Temperature-Responsive Smart Nanocarriers for Delivery Of Therapeutic Agents: Applications and Recent Advances

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Temperature-Responsive Smart Nanocarriers for Delivery Of Therapeutic Agents: Applications and Recent Advances

Mahdi Karimi‡,§,⊥, Parham Sahandi Zangabad#,||, Alireza Ghasemi‖, Mohammad Amir‖, Mohsen Bahrami‖, Hedieh Malekzad§,¶, Hadi Ghahramanzadeh As‖, Zahra Mahdieh□, Mahnaz Bozorgomid○, Amir Ghasemi§,‖, Mohammad Reza Rahmani Taji Boyuk‖, and Michael R. Hamblin*,⊥,△,▲

‡Research Center for Pharmaceutical Nanotechnology (RCPN), Tabriz University of Medical Science (TUOMS), Tabriz, Iran
‖Department of Materials Science and Engineering, Sharif University of Technology, Azadi Avenue, 14588 Tehran, Iran
§Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
¶Department of Chemistry, Kharazmi University of Tehran, Tehran, Iran
○Department of Biomedical and Pharmaceutical Sciences, Material Science and Engineering, University of Montana, Missoula, Montana 59812, United States
□Department of Applied Chemistry, Central Branch of Islamic Azad University of Tehran, Tehran, Iran
△Department of Dermatology, Harvard Medical School, Boston, Massachusetts 02115, United States
▲Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts 02139, United States

Abstract

Smart drug delivery systems (DDSs) have attracted the attention of many scientists, as carriers that can be stimulated by changes in environmental parameters such as temperature, pH, light, electromagnetic fields, mechanical forces, etc. These smart nanocarriers can release their cargo on demand when their target is reached and the stimulus is applied. Using the techniques of nanotechnology, these nanocarriers can be tailored to be target-specific, and exhibit delayed or
controlled release of drugs. Temperature-responsive nanocarriers are one of the most important groups of smart nanoparticles (NPs) that have been investigated during the past decades. Temperature can either act as an external stimulus when heat is applied from the outside, or can be internal when pathological lesions have a naturally elevated temperature. A low critical solution temperature (LCST) is a special feature of some polymeric materials, and most of the temperature-responsive nanocarriers have been designed based on this feature. In this review, we attempt to summarize recent efforts to prepare innovative temperature-responsive nanocarriers and discuss their novel applications.

**Graphical Abstract**

**Keywords**
smart drug delivery systems; temperature-responsive nanocarriers; LCST/UCST behavior; gene delivery; dual/multi responsive; synthesis; characterization; anticancer delivery

1. **INTRODUCTION**

The outstanding area of nanobiotechnology has led to many innovative breakthroughs, and has made tremendous impacts on various fields of basic and applied science ranging from chemistry and physics, to medicine and biomedical engineering. In the biomedical field several nanotechnology-based approaches have influenced the development of therapeutic drugs and pharmaceutical formulations. In particular, the increasing burden to human health caused by serious diseases (e.g., various types of cancers, coronary artery disease (CAD), etc.) has required ongoing innovations in drug delivery and drug eluting systems, many of which rely on nanotechnology. The main applications of these new drug-delivery and targeting systems have been focused on imaging agents for cancer diagnosis, and the development of new anticancer drugs and strategies.1–9 The most utilized nanocarriers in recent years have been based on NPs including liposomes, dendrimers, polymeric NPs, metal NPs, carbon nanomaterials (e.g., fullerenes, carbon nanotubes (CNTs)), solid lipid NPs (SLNs), nanoshells and magnetic NPs, mesoporous silica NPs (MSNs), as well as novel nanostructures based on albumin, chitosan, etc.5,10–17

DDSs for targeted-delivery and controlled-release of drugs and other cargos can be designed to be responsive to a range of different stimuli. These stimuli can either be internal in nature (pH, redox, enzymatic activity, concentration of specific biomolecules) or external in nature.
(magnetic fields, light of various wavelengths, electric fields, ultrasound, mechanical pressure, etc.).\textsuperscript{18–23} Temperature is unique among this array of stimuli as it can be considered to be either internal (some tissues such as tumors and infections are naturally at elevated temperature) or to be external (heat is applied from outside the body). For example, hydrogels can respond to many of these stimuli by undergoing abrupt swelling of their structure leading to volume changes and consequent drug release.\textsuperscript{24,25}

Recently, the introduction of biocompatible temperature-responsive polymeric materials that can be designed to make smart drug nanocarriers, has motivated many investigations.\textsuperscript{26–31} Many studies have confirmed the hypothesis that drug release from these thermoresponsive polymers can be triggered by relatively small variations in temperature.\textsuperscript{26,27,29–32} Various thermosensitive polymers can be used to prepare temperature-responsive hydrogels,\textsuperscript{33,34} such as poly(N-isopropylacrylamide (PNIPAAm) derivatives,\textsuperscript{35} poly(ethylene oxide)-poly(propylene oxide) (PEO–PPO) pluronic copolymers\textsuperscript{36}, core–shell thermoresponsive NPs,\textsuperscript{29,37} polymeric nanotubes,\textsuperscript{28} polymeric micelles,\textsuperscript{10,27,38} layer-by-layer (LBL)-assembled nanocapsules,\textsuperscript{26} microbeads (MBs),\textsuperscript{39} and elastin-like polypeptides (ELPs).\textsuperscript{40} These nanostructures have all been utilized as thermoresponsive carriers in DDSs. Furthermore, other particles formed from polysaccharides (e.g., chitosan and hyaluronic acid (HA)) have been used to modulate the drug encapsulation and release efficiency of thermoresponsive polymeric NPs (e.g., hydrogels).\textsuperscript{41,42} As a consequence of the temperature-sensitivity of these particles (that can respond to only very slight variations in temperature) leading to a subsequent phase transition, the encapsulated cargo can be released.\textsuperscript{43} Thermoresponsive delivery systems can be designed according to the required application, to lead to increased cytotoxicity of the encapsulated drug,\textsuperscript{33,38} or conversely, can be designed to limit the cytotoxicity\textsuperscript{44} until it reaches the targeted site. Recently, some innovative multifunctional nanosystems such as thermoresponsive polymer-coated magnetic NPs (MNs)\textsuperscript{11} and temperature sensitive liposomes (TSLs) encapsulating MRI contrast agents (CAs)\textsuperscript{3} have been developed for simultaneous diagnosis and therapeutic application (theranostics) for cancer treatment. In addition, thermoresponsive nanocarriers have been utilized in multi-stimuli-responsive delivery systems. For example, dual stimuli-responsive systems, e.g., temperature/pH-responsive polymeric NPs,\textsuperscript{43} pH/thermosensitive microcontainers,\textsuperscript{35} pH/thermosensitive core–shell nanostructures,\textsuperscript{39} and triple-stimuli-responsive systems, e.g., thermo-, pH-, and reduction-sensitive polymeric micelles\textsuperscript{46} and glucose-, pH-, and thermoresponsive nanogels\textsuperscript{47} have been reported so far.

Many different synthesis methods have been tested for preparing these thermoresponsive polymeric NPs, such as free radical polymerization followed by hydrolysis,\textsuperscript{48} UV-irradiation-mediated graft copolymerization,\textsuperscript{49} and phase separation, emulsion, and foaming.\textsuperscript{50} Moreover, diverse methodologies have been used for characterization of the thermoresponsive polymeric NPs such as cryogenic transmission electron microscopy (cryo-TEM),\textsuperscript{51} atomic force microscopy (AFM),\textsuperscript{52} nuclear magnetic resonance (NMR) spectroscopy,\textsuperscript{53,54} small-angle X-ray scattering (SAXS),\textsuperscript{51,55,56} Fourier-transform infrared spectroscopy (FT-IR),\textsuperscript{10} UV–vis,\textsuperscript{36} and static and/or dynamic light scattering (SLS/DLS).\textsuperscript{46,57}
Thermoresponsive nanocarriers have mostly been tested for the controlled delivery of drugs\(^{58,59}\) and genes.\(^{60,61}\) However, in recent years, thermoresponsive DDSs have been mainly used for anticancer drugs and imaging agents.\(^{62,63}\) In cancer treatment, improving the efficient and specific targeting of the anticancer therapeutics to cancerous sites is a critical issue.\(^2\) Cancerous cells and tumors have been reported to have particular physical and biological features in comparison with normal cells and tissues, including differences in morphology, permeability, elasticity, blood flow, interstitial pressure, mechanical microenvironment, expression of glycans, and metabolic autonomy. Furthermore, the pH of the cancerous microenvironment found in tumors is significantly lower (more acidic) than that of blood and normal tissues.\(^{64–67}\) Targeted, smart stimulus-responsive multifunctional DDSs are of great importance due to their advantages such as lower systemic toxicity, prevention of overdosing, and the more controlled cancer therapy. Temperature, as external stimulus triggering, can be caused by a light irradiation from outside, applying magnetic fields, electric fields, or external heating.\(^1\) Thermoresponsive intracellular release of the drugs can enhance their cytotoxicity inside the cancer tissue.\(^{38}\) Furthermore, cancer cells are intrinsically susceptible to the heat, and can also be directly eradicated by means of external heating.\(^{68}\)

