with an induced hyperthermia.

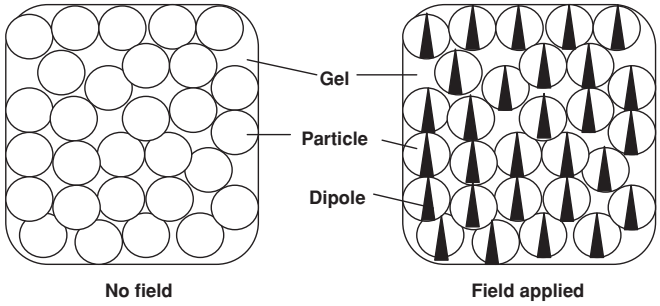

Figure 4. Schematic conceptualization of the electro-responsive effect in a polymer gel. The paths of the particles have been formed prior to application of the electric field (adapted from Shiga [52]).

3.4. Electro-responsive polymers

An electrical field in the form of an external stimulus offers numerous advantages (e.g. availability of equipment). This form of an external stimulus also allows for precise control over the magnitude of the current, the duration of electrical pulses and the interval between pulses [51]. Delivery systems exploiting this external stimulus are prepared from polyelectrolytes, which are polymers that contain a relatively high concentration of ionizable groups along the backbone chain. This property renders these polymers pH-responsive as well as electro-responsive [2, 51]. Under the influence of an electric field, electro-responsive hydrogels generally shrink or swell and this property has allowed its application in drug delivery systems, artificial muscle or biomimetic actuators [2, 52]. The electro-responsive concept is depicted in figure 4. The shape that the hydrogel changes to, i.e. swelling, shrinking or bending, depends on a number of conditions. Partially hydrolyzed polyacrylamide hydrogels which are in direct

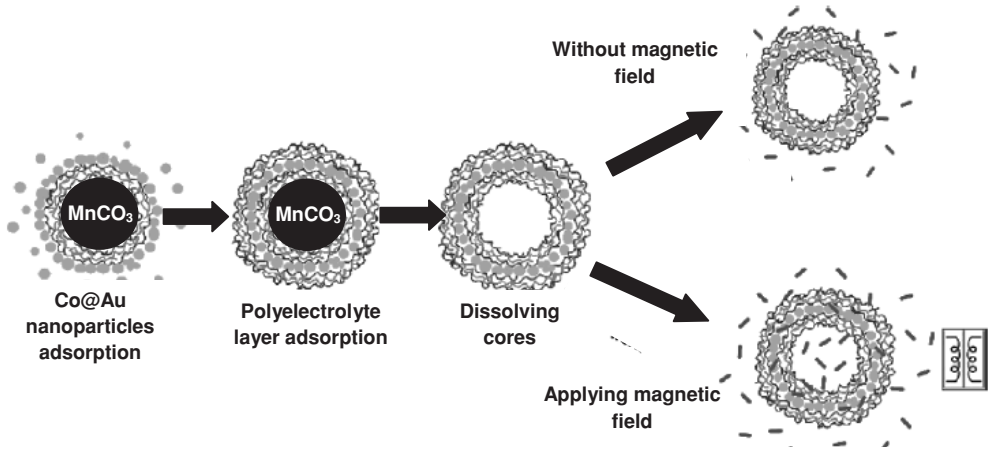

Figure 5. Schematic depicting the assembly and permeability of microcapsules embedded with Co@Au nanoparticles under an oscillating magnetic field (adapted from Lu *et al* [71]).

contact with both the anode and cathode electrodes undergo a volume collapse after a minute change in electric potential is applied across the gel. On application of a potential, H^+ ions migrate to the region of the cathode, which results in a loss of water at the anode side. Simultaneously, the electrostatic attraction that exists between the anode surface and the negatively charged acrylic acid groups creates a uniaxial stress along the gel axis. These two events lead to shrinking of the hydrogel on the anode side [53, 54].

3.4.1. Application of electro-responsive polymers in drug delivery. Electrically controlled delivery systems include technologies such as sonophoresis, iontophoresis and infusion pumps [31]. In most cases, electrical field-responsive polymer matrices are based on electrically driven motility; however, some neutral polymer gels were reported to show electrical field sensitivity. A drug delivery device that consisted of a polymer reservoir with a pair of electrodes placed across the rate limiting membrane was proposed. Controlled and predictable drug release rates were proposed to be modulated by altering the magnitude of the electric field between the electrodes [55, 56]. Another approach to electrically controlled drug delivery is based on polymers which bind and release bioactives in response to electric stimuli. Ideally, the polymer has two redox states, only one of which is suitable for ion binding. The drug ions are bound in one redox state and released from the other [57].