The principal of the thermoresponsive polymer NPs (e.g., hydrogels) is based on their solubility behavior that changes in response to the alterations in the temperature. Most often, the thermoresponsive behavior is obtained by utilizing the LCST polymers by which the solubility behavior of polymeric particles is controlled by temperature changes. The solubility of thermosensitive NPs is increased by decreasing the temperature below the LCST. Subsequently, a volume phase transition and swelling of the hydrogel occurs by means of the formation of hydrogen bonds. Such polymeric NPs are called “negative thermosensitive” polymers.\(^{34,69,70}\) Many studies have been conducted to develop controlled drug/gene delivery systems (DGDSs) (especially for anticancer applications) using the LCST features of polymeric particles.\(^{62,63,71,72}\) However, only a relatively few studies have been done on the application of positive hydrogel polymers possessing an “upper critical solution temperature” (UCST) responsive transition.\(^{73}\) In such polymers, through exploiting the changes that occur while solubilizing the hydrogels above the UCST, they start swelling.\(^{34,69,70}\) Thus, by means of controlling and altering the LCST/UCST transition of the polymers, controlled delivery systems can be acquired.

Thermoresponsive nanostructures are considered as useful drug carriers because of advantages such as low toxicity, being target specific, and prevention of overdoses. Polymers with LCST serve as triggers for these innovative NPs, which can be stimulated with external stimuli such as temperature and can help with release of drugs in cancerous tissues, and increase the cytotoxicity. These nanocarriers have been produced with different shapes for different applications. For example, magnetic nanocarrier-coated polymers can be carriers for MRI contrast agents, or microgels can not only be used as enzymes carriers, but also can be used to study the rheology and the crystallization of concentrated colloidal suspensions.

In this review, the most recent advances in temperature-responsive DDSs will be discussed.
2. LCST/UCST BEHAVIOR OF THERMORESPONSIVE POLYMERIC NPS

Smart polymers undergo fast and reversible structural changes in response to the specific biological stimulus, such as temperature, pH, enzyme activity, hypoxia, and reduction/oxidation (redox) potentials. In particular, the temperature-sensitive features of these intelligent polymeric systems, cause a phase transition that occurs above or below a specific temperature. Thermoresponsive polymers are generally divided into two types according to how the smart polymer reacts to changes in temperature; the first group has a lower critical solution temperature (LCST), whereas the second group has an UCST. As mentioned above, there are only a few reports of polymers that display a UCST, therefore this review mainly focuses on polymers, which display a LCST.74–77

Tunability, versatility in design and site specific phase transition are features of temperature responsive DDSs.78 Among various temperature responsive polymers, PNIPAAm is the most studied polymer with a well-known reproducible LCST behavior.79 The LCST of PNIPAAm can range from 30 to 35 °C depending on the precise solvent and chain modifications.80 However, some researchers,81 have reported an unusual UCST behavior. In the forthcoming section, PNIPAAm will be used to elucidate transitional behavior and delivery features of drug-loaded polymeric carriers.

Below the transition temperature of the LCST, polymers with transitional behavior are soluble and polymeric medium is swollen because of hydrogen bonds formed between molecules of water and the functional groups of incorporated polymer, making them ready to be loaded with drug molecules. When the temperature is increased beyond the LCST, a hydrophilic–hydrophobic transition occurs accompanied by a change in morphology from coil-to-globule. During this transition, the hydrogen bonds and the network collapses, and the polymer becomes insoluble, leading to volumetric shrinkage and squeezing-out of internal water molecules. This transition results in release of the encapsulated cargo from the medium.82–87

Shrinkage of a loaded polymer is shown in Figure 1 as a result of LCST transition. As it is obvious, the shrinkage is accompanied by release of loaded drug.88 Although this scheme refers to a cross-linked PNIPAAm hydrogel network, but same scenario is applicable in the case of polymeric micelles and so on.89 Originally, LCST transition is related to the nature of polymer. Regardless of carrier state, i.e., polymeric hydrogel or micelle and so on, below the LCST temperature, the polymeric medium is hydrophilic in nature, whereas above the LCST, it became hydrophobic. Increasing temperature beyond transitional temperature decreases solubility and volumetric features of carrier medium, leading to release of loaded drug.90 On the other hand, an increase in the polymer solubility can occur when the temperature rises above the UCST, resulting in the swelling of the carrier medium.34,70 It is worth noting that the volumetric change is reversible79,82–84 and it is termed “swelling-shrinkage” behavior.

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3. MISCELLANEOUS TEMPERATURE-RESPONSIVE NANOCARRIERS

3.1. Hydrogels

An important group of materials used in thermoresponsive carriers are hydrogels/nanogels. Thermosensitive nanogels have a volume phase transition at a specific temperature known as the volume phase transition temperature (VPTT), which causes an increase or decrease in particle size. The VPTT and the phase transition temperature range are the key factors in thermoresponsive nanogels suitable for developing a smart DDS. Because the temperature at most disease sites within the body is higher than that at normal sites, the VPTT with slightly higher than biological temperature is preferred.

Hydrogels are physically or chemically cross-linked networks with a high water content. After exposure to external stimulation, a variety of responses may occur in the hydrogel, such as sol–gel phase transition, swelling–shrinking behavior or hydrogel degradation. It is worth noting that these responses generally originate from the effects of specific functional groups. Because of the wide range of applications of thermoresponsive hydrogels, they have been studied more extensively than the other types. Drug/protein delivery, tissue engineering, and sensor films have been proposed as some of their applications.

Among thermally responsive hydrogels, PNIPAAm is the most often studied, because of its unique thermosensitive properties, i.e., a considerable swelling ratio and good thermal reversibility. Copolymerization and grafting of PNIPAAm with other monomers, e.g., polyethylene glycol (PEG) and poly(ethylene oxide) (PEO), are established routes to obtain new hydrogels. Cellulose derivatives, polycaprolactone, polylactic-co-glycolic acid (PLGA), and polyurethane amide (PUA), are other frequently studied polymers that form hydrogels.

Potential cytotoxicity and lack of biodegradability can be considered possible drawbacks of NIPAAm-based materials. Different approaches have been proposed to overcome these problems. The introduction of biodegradable segments into the polymer backbone or into the side chains, or using cleavable bonds as a cross-linking agent can all result in faster degradation and better biocompatibility. In another study, citric acid, PEG, and PNIPAAm were copolymerized. The incorporation of biocompatible polymers such as PEG facilitated degradability and biocompatibility. Additionally, citric acid could act as an antioxidant to reduce side effects in patients.

A biodegradable, specially designed PNIPAAm-g-PEO was synthesized using reversible addition–fragmentation chain transfer (RAFT) polymerization. The hydrophilic constituent had a comb-type structure allowing facile diffusion of water molecules across the “tunnels”. This improved the responsivity to thermal conditions that was achieved in this PNIPAAm-g-PEO copolymer. Figure 2 shows these tunnels that provide facile water diffusion.

Some details concerning temperature-responsive hydrogels, such as LCST/UCST behavior, synthesis method, drug loading, and release profile are listed in Table 1.
3.1.1. Transition Temperature of Hydrogels—Hydrogels that have a transition temperature near to physiological temperature conditions can be used for in situ hydrogel-mediated drug delivery, with many advantages, i.e., they can be used as nonsurgical drug depots and for sustained delivery of drugs and proteins to susceptible living cells.\textsuperscript{81,82,106}

The transition temperature can be readily tuned by the copolymerization conditions and by varying the content of repeating units in the copolymer, for instance, in a study the content of acrylic acid (AAc) in NIPAAm copolymer was indicated to be varied.\textsuperscript{82} The LCST temperature could be changed by changing the AAc content, and at 1.8\% AAc an appropriate hydrogel for physiological conditions (i.e., temperature in the range of 37–38 °C) was obtained. Because of the carboxylic groups in AAc, pH-sensitivity could also be obtained. Therefore, this dual-responsive hydrogel could be a candidate for a hydrophilic drug carrier. A favorable poly(\(N\)-acryloyl glycaminamide) (PNAGA)-based hydrogel was obtained by Boustta et al.,\textsuperscript{73} who used alteration in the molar mass and concentration of the polymer. This polymer, whose UCST transition could be adjusted, was suggested as a delivery system for both neutral and ionic drugs.

Ha et al.\textsuperscript{108} proposed a PEG-based hydrogel as a codelivery system. The length of the PEG chains and cyclodextrin ($\alpha$CDs) acted as transition temperature-determining factors. The transition temperature varied from 30 to 60 °C, thus showing the potential for a variety of different applications. The hydrogel could be loaded simultaneously with two different anticancer drugs, camptothecin (CPT) and 5-fluorouracil (5-FU), to form a smart codelivery system. These are hydrophobic and hydrophilic drugs, respectively. Cellulose derivatives such as methylcellulose (MC) and carboxymethyl cellulose (CMC) can form thermally responsive hydrogels with a LCST. To lower the transition temperature and impart better biocompatibility, researchers have investigated the incorporation of hyaluronic acid (HA),\textsuperscript{93} poly(acrylic acid) (PAAc),\textsuperscript{106} and PEG\textsuperscript{106}.

HA with different molecular weight ranges was introduced into MC.\textsuperscript{93} The LCST was lowered to physiological temperature conditions. A hydrogel with the required rheological properties and good biocompatibility (cell viability assay with HFFF2 cells) was achieved with MC/LMW-HA blends. Lü et al.\textsuperscript{94} developed a CMC-g-PNIPAAm hydrogel in which the PNIPAAm side chains and the CMC backbone provided thermosensitivity and biodegradability, respectively. This hydrogel showed a low gelation concentration, i.e., a concentration of 2 wt \%, that was significantly lower than the normal gelation concentration of PNIPAAm-based hydrogels (Figure 3).

3.1.2. Encapsulation and Membranes—Burst drug-release kinetics may be sometimes observed in the case of hydrogels and porous capsules with low strength that are easily eroded. Additionally, hydrogels can suffer from poor encapsulation of hydrophobic drugs due to the hydrophilic nature of the hydrogel. To overcome these disadvantages, we must take special measures to prolong drug release and better encapsulate hydrophobic drugs.\textsuperscript{86,92,96,98}

Shi et al.\textsuperscript{98} designed hybrid membranes made from alginate/CaCO$_3$/PUA pH-temperature dual-responsive hydrogels, based on interaction of PUA and PAA and with LCST transition
of 55.3 °C. Here, sustained drug-release profile and lowered diffusion rate were observed because of the presence of hybrid CaCO$_3$ microparticles and alginate membranes.

In a similar approach, composites of alginate microspheres combined with PLGA–PEG–PLGA triple coblock hydrogel, were prepared as hybrid-membranes.\textsuperscript{96} Figure 4 shows a schematic representation of these hydrogels loaded with alginate microspheres. A significantly reduced diffusion rate of water-soluble drugs, and more sustained release (the release time was enhanced approximately 4–6 times compared with control) were observed. This system could combine the properties of both barriers, thus achieving prolonged release of hydrophilic drug.

Poloxamer 407-based hydrogels were used to encapsulate etoposide (ETO).\textsuperscript{86} Because of the high molecular weight of the polymer compared to the drug (almost 20 times greater), encapsulation was possible. The in vitro drug release profile showed sustained release, up to 48 h for poloxamer-based hydrogels.

3.2. Polymeric Micelles

The polymeric micelles with specific core/shell architecture generated by self-assembly of amphiphilic block copolymers, have been extensively studied as nanoscaled DDSs.\textsuperscript{109} Polymeric micelles possess unique core–shell structure, in which an inner core entraps hydrophobic drugs as a nanocontainer and is surrounded by an outer shell of hydrophilic polymers, such as poly(ethylene glycol) (PEG), and have proved persistency in the bloodstream and effective tumor accumulation after their systemic administration.\textsuperscript{110}

Polymeric micelles are promising nanocarriers for anticancer agents and are currently being employed in all three stages of clinical trials due to several advantages for drug delivery applications including high payload, ease of administration, reduced systemic toxicity, and improved therapeutic efficacy.\textsuperscript{111}

Thermosensitivity of micelles depends on the LCST or cloud point (CP) of the thermosensitive block of the block copolymer forming micelle. Based on the LCST behavior, several PNIPAAm-containing block copolymers have been reported to prepare thermosensitive micelles for DDSs.\textsuperscript{112}

The mechanism applied for controlled cargo release of thermoresponsive micelle polymers is related to reversible phase transition of PNIPAAm or other thermoresponsive contents in aqueous solution at around 33 °C (LCST); so that it is water-soluble and hydrophilic below its LCST and is insoluble, hydrophobic and aggregates above this point.

Micelles with a LCST of 38 °C would be mostly suitable to ensure constant temperature-dependent drug release in the bile duct. Because the temperature inside the liver is usually around 38 °C and because of the lower temperature of the intestine, drug release from micelles that reach the intestine through the bile duct would be decreased, leading to lower side effects. The bile duct is fully covered by liver tissue and its inner temperature is generally the same.\textsuperscript{113}
As an alternative to PNIPAAm, the oligomers containing oligo(ethylene glycol) (OEG) with amphiphilic characteristics that integrate the biocompatibility of PEG with a versatile and controllable LCST behavior have been used to develop polymeric micelles, such as copolymers of 2-(2-methoxyethoxy) ethyl methacrylate (MEO2MA) and OEG methacrylate (OEGMA), (P(MEO2MA-co-OEGMA), which self-assemble into spherical nanomicelles in water and exhibit a LCST, which can be tuned between 26 to 90 °C by altering the ratio of MEO2MA and OEGMA.\textsuperscript{114–116} The thermoresponsive biodegradable copolymer of poly(DL-lactide-co-glycolide)-poly-(ethylene glycol)-poly(DL-lactide-co-glycolide) (PLGA-PEG-PLGA) is proved to overcome shortcomings of the PNIPAAm copolymers, i.e., lack of biocompatibility and biodegradability, poor micellar stability.

### 3.3. Core–Shell Structures

Besides temperature-sensitive polymers such as hydrogels and micelles, core–shell nanostructures and even films have been also designed. Figure 5 illustrates a commonly employed core–shell structure. The main polymer network that has been of particular interest is formed from poly(N-isopropylacrylamide) (PNIPAAm). This polymeric network can undergo a volumetric phase transition after temperature elevation or by solvent uptake at low temperatures followed by swelling. After that phase transition, the swollen network then expels water and shrinks.

Another common structure that has been used in drug carriers, is that of thermoresponsive star block copolymers that can encapsulate the drug between their branches. Xiaojie Li and his group\textsuperscript{117} developed a star-shaped block copolymer H40-PCL-b-P(OEGMA-co-AzPMA). The polymer micelle showed satisfactory drug-release properties, with controlled release at temperatures higher than the LCST temperature.

In another study, hydrogen-bonded layer-by-layer films were utilized. These films could serve as a robust platform that could be triggered by temperature changes to release drugs. These films were assembled with temperature-responsive block copolymer micelles and combined with tannic acid. The polymeric micelles were made by heating solutions of a neutral diblock copolymer, poly(N-vinylpyrrolidone)-b-poly(N-isopropylacrylamide) (PVVPON-b-PNIPAAm), to a temperature above the LCST of PNIPAAm. Films and micelles made from PNIPAAm core–shell structure did not lose their structure after repeating the drug loading and releasing processes for 15 times.\textsuperscript{118}

### 4. SYNTHESIS METHODS OF THERMOSENSITIVE NPs

#### 4.1. Core–Shell Microgel Particles

Core–shell microgel particles that are thermosensitive can be synthesized by various methods, which depends on the nature and structure of the particles. Usually, the synthesize methods have a common path, that at first the core will be synthesized and then the shell is either synthesized by polymerization on the core or another step (usually surface modification) is required before the core will be covered with the shell. As follows, some cases has been mentioned to clear the synthesis method.
a. Two-step synthesis, that at first the core is synthesized and then the shell is prepared by polymerization on the core. One of the examples of this method is to prepare a core–shell particle with polystyrene (PS) as core and PNIPAAm as the shell. First, a seed latex component is synthesized by emulsion polymerization of PS. A thin shell of PNIPAAm is then generated around the core particles by copolymerization of NIPA monomer. Using seeded emulsion polymerization, the PNIPAAm network is polymerized onto the PS-cores (at $T > \text{LCST}$). Gel-like PNIPAAm particles form when the polymerization of NIPA and the cross-linker starts in the aqueous phase. Finally, these particles are precipitated onto the surface of the core particle.\textsuperscript{119}

Another example of two-step synthesis is when nonspherical systems or more accurately dumbbell-shape core–shell systems are made. For example, by using a precipitation polymerization method, a cross-linked PNIPAAm-shell is grafted onto the paired spheres each consisting of a PS core and a PMMA shell. Also, another example is when functional monomers have been used, and in these cases, it is possible to make either neutral shells with charged core or neutral core with charged shell. Richtering et al.\textsuperscript{120} developed core–shell microgels, utilizing temperature-sensitive PNIPAAm. Two different types of microgels could be produced, (a) neutral core and charged shell, and (b) charged core and neutral shell. In the microgel core, polyelectrolytes could be encapsulated and would be protected by the shell.\textsuperscript{119}

b. Three-step synthesis is when there is another step between synthesizing the core and putting a shell on it, so-called photoemulsion polymerization. This synthetic approach is a way to overcome a problem with the polymerization rate of the cross-linker, N,N$'$-methylenebis(acrylamide) (MBAm), which is higher than that of NIPA.\textsuperscript{121} Therefore, the growth of the microgel particles may not be uniform. As shown in Figure 6, this synthesis was accomplished in three steps: (1) Using a conventional emulsion-polymerization method, the PS-core particles containing 5 mol % NIPA were synthesized. (2) The photoinitiator 2-[p-(2-hydroxy-2-methylpropiophenone)]-ethylene-glycol-methacrylate (HMEM) is used to cover these core particles. (3) By means of light irradiation of a suspension of these particles, free radical generation occurs, leading to photoemulsion polymerization.\textsuperscript{122}