3.5. Magnetically responsive polymers

When designing a magnetic-responsive delivery system several factors need to be considered, including the magnetic properties of the carrier particles, field strength, field geometry, drug/gene binding capacity and physiological parameters such as the depth to target, the rate of blood flow, vascular supply and body weight [58]. Magnetic targeting is based on the attraction of magnetic micro- and nanoparticles to an external magnetic field source. In principle, in the presence of a magnetic field gradient, a translational force will be exerted on the particle/drug complex. This effectively traps the complex in the field at the target site and pulls it toward the magnet [59, 60].

Superparamagnetic iron oxide nanoparticles (SPION) are small synthetic γ-Fe_2O_3 or Fe_3O_4 particles that have a core size of <10 nm and an organic or inorganic coating. In the presence of an external magnetic field, these SPIONs are capable of delivering drug particles and fixing them at the intended site while the drug is being released. This is termed magnetic drug targeting (MDT) [61–63]. It is visualized that the SPIONs will prove to be immensely beneficial in postoperative prophylaxis of infections around surgical implants and prosthesis. In this case, it is proposed that the implants are combined with soft ferromagnetic materials so that if complications develop after implantation, such as infection, thrombosis, rejection or calcification, the appropriate medication could be delivered to the implant site [58].

Several forms of magnetic targeting have been synthesized by various different methods e.g. magnetic nanoparticles with a magnetic core and a polymer shell; and magnetoliposomes which have a magnetic core and an artificial liposomal shell [64–68]. These magnetic nanoparticles may also be embedded in hydrogels which can carry therapeutic agents that are released upon heating [60, 70]. Lu *et al* [71] explored the institution of a magnetic field to modulate the permeability of polyelectrolyte microcapsules prepared by layer-by-layer self-assembly. Ferromagnetic gold-coated cobalt (Co@Au) nanoparticles were embedded within the capsule walls. External alternating magnetic fields were applied to rotate the embedded Co@Au nanoparticles, which subsequently disturbed and distorted the capsule wall and drastically increased its permeability to macromolecules (figure 5). A theoretical explanation was proposed for the permeability control mechanisms; the 'switching on' of the microcapsules via a magnetic field purportedly makes this method a good candidate for controlled drug delivery in biomedical applications.

3.5.1. Application of magnetism in drug delivery. A major drawback of chemotherapeutic approaches to cancer tumor

treatment is that they are highly non-specific. Cytotoxic drugs are generally administered intravenously and the systemic circulation of these agents indefinitely results in numerous adverse effects as a result of healthy cells being targeted along with cancer cells. In the late 1970s, magnetic micro- and nanoparticles were explored as possible drug carriers for site-specific drug targeting [72, 73]. Cytotoxic drugs were attached to these carriers and injected into the subject either via intravenous or intra-arterial injection. High-gradient, external magnetic fields generated by rare earth permanent magnets were then used to guide and concentrate the drugs at the tumor sites [74].

Although theoretically very effective, magnetic drug carriers still have numerous obstacles. In order to retain the magnetic carrier–drug complex at a specific location, the externally applied field must have a relatively strong gradient. Additionally, as soon as the drug is released from the magnetic complex, it is no longer responsive to the applied field. It is then free to resume its normal distribution patterns in the body and if the drug is released while the complex is still within the vasculature, even if they are held at the target site, there will then be some degree of systemic distribution. There is also the potential for embolization as the particles are capable of accumulating and blocking blood flow or they may also concentrate in the liver [75]. There is also the problem as to the depth that the magnet may function, as is encountered when scaling up from small animals with near-surface targets to larger animals and humans.