The core can also be made with inorganic materials. Fe$_3$O$_4$ is one of the materials that can be used as the super paramagnetic core. In the study by Zeng and his group, a core–shell NP with Fe$_3$O$_4$ was provided as the core and the next layer was poly(methacrylic acid) (PMAA), which is dual-responsive, and the final layer was PNIPAAm, which is thermal-responsive and works as a “gatekeeper”. The Fe$_3$O$_4$ NPs has been made with the help of copercipitation of ferric and ferrous ions in alkali solution. After the core was made, the Fe$_3$O$_4$\textsuperscript{−} PMAA NPs were made by
distillation precipitation polymerization. The latest layer was made by two-stage distillation precipitation polymerization with Fe₃O₄−PMAA as seeds.

c. Another method to synthesize core–shell particles is to graft copolymers. In this case, core and shell are created at the same time. In the study by Zhou and his group, the graft copolymer CS-γ-PSBMA is synthesized under gamma-ray irradiation with graft polymerization, and folic acid was loaded into the hydrophobic CS core with the help of self-assembly (Figure 7).

In a research by Huang and his group, the same method was used to graft chitosan to N-(2-hydroxyethyl) prop-2-enamide (HEPE) and produce another core–shell particle that could be responsive both to pH and temperature. Here, pH and temperature equal to 5.4 and 37 °C, respectively, showed the best drug release rate.

4.2. Hydrogels

Hydrogels are three-dimensional polymeric networks dispersed in water and make semi solid states containing more than 99% water in their composition. There are two main kinds of hydrogels: physical hydrogels formed by several physical entanglement between the polymer chains and/or micelle ordering in solution, and covalently (chemically) linked hydrogels that are synthesized by cross-linking of polymer chains with covalant bonds.

Over the past two decades, both groups of cohydrogels have been under intense development for such applications as drug delivery and tissue engineering. The transition from a polymer solution to a gel resulting from exposure to the thermal environment like temperature are initiated in a certain temperature. In other words, the hydrophilic groups in the polymeric network become hydrated in aqueous media and this prepares the mentioned transition from a polymer solution to a hydrogel.

Hydrogels may be explored by number of classical chemical methods including one-step procedures and multiple step ones. In the one-step method a hydrogel is synthesized by polymerization and parallel cross-linking of multifunctional monomers. However, in the multiple step method, a hydrogel can be prepared by reaction of polymers with suitable cross-linking agents, and possibly by synthesis of polymer molecules with reactive groups and the subsequent cross-linking of the polymers.

Hydrogel-containing polymersomes can act as thermosensitive nanocarriers. Lee et al. developed novel bilayer-enclosed nanostructures containing hydrosomes (a thermosensitive hydrogel). Such NPs were prepared by incorporation of PNIPAAm into the mPEG–PDLLA particles by injection of a THF solution into the water. The hydrogel-containing polymersomes were obtained at 37 °C. By using mPEG–PDLLA labeled with lissamine rhodamine B (RB) and fluorescein isothiocyanate (FITC)-labeled PNIPAAm dual fluorescent NP were prepared. Confocal microscopy showed the colocalization of both fluorophores in the particles. By using CHCl₃ as the organic phase, “giant” polymersomes with a diameter of 5–10 μm were formed that underwent phase separation of the internal FITC-NP above the LCST.
4.3. Thermoresponsive Composite Films

Thermoresponsive composite films can be prepared by grafting hydrogel layers (submicron thickness to several microns thick) onto a polycarbonate (PC) support layer in order to achieve controlled drug release. The grafting methods that can be used to prepare composite films include plasma modification,\textsuperscript{129,130} ionizing irradiation,\textsuperscript{131} redox polymerization,\textsuperscript{132,133} UV photografting,\textsuperscript{134} and atomic-transfer radical-polymerization (ATRP).\textsuperscript{135} To adjust the LCSTs of the products, NIPAAm and AAc copolymer hydrogels were formulated in grafted films. At temperatures near their LCST, the films showed swelling or shrinking of the poly(NIPAAm-co-AAc), and the PC support exhibited straight cylindrical pores.

The PC-hydrogel composite films were characterized in terms of film thickness and grafting yield by employing X-ray photoelectron spectroscopy (XPS) and FTIR techniques, and the LCSTs were calculated from effective pore diameter, water permeability and drug permeability data. The LCST increased from 34.0 to 41.8 °C, and the grafting yield and film thickness increased, as the concentration of AAc in the preparative solution was increased. With on–off ratios between 1.4 and 3.2, the films showed a good permeability to drugs (3.22 × 10\textsuperscript{-7} to 4.64 × 10\textsuperscript{-7} cm\textsuperscript{2}/s). With 1.8% AAc, the film exhibited an LCST close to human body temperature.\textsuperscript{133} In these composites citric acid, 4-acetamidophenol, KCl, and methyl orange could be used as model drugs. The larger molecules exhibited higher on–off ratio and lower permeability than the smaller model drugs (for neutral compounds). Lower permeability was found with ionic compounds with large hydration shells, such as the strong electrolyte (KCl). Increasing on–off ratio and drug permeability was found for the acidic compound (citric acid), promoting gel shrinkage. Figure 8 illustrates an on–off valve mechanism designed through grafting a P(NIPAAm-co-AAc) hydrogel onto microporous films. Thus, as the environmental temperature changes, drug permeation can be regulated.\textsuperscript{136}

4.4. Smart Three Dimensionally Ordered Porous (3DOM) Materials

In recent years, three-dimensionally ordered macroporous (3DOM) structures have attracted the attention of great researchers because of their interconnected porous structures, well-defined surfaces, and precisely controlled pore sizes in submicrometer range.\textsuperscript{137,138} Such materials have accessible macroporous networks, which can increase mass transport by reducing diffusion resistance. With this in mind, 3DOM materials are widely used as a drug/gene carrier in an efficient manner.\textsuperscript{139,140}

Although some of 3DOMs are synthesized by synthetic polymer, inorganic oxides, and semiconductor materials, 3DOMs are generally synthesized by the colloidal crystal template method. This method is based on such templates including monodispersed as PMMA, PS, which can be assembled into colloidal crystals by several methods like vertical deposition, gravitational sedimentation, and centrifugal sedimentation. Then, the interstitial areas of the colloidal crystal template are filled with a precursor solution. In the next step, a subsequent transformation occurs in the colloidal crystals, and the template is removed by roasting or dissolving. Through this process, 3DOM materials with a wide range of pore sizes, different components materials, and different properties can be prepared.\textsuperscript{137,141,142}
Among the many prospective materials, chitosan is a promising candidate for developing a porous 3D chitosan (CHT)-matrices. These kind of 3DOM materials attracted abiding interest not only for their good film forming properties but also for sticking of polymer layers on the surface of the sacrificing spheres of chitosan. It is worth mentioning that the shrinkage and deformation took place in drying of polymer into the corresponding gel structures are the main challenges in 3D CHT matrices. So, many researchers increased the mechanical, thermal, and physical properties of polymers by such inorganic compounds as silica NPs. These matrices can be produced through microscale technologies, and show dual pH-responsive as well as temperature-responsive behaviors. Such materials have also possible applications as switchable devices for release of bioactive molecules and uses in tissue engineering. For instance, using in situ synthesis of PNIPAAm within CHT micropores, CHT-scaffolds (the pH sensitive component) were coated/impregnated with PNIPAAm (the thermoresponsive component). A fine balance between hydrophobic and hydrophilic moieties in the molecular structure determined the LCST of this polymer (between 30 and 31 °C).

To impregnate the polymeric devices, two different strategies were used: (1) bulk loading to impregnate a model protein (e.g., bovine serum albumin (BSA)); (2) supercritical fluid impregnation with a model drug (i.e., ibuprofen(Ibu)) for scaffold uptake. BSA release was controlled by the PNIPAAm temperature-responsiveness (e.g., in temperature 20 and 37 °C), whereas the release of Ibu was responsive to changes in environmental pH (e.g., pH 5.4 and 7.4) conditions.

4.5. Thermosensitive Micelles

According to their formation mechanism, it is possible to categorize the polymer micelles into three types of block copolymer micelles, graft copolymer micelles, polyelectrolyte micelles or hybrid polion complex micelles. Prior to predicting and controlling the properties of a micellar system, the copolymers utilized should be well-defined. Depending on the nature of the polymer to be synthesized, the most appropriate polymerization reaction to use, such as anionic, ring-opening polymerization (ROP), or radical polymerization is determined. Currently, RAFT polymerization and ATRP are the most common controlled radical polymerization techniques used for building thermoresponsive block copolymers.

Hu et al. synthesized thermoresponsive brushlike amphiphilic poly[2-(2-methoxyethoxy)ethyl methacrylate-co-OEG methacrylate]-b-poly(L-lactide)-b-poly[2-(2-methoxy-thoxy)ethyl methacrylate-co-OEG methacrylate] [P-(MEO₂ MA- co -OEGMA)- b -PLLA- b - P(MEO₂ MA- co -OEGMA)] triblock copolymers by ATRP of MEO₂ MA and OEGMA comonomers using a α,ω-bromopropionyl poly(L-lactide) (Br-PLLA-Br) macroinitiator and the polymeric micelles were generated by self-assembly of copolymers in aqueous medium.

In another study, thermosensitive polyelectrolyte complex (PEC) NPs assembled from two oppositely charged poly-electrolytes, chitosan-graft- PNIPAAm (CS-g-PNIPAAm) and sodium alginate-graft- PNIPAAm (SA-g-PNIPAAm) were prepared with size range of 130–180 for entrapment and release of 5-fluorouracil (5-FU). The CS-g-PNIPAAm and SA-g-PNIPAAm graft copolymers were synthesized by employing free radical polymerization.
using cerium ammonium nitrate (CAN) and potassium persulfate (KPS), sodium sulphite (SDS) initiators, respectively.\textsuperscript{150}

Luo et al.\textsuperscript{151} fabricated triarmed star-shaped s-P(NIPAM-co-DMAM) random copolymers with tunable CP values via one-pot ammonolysis reaction between triethyl 1,3,5-benzenetri-carboxylate and P(NIPAM-co-DMAM)-NH\textsubscript{2}. And, copolymers formed thermosensitive micelle aggregates through hydrophobic interactions among the isopropyl groups of PNI\textsubscript{PAAm} chains and interstar association at a polymer concentration above critical aggregation concentrations from 4.06 to 6.55 mg L\textsuperscript{−1}, with a CP range from 36.6 to 52.1 °C, and micelle size below 200 nm.

The combination of ROP and ATRP was applied to synthesize a thermosensitive diblock polymer based on complex polymeric micelles of PNI\textsubscript{PAAm} and biotin, which is a cell-interacting ligand. The obtained nanocarrier demonstrated targeted delivery with enhanced cellular uptake properties and prolonged blood circulation time.\textsuperscript{152}

\section*{4.6. Thermosensitive Nanogels}

Temperature-responsive nanogels with a VPPT\textsuperscript{91} can be prepared via different methods. The techniques of reverse microemulsion combined with thermally induced gelation were used to prepare cross-linked \(\kappa\)-carrageenan hydrogel NPs (i.e., nanogels). They were prepared via water-in-oil microemulsions with average diameters smaller than 100 nm. In a temperature range that was tolerable for living cells (37–45 °C), the nanogels were found to be thermosensitive with temperature-responsive reversible volume transitions. These nanogels could be applicable as smart thermosensitive drug carriers.\textsuperscript{153}

Since 1986 when Pelton synthesized NIPAAm for the first time, it has become the most-reported water-based thermo-sensitive nanogel. Generally, the phase transition temperature of such nanogels is fitted by copolymerizing ionic monomers with NIPAAm.\textsuperscript{154,155} Nanogels of NIPAAm are synthesized in a one-step polymerization procedure, in which a divinyl cross-linker copolymerizes enables polymer chains into a porous 3D nanospherical network.\textsuperscript{156}

\section*{4.7. Thermosensitive Microcontainers (MCs)}

In the recent decade, the synthesize of multifunctional MCs has been considerably increased thanks to their unique advantages in such applications as DGDSs, water treatment, and catalysis.\textsuperscript{157} MCs have potential to encapsulate and release such biomolecules as drugs/genes, and peptides in a controlled manner. In addition, MCs have better chemical resistance, increased colloidal stability, and easier formation compared with other reviewed NPs in previous parts.\textsuperscript{158} There are several strategies that have been reported to synthesize polymeric microcontainers including layer-by-layer techniques, emulsion/interfacial polymerization, and precipitation polymerization onto templates, which is the most widespread method.\textsuperscript{159–161}

The fabrication of the MCs had two steps including the core synthesis, as well as the shell formation. For example, the shell consisted of PNIPAAm, PMMA, polydivinylbenzene (PDVB), and PAA, whereas the core consisted of PMMA. PMMA seeds were synthesized
using the emulsifier-free emulsion polymerization method.\textsuperscript{162} Daunorubicin (DNR) was selected as a model drug for studying loading and release under different pH (i.e., physiological pH of 7.4 and an acidic pH of 5.4) and thermo-conditions (i.e., 25 and 45 °C). Two different cell lines (3T3 mouse embryonic fibroblast cells and MCF-7 breast cancer) were subjected to cytotoxicity studies. Hollow MCs possessed different temperature sensitivities and were suggested as a potential smart anticancer drug delivery system using traditional highly cytotoxic drugs to reduce side effects.\textsuperscript{45}

5. CHARACTERIZATION OF THERMOSENSITIVE NPS

Temperature-responsive nanocarriers are characterized using several different techniques which are described as follows.

5.1. Transmission Electron Microscopy (TEM)

TEM analysis is utilized for characterizing the size and size distribution, structure, shape, morphology, and chemical composition of thermos-sensitive NPs.\textsuperscript{163–170}

Besides, cryogenic-TEM is used for the characterization of colloidal DDSs. As well as being used for studying the overall colloidal composition of the corresponding dispersions, it can also be applied to study the internal structure, shape morphology, and size of the nanoparticulate carrier systems.\textsuperscript{171} In an amorphous water phase, the particles are embedded and the aqueous phase is converted into hyper quenched glassy water.\textsuperscript{172}

5.2. Scanning Electron Microscopy (SEM) and AFM

SEM and AFM are used to observe the surface morphology, average diameter, particle size, and pore volume of the NPs. Moreover, SEM observation is performed using a cold field-emission scanning electron microscope (FESEM) and energy-dispersive X-ray (EDX) analysis to demonstrate overall elemental composition.\textsuperscript{173–176}

5.3. X-ray Diffraction (XRD) Spectroscopy

Purity and structural properties of samples are determined by XRD system. Specially, the peaks of XRD pattern are used to find the existence of crystalline structure. Moreover, from XRD data, the crystallite size (Dc) of the NPs is calculated using the Debye–Scherrer equation.\textsuperscript{164,167,177}

5.4. Fourier Transforms Infrared (FTIR)

The chemical structure of the synthesized thermoresponsive nanocarriers, are examined by FTIR. According to absorption peaks which correspond to the frequencies of vibrations between the bonds, useful information can be obtained, such as component confirmation, stretch and vibration of bands, bonds interaction, and binding of coating on surface of NPs.\textsuperscript{167,170,174,177–180} For instance, by means of FTIR spectra and calculation of Gibbs free energies for the hydrogen-bonded intermolecular complexes, the importance of hydrogen bonds in creating the NPs is realized.\textsuperscript{181} In addition, cross-linked and non-cross-linked states of the polymers can be studied by FTIR.\textsuperscript{182}
5.5. Gel Permeation Chromatography (GPC) and NMR Spectroscopy

GPC and hydrogen or carbon NMR spectroscopy implement to examine the chemical structure besides the molecular number and molecular weight ($M_n$, $M_w$), purity and polydispersity index (PDI, $PDI = M_w/M_n$) of synthesized thermosensitive NPs. Also, these analyses are utilized in order to understand the environmentally responsive mechanism at different temperatures.\textsuperscript{166,173,180,183–185} The visible peaks of NMR spectroscopy and their movement in respect of temperature changes, indicating the thermal-responsive behavior of NPs such as breakdown of polymer–water hydrogen bonding (water solubility of the polymers).\textsuperscript{183}

5.6. SAXS and small angle neutron scattering (SANS)

SAXS and SANS are both well-suited for studying microgels with sizes below 200 nm. The size of the particles and their interaction at low q (magnitude of scattering vector) can be determined from the scattering intensity $I(q)$.\textsuperscript{172} Through using a range of large scattering angles, network fluctuations can be analyzed quantitatively and small-scale details can be probed. Also, the early stages of NPs creation and closer insight into the particles interior are realized via SAXS and SANS.\textsuperscript{181}

5.7. Scanning X-ray Microscopy (STXM)

STXM has high chemical sensitivity, and it is possible to monitor wet samples directly using the osmotic deswelling of the particles, which results from high salt concentrations. Therefore, for in situ study of microgels, this technique is very well suited. Recent improvements have led to a resolution of 14 nm.\textsuperscript{172}