The quest to achieve an optimal release of insulin has occurred in a sequential process. From previous studies, it was established that insulin and other molecules were capable of being continuously released by embedding the hormone in a carrier such as an ethylene vinyl acetate copolymer (EVAc) [76]. The critical parameters affecting these release rates were then characterized by conducting *in vitro* studies [77]. Using this information, single subcutaneous implants of EVAc-insulin was designed. These implants were capable of decreasing glucose levels in diabetic rats for 105 days [78]. EVAc-protein matrices that contained magnetic beads exhibited enhanced release rates when placed in an oscillating external magnetic field [79–81]. *In vivo* studies on implanted polymeric matrices that contained insulin and magnetic beads showed that glucose levels could be repeatedly decreased on demand by applying an oscillating magnetic field [82].

3.6. Ultrasonically responsive polymers

One of the pioneering approaches of exploiting ultrasound in drug delivery involved directing the ultrasonic waves directly at the polymers or the hydrogel matrix [83]. This approach of ultrasound-responsive drug delivery achieved a 27-fold increase in the release of 5-fluorouracil from an EVAc matrix [84]. Ultrasonically regulated drug delivery in which release rates of substances are repeatedly modulated at will from a position external to the delivery system was suggested as being highly feasible by Kost *et al* [85]. Bioerodible polymers that were evaluated as drug carrier matrices include polyglycolide, polylactide, poly(bis(*p*-carboxyphenoxy))alkane-anhydrides and their copolymers with sebacic acid. The releasing agents were *p*-nitroaniline, *p*-amino-hippurate, bovine serum albumin and insulin. When exposed to ultrasound, these bioerodible polymers responded rapidly and reversibly. It is believed that the ultrasound causes an increase in temperature in the delivery system, which facilitates diffusion [86, 87].

3.6.1. Application of ultrasound in drug delivery. The use of ultrasound in drug delivery applications proves to be highly advantageous as it is non-invasive and capable of penetrating deep into the interior of the body and thus drug delivery can be focused and carefully controlled through a number of parameters including frequency, power density, duty cycles and time of application [88, 89]. The main mechanism of the biological action of ultrasound is related to the generation of thermal energy, perturbation of cell membranes under the influence of micro-convection or inertia cavitation, and enhanced permeability of blood capillaries [90].

Numerous carriers for ultrasonically enhanced drug delivery have been explored including polymeric ultrasound contrast agents with targeting potential, modified lipospheres and nano-/micro-bubble-enhanced chemotherapy [91–95]. Tumor targeting via carrier-encapsulated drug delivery followed by ultrasonic irradiation of the tumor has been achieved by Kruskal *et al* [96]. In this study a human liver tumor model in mice was used and liposome-encapsulated doxorubicin (DOX) was injected systemically. Thereafter, a burst of ultrasound was directed to the tumor. Suzuki *et al* [97] achieved tumor-specific ultrasound-enhanced gene transfer with novel liposomal bubbles, which entrapped an ultrasound imaging gas. The bubble liposomes efficiently transferred genes, only at the site of ultrasound exposure, into tumor cells and solid tumor tissue. Ultrasound should be applied at the time when a sufficient quantity of a micellar-encapsulated drug has accumulated in the tumor interstitium. This time required for drug accumulation in the tumor volume depends on the pharmacokinetics of the particular delivery system [85, 98–100].

3.7. Light-responsive polymers

Since the stimulus of light can be imposed instantaneously and can be delivered in specific amounts with high accuracy, it renders light-responsive hydrogels highly advantageous over others. Also, the capacity for instantaneous delivery of the sol–gel stimulus renders light-responsive polymers potentially applicable for the development of optical switches, display units and ophthalmic drug delivery systems [2]. Light-responsive polymers may be UV or visible light sensitive; however, visible light-responsive polymers and hydrogels are more beneficial in that they are safe, inexpensive, readily available, clean and easily manipulated [2]. Bis(4-dimethylamino)phenylmethyl leucocyanide, a leuco derivative molecule, was introduced into a polymer network to produce a UV light-responsive hydrogel. Triphenylmethane leuco derivatives are normally neutral but dissociate into ion pairs under ultraviolet irradiation producing triphenyl methyl cations. At a fixed temperature, the hydrogels discontinuously