5.8. DLS and Depolarized Dynamic Light Scattering (DDLS)

To investigate the hydrodynamic diameter ($D_h$) of the NPs, size distribution and PDI, samples are characterized by DLS.\textsuperscript{163–170,178,185,186} It should be noted that the diameters are obtained from SEM/TEM measurement could be equal or different with the size measured by DLS method. This phenomenon could be due to morphological changes during preparation of samples. Furthermore, the data provided by DLS are related to the particles swollen in the solution, whereas SEM/TEM images are related to dried particles.\textsuperscript{179,180,187,188}

Moreover, DLS and DDLS (characterization in dilute solution) can characterize microgels in dilute solution, and is used to measure the diffusion coefficient and the overall size of core–shell microgels \cite{1}. Previously, DDLS techniques were developed for characterization of anisometric particles but recently they have been used to study microgels.\textsuperscript{172} Also, by using the results of angle-dependent DLS, self-assembly behavior of the brush that could self-assemble into thermosensitive micelles in water is explored.\textsuperscript{189} To evaluate the NPs’ colloidal stability, researchers used the average diameter of TEM and zeta potential, which is obtained by zeta potential analyzer with a DLS detector.\textsuperscript{163,190}
5.9. Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)

Thermal properties of the nanocarriers are measured using TGA and DSC.\textsuperscript{164,177,183,187,191} By means of TGA analysis, the rate of weight loss of the samples as a function of temperature assists to define the thermal degradation mechanism of polymeric particles, strength of intermolecular interactions, and finally the thermal stability are exploited.\textsuperscript{177,192} Also, the chains flexibility and grafted polymer density ($\rho_{\text{graft}}$) is estimated from the weight loss of polymeric grafts which is measured by TGA. In addition, the LCST transitions of the synthesized copolymers and $T_g$ of the dried samples are characterized by DSC. For instance, DSC thermograms of the hydrogel nanocarriers indicate the miscibility of them. Single $T_g$ in the thermograms means that the samples have a good miscibility.\textsuperscript{192}

For a brief look at the recent studies regarding various thermosensitive DDSs, several different synthesis and characterization methods are provided in Table 2.

6. TEMPERATURE RESPONSIVE NANOCARRIERS FOR CARGO DELIVERY

One of the challenges of DDSs is drug delivery and release at the right time and right concentration (i.e., spatiotemporal control). Temperature-responsive polymers NPs can be used as biocompatible nanocarriers for drugs and other cargos. Using these nanocarriers, it is possible to control drug release with the aid of changes in environmental parameters such as temperature. These kinds of nanocarriers were initially studied as drug-delivery systems to treat cancer, because they could reduce the side effects of chemotherapy drugs. Also, they are good candidates for gene delivery vehicles. There are also many common diseases like diabetes and hypercholesterolemia, which need repeated daily doses of the appropriate drugs. Therefore, another application that was proposed for these kinds of NPs was to carry the drugs capable to treat diseases requiring a regular repeated dose of the drug, because of their delayed drug-release properties. Therefore, such NPs not only are used for carrying genes or cancer drugs such as Paclitaxel, but also for carrying common drugs like Atorvastatin, a statin for cholesterol regulation, or antiflammatory drugs.

6.1. Anticancer Drug Delivery

Thermoresponsive DDSs can be aimed to strengthen the efficacy of anticancer drugs against cancerous sites, and obtaining controlled drug delivery/release. Enhancing cancer chemotherapy using thermoresponsive NPs such as hydrogels loaded with etoposide (ETO),\textsuperscript{86} and with the combination of camptotheacin and 5-fluorouracil (CPT/5-FU),\textsuperscript{108} has been a field of interest. Another effort that was made using PNIPAAm was to prepare a graft copolymer from sodium alginate-$g$- PNIPAAm (ALG-$g$- PNIPAAm) with multivalent metal ions (Ca$^{2+}$ and Al$^{3+}$) and 5-fluorouracil (5-FU) anticancer drug.\textsuperscript{211} In this study, Guiying Li and his group prepared a hollow structure with a size of about 100 nm. The swollen polymer chains formed the neutral part, and the complex bonds between the sodium alginate chains and the metal ions imparted stability to the micelles. The polymer vesicles had a LCST temperature around 37 °C. It was observed that the hollow structure of the particles had large cavities that enabled chemical attraction between the surface of the cavities and the drug, thus helping the drug-loading efficiency.
A thermoresponsive polymer system with a LCST close to body temperature is Pluronic F127-poly(D.L-lactic acid) (F127-PLA, abbreviated as FP). The copolymer micelles were decorated with folate (FA) to impart active targeting. Xing Guo et al. reported that using this polymeric micelle system, a small amount of the encapsulated DOX was released as soon as the temperature reached the LCST (39.2 °C) and, at a temperature only just a little bit higher (40 °C), the polymer shells released the drug rapidly.

Thermoresponsive micelle systems are promising for anticancer therapy, because they respond to the demands of active tumor targeting and considerations related to tumor tissues’ abnormal temperature gradients as compared with those of normal ones. Micelles can be specifically taken up by folate receptor (FR)-overexpressed tumor cells through receptor-mediated endocytosis, and rapidly release the cargo inside cells at low hyperthermia (40 °C) because tumor tissues are much more sensitive to high temperatures and effectively terminating the tumor by hyperthermia is obtained in the range of 40–43 °C (Figure 9).

The drug loading and release behavior for the (P(MEO2MA-co-OEGMA) copolymer was assessed using chlorambucil (CBL), as a model hydrophobic anticancer agent under various temperature conditions. Under the LCST (28 °C), the highly hydrated micellar polymer segments stabilized the hydrophobic–hydrophilic core–shell structure of micelles of dendrimer-star copolymer with Y-shaped arms, hence the drug release was slow and about 78% drug still remained in the core of the micelles after 40 h. But, after the temperature was raised above the LCST (40 °C), and because the polymers shell became hydrophobic, the temperature-induced structural changes of the micelles were led to deformation of the micellar core–shell structure and the drug release was accelerated.

6-2. Gene Delivery via Thermoresponsive Nano-carriers

Gene therapy is a technique in which genetic materials (nucleic acids) are delivered to the nuclei of diseased cells or tissues. Different forms of exogenous nucleic acids (such as plasmid DNA, minivector DNA, siRNA, antisense oligonucleotides, etc.) are used in order to treat pathological conditions and remedy genetic disorders. The issue of gene delivery into the cell nuclei is one of the critical steps and is the most important barrier to successful gene therapy. The existence of several obstacles that need to be overcome before genetic materials arrive in target cells has hindered efficient gene delivery so far.

6.2.1. Nonviral Gene Delivery—Considering the fact that naked DNA does not enter cells efficiently, researchers have investigated two different broad types of gene delivery system, namely viral and nonviral vectors (natural or synthetic). The pros and cons of the above-mentioned approaches can be considered in three areas: production, safety, and efficiency, which are described in Table 3.

The concerns over safety issues that have been reported to have occurred with viral vectors have limited their routine utilization in both basic research laboratories, as well as in clinical trial applications. In contrast, nonviral gene delivery methods are characterized by relatively favorable features such as a lack of safety concerns, and the potential ability to carry a wider variety of different nucleic acids and genetic materials. Furthermore, nonviral vectors can be selective targeted toward specific cell types by attaching molecular recognition ligands.
In nonviral gene delivery systems, a synthetic vehicle is used to encapsulate the nucleic acid until it reaches its target cell. These vehicles can be mainly divided into two different broad groups. The first group is composed of organic nanostructures, which include cationic lipids and various polymers (the most common and efficient nonviral gene vectors), and also peptides or carbon nanotubes. The second group is based on NPs formed from inorganic components such as gold NPs, quantum dots and calcium phosphate particles.\textsuperscript{213–215,219–222}

Among the above-mentioned groups, polymeric gene delivery systems have received particular attention thanks to their stimuli-responsive features. It is ideal for a polymeric nanocarrier with gene delivery purpose to exhibit (1) efficient protection of nucleic acids from degradation and clearance during circulation, (2) specific delivery of nucleic acids to target tissues/cells, and (3) readily nucleic acids release in the target intracellular compartments after overcoming multiple intra-cellular barriers. Those requirements are achievable by employing molecularly engineered nonviral vectors to transform their physicochemical properties in response to various extra and intracellular stimuli as well as external triggers.\textsuperscript{223}

Here, in the same manner as for drug delivery, cargo release occurs as a result of their phase transition at certain temperatures (LCST or UCST). These polymers should have the potential to form strong complex binding with DNA while being transported to the target sites at a temperature below their LCST. On the other hand, the binding in these complexes should become loose enough at the target cells and tissue sites to release the DNA at temperatures above their LCST. In this way, this smart stimulus-responsive system enhances the efficiency of transfection.\textsuperscript{74–77}

PNIPAAm is the most extensively investigated thermoresponsive polymer used in gene delivery systems. PNIPAAm is a water-soluble polymer that is hydrophilic below 32 °C (its critical solution temperature) due to the hydrogen bonding between its amide groups and water molecules. When the temperature reaches this critical point (LCST or “cloud point”), the hydrogen bonds are broken and the structure of the polymer chains changes from water-soluble coils (hydrophilic) to a globular structure (hydrophobic). It is considered that PNIPAAm may have some slight toxicity after long-term exposure, in that its accumulation in the body could be injurious because of its nonbiodegradability. Copolymerization of PNIPAAm with other biodegradable polymers (hydrophilic or hydrophobic blocks) would not only partially overcome this drawback but also could alter the LCST of PNIPAAm.\textsuperscript{74,76,216,224–226} A short summary of examples of thermosensitive polymers as gene delivery systems including their features and applications is provided in Table 4.

Feng et al.\textsuperscript{239} developed an efficient nonviral cationic block copolymer gene delivery system to deliver therapeutic plasmid DNA (pDNA) for nucleus pulposus regeneration toward Disc degeneration disease. The copolymer was prepared via complexation between the mixed cationic block copolymers and pDNA. The obtained mixed polyplex micelles (MPMs) containing thermoresponsive heterogeneous coronas with hydrophobic and hydrophilic microdomains coexisting could be achieved upon heating from 25 to 37 °C, which demonstrated high tolerability against nuclease, strong resistance toward protein adsorption, high gene transfection efficiency, and low cytotoxicity.
The polyplex micelles have also been investigated for antitumor gene delivery. The rod-shaped ternary polyplex micelles (TPMs) was synthesized via complexation between the mixed block copolymers of poly(ethylene glycol)-b-poly(N′-[N-(2-aminoethyl)-2-aminoethyl]aspartamide) (PEG-b-PAsp-(DET)) and PNIPAAm-b-PAsp(DET) (PNIPAAm-b-PAsp-(DET)) and plasmid DNA (pDNA) at room temperature. The formation of a hydrophobic intermediate layer between PEG shells and pDNA cores demonstrated thermoresponsive behaviors through facile temperature increase from room temperature to body temperature (~37 °C). The results revealed that incorporating the thermoresponsive PNIPAAm content as an intermediate barrier provided prolonged blood circulation and the TPMs loading therapeutic pDNA encoding an antiangiogenic protein remarkably suppressed tumor growth following intravenous injection into H22 tumor-bearing mice.

The differences between the physicochemical structures of DNA and nucleic acids and those of small molecule drugs, makes it impossible to use similar nanocarriers to deliver both of them. Gaspar et al. reported on the preparation of nanocarriers capable of efficient gene delivery. They synthesized amphiphilic triblock poly(2-ethyl-2-oxazoline)-PLA-g-PEI (PEOz-PLA-g-PEI) micelles for the delivery of minicircular DNA (mcDNA) vectors. In this copolymer, a hydrophilic shell was constructed by replacing the PEG blocks with PEOz. Additionally, the concept of codelivery of mcDNA and doxorubicin (DOX) was demonstrated by their simultaneous encapsulation in PEOz-PLA-g-PEI carriers with high efficiency.

6.2.2. Gene Transfection by Thermoresponsive Nano-carriers—Gene transfection is the main goal of nucleic acid delivery using polymeric nonviral carriers (Figure 10), which involves four stages: (1) complexation of plasmid DNA and the polymer that is usually performed at room temperature; (2) addition of the complex of DNA with the polymer (polyplex) into cell culture medium for a period of time (transfection time) which is carried out at 37 °C; (3) DNA release into the cytoplasm, when the polyplex is eliminated from the cell; and (4) the cells are left to incubate for a period of time (incubation time) in order to transfer the DNA into nucleus. One strategy to increase the transfection efficiency is changing the temperature during each of the steps (complexation, incubation or transfection period). Another method to enhance the transfection efficiency is increasing the weight ratio of polymer to DNA in the complexes.

For instance, Wenguang Liu et al. used PEG polymers with grafted PEI chains and tested changing the temperature of complexation as well as of transfection, and improved transfection efficiency. They could increase efficiency of transfection by increasing the weight ratio of PEIMH/DNA from 5:1 to 30:1. In another study, Zhang et al. employed a cationic lipid as a nonviral vector for gene delivery and they could show that cationic lipids with a shorter chain length had a higher transfection efficiency. In an interesting study, Freitag et al. found that a minimum molecular weight for the polymers was necessary for high transfection efficiency, and also with increased branching (e.g., arm number) better transfection could be observed. In this regard, they suggested star-shaped polymers, which not only had several arms (more than 5), but also possessed intermediate molecular weight to obtain the appropriate transfection efficiency.
6-2-3. Gene Expression by Thermoresponsive Nano-carriers—Gene expression is a complex process, which requires the nucleic acids to cross the cellular membrane, to undergo appropriate intracellular trafficking, and finally nuclear delivery of the cargo of genetic material. When the DNA is transcribed in the nucleus it produces mature mRNA, which is released and exported to the cytoplasm for translation. At this stage, mRNA engages with the ribosomes at initial step of translation, and after elongation and termination (as intermediate steps), the ribosome disengages from the mRNA, and then its subunits dissociate to repeat the cycle. In other words, at translation step, the genetic code is read, and the formation of the polypeptides builds up stepwise and finally the completed polypeptide chain is released.246,247

7. DUAL STIMULI IN TEMPERATURE-RESPONSIVE SYSTEMS

7.1. pH/Temperature

Multistimuli-responsive hydrogels (MSRHs) can be produced by taking advantage of the interaction between various functional groups that exist in macromolecules. MSRHS, particularly those that respond to changes in pH and temperature, have been reported extensively, focusing on their in situ hydrogel-forming abilities and site-specific applications.

Némethy et al.53 reported a pH-and temperature-responsive poly(aspartic acid)-l-poly(N-isopropylacrylamide) conetwork hydrogel (PASP-l-PNIPAAm) in which PASP and PNIPAAm imparted pH- and thermoresponsivity, respectively. The hydrogel formed a conetwork and made it possible to absorb more water. The in vitro release rate of the loaded anti-inflammatory drug, diclofenac sodium (DFS), was reported to be slower at 37 °C and pH 7.6 after preconditioning in an acidic milieu (with pH 1.2) compared to the case of purely alkaline milieu. In another study, the introduction of carboxylated nanocrystalline cellulose (CNCC) into PNI-PAAm, resulted in generation of a pH-thermoresponsive PNIPAAm-based hydrogel.84 The carboxyl groups of CNCC imparted hydrophilicity, improved swelling ability, better swelling rate, and pH responsiveness.

The urea groups present in poly(amine urea urethane) (PAUU) contained in PEG-g-PAUU, enhanced the hydrogel interaction with drugs, reduced the critical copolymer concentration needed for gelation, lowered toxicity, and imparted pH-temperature dual responsivity.248 By studying the long-term release of FITC-BSA-loaded protein, the research team proposed PEG-g-PAUU hydrogel as a protein-release depot that could be injected.

Dual-responsive polymeric micellar NPs have been also reported. Herein, to develop more advanced micellar polymers that can respond both to temperature and pH variations, the pH responsive poly(L-histidine) was integrated to PLGA-PEG-PLGA forming dual-responsive copolymer for more efficient drug delivery. The lower pH amounts (e.g., 6.0 and 5.0) and higher temperatures (above LCST e.g. 41 °C) showed higher rates of DOX release (Figure 11a–c).249

7.1.1. pH/Temperature for Anticancer Drug Delivery—In a study using PNIPAAm, two new formulations were designed for dual temperature and pH-responsive nanogels.

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PNIPAAm was used as the basis of the hydrogel structure. They introduced PNIPAAm to N-hydroxyethyl acrylamide (HEAA) and tert-butyl 2-acrylamidoethyl carbamate (2AAECM) to form a nanocarrier to deliver the common and well-known anticancer drug, Paclitaxel. They found out that at a pH value around 5, a faster drug-release was obtained. The thermo-induced changes were also investigated in these polymeric micelles by observing the optical absorbance as a function of temperature. The temperature of the solution was varied between 15 and 45 °C and it was observed that the absorbance increased when the temperature of solution was between 28 and 33 °C, which is the temperature range in which the phase change occurs in PNIPAAm, and it releases the water absorbed by PNIPAAm.

In another effort to prepare polymeric NPs that were responsive to both temperature and pH, Yongkyun et al. used a fluorescent cross-linked PNIPAAm-based probe. They studied the influence of the temperature and pH on the following the opening of the lactone ring of fluorescein and the lactam ring of rhodamine and the subsequent fluorescence emission at 514 and 586 nm. They showed influence of temperature changes (i.e., between 25 and 42 °C) and pH changes, (i.e., acidic (2.3–5.3) and basic (7.4–12.4) conditions) on the turbidity of the microgels, above the LCST transition temperature, and the fluorescence intensity.