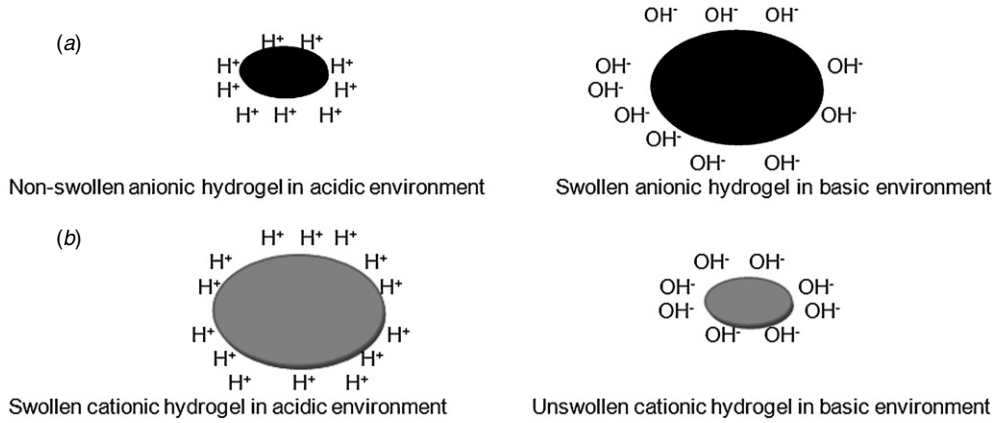

Figure 6. The pH-responsive swelling of (*a*) anionic and (*b*) cationic hydrogels (adapted from Gupta [1]).

swell in response to UV irradiation but shrink when the UV light is removed. The swelling is due to an increase in osmotic pressure within the gel due to the appearance of cyanide ions formed by UV irradiation [101].

By introducing a light-sensitive chromophore, e.g. trisodium salt of copper chlorophyllin to poly(*N*-isopropylacrylamide) hydrogels, visible light-responsive hydrogels can be prepared [102]. When light is applied to the hydrogel, the chromophore absorbs the light which is then dissipated locally as heat by radiationless transitions, increasing the 'local' temperature of the hydrogel. This increase in temperature alters the swelling behavior of the hydrogel. By addition of another functional group, such as an ionizable group of polyacrylic acid, the light-responsive hydrogel can be rendered pH-sensitive as well and may be activated (i.e. induced to shrink) by visible light and can be deactivated (i.e. induced to swell) by increasing pH [103]. Although the action of the light stimulus is instantaneous, the reaction of the hydrogels to the action is slow since light energy first has to be converted into thermal energy.

4. Internally regulated drug delivery systems

Internal stimuli-responsive systems have gained wider attention compared to systems responding to external stimuli due to their feasibility in therapeutic applications, product scale-up and cost considerations [1].

4.1. pH-responsive polymers and drug delivery systems

4.1.1. Physiological considerations. For the oral delivery of any drug, certain physiological aspects of the body and more specifically the gastrointestinal tract has to be kept in mind. For instance, an obvious pH change occurs along the gastrointestinal tract [6, 17]; however, more subtle changes occur within the various bodily tissues (table 2). Chronic wounds have a pH between 7.4 and 5.4 and cancer tissue has been reported to be acidic extracellularly [17, 104]. The same is true for the different cellular compartments in the body [105]. Since variations in pH occur within the body, unlike temperature changes, this property can be exploited to direct a response to a certain tissue or cellular compartment. Also, local pH changes in response to specific substrates can be generated and exploited for modulating drug release.

Table 2. pH in various tissues and cellular compartments (adapted from Schmaljohann [6] and Watson *et al* [105]).

Tissue/cellular compartment	pH
Blood	7.35–7.45
Stomach	1.0–3.0
Duodenum	4.8–8.2
Colon	7.0–7.5
Early endosome	6.0–6.5
Late endosome	5.0–6.0
Lysosome	4.5–5.0
Golgi	6.4
Tumor, extracellular	7.2–6.5

4.1.2. Properties of pH-responsive polymers. All pH-responsive polymers contain pendant acidic (e.g. carboxylic and sulfonic acids) or basic (e.g. ammonium salts) groups that are capable of either accepting or releasing protons in response to environmental changes in pH [1, 2, 6]. Changes in the environmental pH thus lead to conformational changes of the soluble polymers and a change in the swelling behavior of the hydrogels when ionizable groups are linked to the polymer structure. The most commonly studied ionic polymers for pH-responsive behavior include poly(acrylamide) (PAAm), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(diethylaminoethyl methacrylate) (PDEAEMA) and poly(dimethylaminoethyl methacrylate) (PDMAEMA). But polymers containing phosphoric acid derivatives have also been reported [106, 107]. The swelling of pH-responsive hydrogels is governed by their degree of ionization i.e. protonation or deprotonation (figure 6). On exposure to aqueous media of appropriate pH and ionic strength, pendant groups ionize and develop fixed charges on the polymer network, causing electrostatic repulsive forces responsible for pH-dependent swelling or deswelling of the hydrogel, which ultimately controls drug release [1].

Various natural polymers such as albumin and gelatin have also shown pH-responsive swelling behavior. Under appropriate conditions, these linear polymers form helices

that function as cross-links that hold the amorphous regions together. These proteins have a minimal surface charge at their isoelectric point (p*I*) and show extensive swelling at a pH away from their p*I*. This occurs due to the development of a high surface net-charge and increased electrostatic repulsive forces [108, 109].

4.1.3. pH-responsive cancer targeting. Drug molecules that are conjugated to a polymer are usually inactive. These conjugates are therefore known as prodrugs. The use of prodrugs is particularly advantageous for cytotoxic drugs e.g. the incorporation of a targeting system in cancer chemotherapy may avoid or minimize adverse reactions that may lead to non-specific toxicity. However, only an optimal release of the drug at the site of action gives these prodrugs the full advantage. The hydrostatic pressure inside a tumor mass is significantly higher than it is in the vasculature; therefore, many drugs are incapable of diffusing evenly within the interstitial matrix. Also, the tumor metabolic profile is different within the interstitial matrix because of poor oxygen perfusion which results in elevated levels of lactic acid and a reduction in pH from 7.4 to 6 [110]. The triggering, targeting and controlling of drug release according to variations in pH can be achieved in two ways. Firstly, the extracellular tissue can be targeted: tumor tissue has an extracellular pH between 6.5 and 7.2, which is slightly lower than the normal pH of 7.4. Secondly, the lysosomes may be targeted: after cellular uptake, the drug conjugate reaches the lysosome which has a pH of 4.5–5.0, and in this case hydrolytic enzymes such as cathepsin B may also be utilized to release drug [111].

It has been established that nanocarriers can concentrate preferentially on solid tumors, by virtue of the EPR mechanism [112]. Once accumulated at the tumor site, they can act as a local drug depot depending upon the makeup of the carrier [113, 114]. Shenoy *et al* [115] created pH-sensitive nanoparticles that are capable of boosting the delivery of the anticancer drug paclitaxel to tumor cells. A representative poly(β-amino ester) (PbAE) with biodegradable and pH-sensitive properties was used to formulate this delivery system. It was found that this nanoparticle-paclitaxel formulation was more effective at killing cancer cells than was free drug. Investigators have also developed and characterized nanoparticles using poly(ε-caprolactone) (PCL) to increase the local concentration of tamoxifen in estrogen receptor (ER)-positive breast cancer [116]. Poly(l-histidine)-*b*-PEG in combination with PLLA-*b*-PEG and adriamycin (ADR) as drug was also studied for extracellular tumor targeting [117]. Ionized destabilized micelles triggered adriamycin release.

4.2. Ionic strength-responsive polymers

Stimuli-responsive polymers may respond to applied stimuli in various different ways. The responsiveness to ionic strength is a typical property of polymers containing ionizable groups. Changes in ionic strength may cause changes in the size of the polymeric micelles, polymer solubility and the fluorescence quenching kinetics of the chromophores bound to electrolytes [118–120]. Polyampholytes incorporate both anionic and cationic charged moieties into a single polymer chain. These polymer systems exhibit unusual rheological behavior as a result of the attractive Coulombic interactions between oppositely charged species. The behavior of polyampholytes in solution is also related to the ratio of the ionic species incorporated into the polymer [119, 121, 122]. This ratio can be altered through synthetic methods and through extrinsic changes in the aqueous environment, particularly changes in pH. In the presence of an excess of either anionic or cationic groups, the systems behave as polyelectrolytes. However, as the ratio of anionic to cationic species approaches unity, the solution behavior is dominated by Coulombic attractions. These attractions may render the polymer insoluble in deionized water but soluble in the presence of a critical concentration of added electrolytes as the attractive charge/charge interactions are shielded.

Different concentrations of salts, which determine the ionic strength, may cause phase transitions in ionic strength-responsive polymers. Cu(II) metal ion was immobilized on poly(NIPAAm-*co*-vinylimidazole) for protein separation using the affinity binding of specific proteins to Cu(II) [123]. With an increase in the ionic strength, the polymer chains binding the proteins precipitated. The high salt concentration reduced the repulsive electrostatic strength of the copolymer, which resulted in an increase in the hydrophobic interactions and thus lead to precipitation.

4.3. Glucose-responsive polymers

4.3.1. Physiological implications of uncontrolled glucose levels. The treatment for insulin-dependent diabetes mellitus (IDDM) is currently limited to insulin injection. This treatment modality however significantly burdens patients and more importantly insulin modulation in this manner remains unstable [124]. An overdose of insulin may send a patient into a hypoglycemic coma or when insufficient insulin is injected hyperglycemia may result [15]. Regular glucose testing is not only critical in diabetic patients but is also of immense value in monitoring cell growth since glucose is the primary carbon source in most fermentation processes [125]. It is for this reason that precisely engineered glucose-sensitive/glucose-responsive delivery systems promise to have huge potential in the quest for maintaining appropriate glucose levels. It may also facilitate the construction of an artificial pancreas that may be capable of functioning in a manner similar to the beta cells of the pancreas [17].

4.3.2. Glucose-responsive insulin delivery. Glucose-responsive hydrogel systems have the ability to provide self-regulated insulin release in response to the concentration of glucose in the blood, thereby controlling the concentration of insulin within a normal range [20]. The most common of these hydrogel systems utilize immobilized enzymes or biocatalysts, more specifically glucose oxidase [126–128]. The rationale behind this is that generally when an enzyme is covalently coupled to a smart polymer, environmental changes result in drastic changes in the polymer conformation which significantly affects the enzyme activity and substrate access

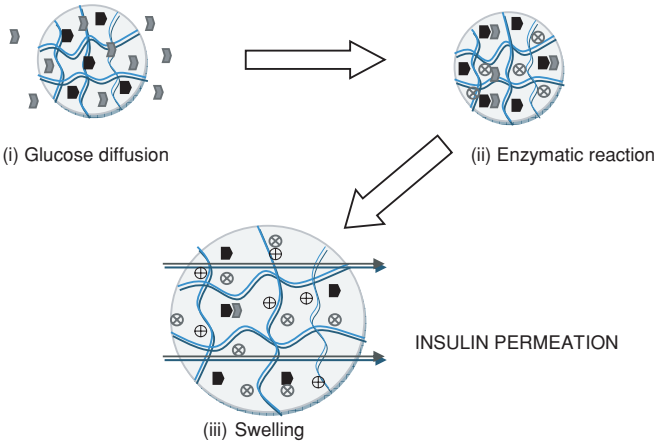

Figure 7. Schematic representation of a glucose-responsive glucose-oxidase-loaded membrane. ◗ = glucose oxidase, ◗ = glucose, ⊗ = gluconic acid (adapted from Miyata *et al* [132]). (This figure is in colour only in the electronic version)

to the enzyme molecule. These biocatalysts act by catalyzing an enzymatic reaction in their soluble state and the products of this enzymatic reaction then triggers the gel's phase transition. Studies have shown that conjugating the biopolymer chitosan using carbodiimide conjugation formed gel beads of the enzyme–biopolymer complex. A sulfonamide-based glucose-responsive hydrogel with covalently immobilized glucose oxidase and catalase was also synthesized and evalvated [126, 128].

In the case of insulin delivery, glucose oxidase exploits the pH sensitivity of the polymer used to immobilize the enzyme (figure 7). The glucose oxidase oxidizes glucose to form gluconic acid which causes a pH change in the environment [2]. The pH-sensitive hydrogel then exhibits a volume transition in response to the lowered pH, which occurs due to the formation of gluconic acid. Therefore, the swelling ratio of the hydrogel is regulated by the body's glucose levels [17].

In the case of pH-sensitive membrane systems made of polycations such as poly[(*N,N*-dimethylamino)ethyl methacrylate] (PDEAEM), a lowering in the pH results in swelling of the membrane which tends to cause an increase in drug release e.g. insulin. This occurs due to ionization of the polymer in the acidic medium [2, 129, 130]. In the case where the hydrogels are made of polyanions, insulin release is regulated by different mechanisms. These polyanions can be grafted to a porous filter, e.g. poly(methacrylic acid-co-butyl methacrylate) to form grafted polyanion chains that are expanded at pH 7. This occurs due to electrostatic repulsive forces among the charges on the polymer chains. However, when gluconic acid is produced, the chains collapse due to protonation of the carboxyl groups of the polymer which results in the opening of the pores and insulin diffusion [131]. A dry pH-responsive polymer, poly[(*N*, *N*-dimethylamino)ethyl methacrylate-*co*-ethylacrylamide] was combined with glucose oxidase, bovine serum albumin and insulin and compressed into an insulin-loaded matrix. The delivery system resulted in the oxidation of glucose to gluconic acid due to the presence of glucose oxidase. This in turn caused a decrease in the pH, protonation and swelling of the polymer, and ultimately insulin release.

Concanavalin A (Con-A) is a glucose-binding protein capable of holding up to four glucose units per molecule. It is obtained from the jack bean plant, *Canavalia ensiformis*, which is frequently being used in modulated insulin delivery. Pioneering studies in glucose-responsive insulin delivery focused on the synthesis of stable, biologically active glycosylated insulin derivatives capable of forming a complex with Con-A [133–135].

4.4. Protein-responsive polymers

4.4.1. Enzyme-responsive polymers. Enzyme-responsive polymers form the basis for hydrogels that are responsive only to specific enzymes. These enzymes are used as signals for monitoring several physiological changes and have very successfully been used as signals for the site-specific delivery of various drugs to specific organs. The microbial population of the colon has been extensively exploited for targeting drugs specifically to this region of the gastro-intestinal tract (GIT). Colonic drug delivery is intended for the local treatment of irritable bowel syndrome (IBS) and can potentially be useful for the treatment of colon cancer or the systemic delivery of drugs that are adversely affected by the upper gastro-intestinal tract [136]. Except for the reduced incidence of adverse effects, reduced dosages required and improved patient compliance with treatment, colon-targeted drug delivery is also known to be an ideal site for protein and peptide absorption, which would ordinarily be degraded by acid and enzymes in the upper GIT [137–139]. Precise colonic delivery requires that the triggering mechanism/stimuli in the delivery system only respond to the physiological conditions particular to the colon. Colonic microflora may include reductive enzymes (e.g. azoreductase) or hydrolytic enzymes (e.g. glycosidases) and they are also capable of degrading various types of polysaccharides, e.g. pectin, chitosan, cyclodextrin, dextrin. Since the microbial enzyme dextranase can degrade the polysaccharide dextran it has been exploited by formulating hydrogels cross-linked with diisocyanate for colonic delivery [140]. *In vitro* studies in a human colonic fermentation model demonstrated the degradation of these hydrogels by dextranase.

Researchers have used azoaromatic bonds which are sensitive to azoreductase degradation to target drug delivery to the colon [141–145]. Azoaromatic bonds were used as cross-linking agents to produce hydrogels which were pH-sensitive due to acidic co-monomers. On passage through the GIT, the hydrogels swell due to the ionization of carboxylic acid groups. In the colon, azoreductase can access the cross-links of the swollen hydrogels and degrade the matrix to release the protein drugs. This combination of pH and enzyme sensitivity enables a site-specific colonic drug delivery.

4.4.2. Inflammation-responsive polymers. The inflammatory process is initiated by T- and B-lymphocytes, but polymorphonuclear leukocytes (PMNs) and macrophages mediate amplification and perpetuation of the inflammatory

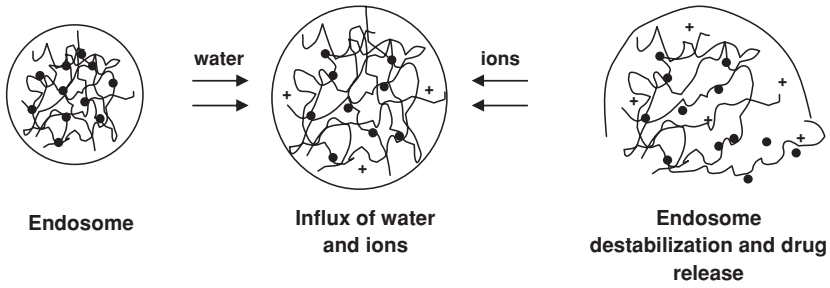

Figure 8. Schematic representation of endosomal escape and drug release of *N*-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles (adapted from Park *et al* [158]).

process. The tissue damage is caused by various chemical mediators including arachidonic acid metabolites, proteolytic enzymes and oxygen metabolites. Attention has been directed to reactive oxygen metabolites (oxygen free radicals) released by PMNs and macrophages during the initial phase of inflammation [146]. Such chemical mediators warrant attention as stimuli for responsive drug delivery. Previous groups have demonstrated the degradation of hyaluronic acid (HA) hydrogels cross-linked with glycidylether by hydroxyl radicals *in vitro* and by inflammation *in vivo*. *In vitro*, hydroxyl radicals induced a rapid but limited degradation of the cross-linked HA gels. *In vivo* implantation experiments revealed the degradability of the HA gel in response to inflammation [147].

4.5. Functional polymer blends

Recently, scientists have opted for the design of functional polymer blends from existing polymers. This principle involves the blending of minor fractions of functional additives with inert matrix polymers instead of synthesizing new, complex functional macromolecules from scratch, thus creating new and novel materials with unique and unusual property matrices. These polymer blends may have integrated sensors in their materials which are capable of visually detecting stimuli, e.g. mechanical deformation [148]. This is based on the incorporation of small amounts of specially tailored fluorescent dyes into conventional polymers and relies on the formation of nano-scale aggregates of the sensor molecules in the polymer matrix. This type of sensing scheme may be useful in a wide variety of applications such as tamper-evident packaging materials and smart fishing lines. Research is also being conducted on the general idea of incorporating stimuli-responsive molecules into nano-scale templates which feature periodic structures. This results in hybrid materials in which the functionality of small organic molecules is combined with the geometric effects of the template. These hybrid materials display properties that are then absent in their respective constituents [149].

4.6. Self-assembled nanoparticle systems

There is continued investigation of polymeric nanoparticles for the intracellular delivery of different classes of therapeutic agents (e.g. chemicals, oligonucleotides, siRNA, DNA and proteins) [150]. Nanoparticles are thought to enter cells via an endocytotic pathway through either specific (e.g. receptor-mediated endocytosis) or non-specific interactions (e.g. adsorptive endocytosis) with cell membranes. Following cellular endocytosis of a nanoparticulate system, efficient delivery of the therapeutic load necessitates that these systems overcome intracellular barriers, such as endosomes, and release the agent into the cytosol [151, 152]. In the absence of a mechanism for endosomal escape, the nanoparticulate system is localized in endosomes for ultimate trafficking to lysosomes. Research is being focused on the development of supramolecular assemblies, such as polymeric micelles and nanoparticles, which selectively release drugs or genes into the cytosol by sensing a low pH in endosomes and lysosomes [153–157].

Park *et al* [158] described *N*-acetyl histidine-conjugated glycol chitosan (NAcHis-GC) self-assembled nanoparticles as a promising system for intracytoplasmic delivery of drugs. Because *N*-acetyl histidine (NAcHis) is hydrophobic at neutral pH, the conjugates formed self-assembled nanoparticles. When exposed to the slightly acidic environment in endosomes, the nanoparticles were disassembled due to breakdown of the hydrophilic/hydrophobic balance by the protonation of the imidazole group of NAcHis (figure 8). Flow cytometry and confocal microscopy showed that NAcHis-GC nanoparticles released drugs into the cytosol and cell cycle analysis demonstrated that their paclitaxel-incorporated NAcHis-GC nanoparticles were effective in inducing arrest of cell growth.

5. Conclusions

Although possibly expensive at the beginning, chronotherapy can ultimately be cost effective in the long run. The delivery of an effective concentration of drug at the right time and at the right place can minimize systemic adverse reactions, reduce drug dosage and eventually lower the cost and improve therapeutic outcome and patient compliance. Research into stimuli-responsive polymers as a means of achieving this is steadily gaining momentum and more novel polymers are being synthesized to be incorporated into innovative delivery systems intended for site-specific and self-regulated drug delivery. These stimuli-responsive polymers are also immensely valuable in applications such as bioseparation of proteins and other bio-particles for basic life science research as well as industrial applications. Recent research trends in stimuli-responsive systems are focused on the employment of

smart polymers in order to achieve site-specific drug release in specific areas of the gastro-intestinal tract relying solely on a stimulus specific to that region.

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