### 7.1.2. Stability of Particles and Controlled Drug Release

One of the challenging issues with polymeric micelles is the required balance between the stability needed during prolonged blood circulation, and active drug-release when the nanocarrier arrives at the tumor site. Stimuli-responsive materials including a pH-responsive element provide a mechanism for triggered drug-release in the acidic tumor and intracellular microenvironments. Ching-Yi Chen et al. synthesized a series of dual pH- and temperature-responsive block copolymers, with the optimized LCST transition ~35 °C, containing a poly(ε-caprolactone) (PCL) hydrophobic block with a PEG block. The block copolymers formed micellar structures in aqueous solutions. The optimized polymer was stable at blood pH (~7.4), whereas they showed pH-responsive phase transitions at mildly acidic pH (~5.3) and at body temperature. These micelles showed better anticancer activity than free DOX. Yukun Wu et al. tested a method to increase the stability of an amphiphilic block copolymer by developing novel core cross-linked micelles. They synthesized poly(ethylene glycol)-block-poly(N-isopropylacrylamide-co-N-(4-hydroxyphenethyl) acrylamide) diblock copolymer (PEG-b-P(NIPAAm-co-NHPAAm)). The diblock copolymer had a phase transition into non-cross-linked (NCL) micelles. When the temperature exceeded the LCST (about 32 °C), at the temperature about 37 °C, the NCL micelles exhibited a better performance for drug release in comparison with core cross-linked micelles (Figure 12).

Agut et al. fabricated polypeptide-based double hydrophilic block copolymers (DHBCs), namely, poly[2-(dimethylamino)ethyl methacrylate]-b-poly(glutamic acid) (PDMAEMA-b-PGA) with dual temperature and pH sensitivity. At pH values close to the isoelectric point, the polymeric micelles had a spherical morphology. The thermo-responsivity of the double-hydrophilic block copolymers (DHBC) was evident around the LCST (about 40 °C). Around pH 11 and when the temperature was below the LCST, the DHBC polymer was present as free chains, whereas at temperatures higher than the LCST, the polymers changed their...
conformation and assembled into spherical polymeric micelles. Moreover, by varying the length of the PGA blocks, a variety of different sizes, shapes, and morphologies could be produced.

Zhang et al. synthesized double-block hydrophilic copolymers composed of PEG blocks and bicyclodextrin (b-CD) blocks to form PEG-b-PCD. Next a drug carrier was synthesized as a core–shell structure using poly(b-benzyl L-aspartate) (PBLA) as the core, and the hydrophilic copolymer PEG-b-PCD was the shell of this core–shell structure. The hydrophobic drug could interact with the b-CD and the hydrophobic benzyl groups to form stable assemblies. In vivo and in vitro drug release studies confirmed the ability of the polymeric core–shell nanostructure to mediate controlled drug delivery.

7.2. Magnetic/Temperature Dual Stimuli-Responsive Nanocarriers

Using magnetic properties to control and improve the drug-release profile is another common method that has been used. Sundaresan et al. used PNIPAAm that was coated onto MNPs to achieve better and more precise control, over drug release. The core of the ~150 nm sized core–shell structure was Iron oxide with superparamagnetic properties. The dual-responsive functionality of these NPs was observed, and it was reported that at 40 °C and pH 6, the release of anticancer drug DOX was at a maximum level. The drug-release rate was increased when the NPs were exposed to a 1.3T magnetic field. In the study conducted by Pernia Leal et al., they produced thermosensitive polymer shells on the surface of superparamagnetic iron oxide particles that were embedded onto the surface of poly(maleic anhydride-alt-1-ocatadecene) polymeric nanobeads. The phase transition temperature of the PNIPAAm-magnetic nanobead NPs, which was initially reported to be (about 32 °C), can differ depending on the composition of the comonomers and their relative concentrations; however, in the range between 26 and 47 °C it was observed that nanobeads formed a stable suspension below the LCST, and magnetic field could not be easily applied to trap the beads. However, at temperatures higher than the CST, the nanobeads agglomerated and formed clusters, with magnetic moments high enough to be trapped by the magnetic field. At the same time, because the temperature was higher than the LCST, the swollen polymer started to release the drug. Another magnetic core structure that can be used in core–shell structures is Fe₃O₄. Haie Zhu and his group developed a novel core–shell structure with Fe₃O₄ as the core and PNIPAAm as the shell. This new core–shell structure demonstrated unique triple features, fluorescence, superpara-magnetism and thermoresponsivity. They synthesized this core–shell structure using Fe₃O₄/poly(Styrene (St)-NIPAM) NPs, which acted as nucleation sites for polymerization of Eu(AA)₃Phen with the addition of St and NIPAM to form an outer fluorescent layer. The fluorescent layer on the NP was employed as a tracer to follow the NP distribution.

7.3. Biomolecule/Temperature Dual Stimuli-Responsive Nanocarriers

Thermoresponsive polymeric nanocarriers can also be designed to be glucose-responsive, thus they can be used as insulin carriers, and could release insulin in response to varying glucose levels in the body. The fact that the cores in core–shell structures can be made from inorganic materials, gives the opportunity to produce a nanocarrier that is responsive not only to temperature but also to magnetic fields, and consequently the drug-release process...
could be managed more effectively. Yuan Yao et al.\textsuperscript{259} attempted to prepare a glucose-responsive carrier for controlled release of insulin by combining PNIPAAm with poly(ethylene glycol)-\textit{block}-poly-(phenyl bromate ester). They prepared polymeric micelles with a core–shell–corona structure comprising glucose-responsive PPDEMA as the core and PNIPAAm as the shell components. They could control the structure by varying the temperature between 15 and 37 °C. As a result, they achieved much better stability for insulin encapsulation in the polymeric micelles by introducing PNIPAAm as the shell in the core–shell structures. In another study, thermo- and enzyme dual-responsive polymeric amphiphiles rendered by Kashyap et al.\textsuperscript{260} In this approach, tailor-made polymer of 3-pentadecylphenol (PDP) copolymerized with oligoethylene glycol acrylate by both radical and reversible addition–fragmentation chain transfer (RAFT) synthesis methods and spherical core–shell nano-particles were self-assembled in aqueous medium and doxorubicin (DOX) was loaded. This assembly showed tunable LCST behavior at the tumor site and sensitivity to esterase enzyme at the intracellular environment.

### 7.4. Redox/Temperature Dual Stimuli-Responsive Nanocarriers

Cleavable diselenide bonds\textsuperscript{87} as well as cleavable disulfide bonds\textsuperscript{261,262} can be introduced into hydrogels to achieve better degradability. Diselenide bonds have been used as a cross-linker in NIPAAM and HEA hydrogels. In an environment containing oxidizing agents such as enzymes and ROS, Se–Se bonds can be cleaved, thus achieving an oxidation-responsive hydrogel. Huo and his group\textsuperscript{263} developed a novel dual oxidation-thermoresponsive PNIPAAm-based hydrogel with enhanced degradability.

### 7.5. Triple Stimuli-Responsive Nanocarriers

Temperature can be employed in triple stimuli-responsive DDSs in order to obtain highly controlled drug delivery and release.\textsuperscript{46,264–266} Zeng et al.\textsuperscript{267} report a strategy for formation of a multiresponsive Fe\textsubscript{3}O\textsubscript{4}@PMAA@PNIPAM magnetic core, inner shell (reduction/pH-responsive), and outer shell (thermal-responsive), respectively, core–shell NP. A model drug, DOX, showed efficiency of these NPs for controlled releasing purposes.

### 8. LIGHT-INDUCED THERMORESPONSIVE NANOCARRIERS

Light is an appreciable source to be exploited in nanomedicine and therapies and especially DDSs.\textsuperscript{268–272} Here, an important group of the temperature-responsive nanocarriers includes the nanocarriers having photoinduced temperature-responsive drug release capability for controlled drug delivery. In the structure of these nanocarriers, photothermal inducing agents can be employed, which absorb the irradiated light and convert it into a localized heat. The induced heat can be employed to induce temperature-triggered drug/gene release inside the biological milieu.\textsuperscript{273}

Noble metal nanomaterials (Au, Ag, Pt, and Ge) are one of the most studied agents for photothermally induced drug release.\textsuperscript{274} Noble metals exhibit remarkable optical-electronic properties because of their surface plasmon resonances (SPRs). SPR is the free electrons resonant oscillation at the interface of a negative and positive permittivity material stimulated by external electromagnetic fields. SPR in a material results in dipole oscillations.
along the direction of electromagnetic field. At the SPR frequency, the amplitude of oscillation reaches a maximum, inducing high absorption of the electric field of the incident light. Noble metals exhibit strong SPR when stimulated by visible light, which could be used in photothermal therapy (PTT) applications.

Other groups of materials including CNTs; graphene, and GO; transition metal and semiconductor based NPs have been also suggested to be efficiently used for controlled release of therapeutic agents from thermoresponsive nanocarriers. Transition-metal dichalcogenides (TMDCs), such as MoS$_2$ and WS$_2$, are new groups of materials being proposed as photothermal agents. These materials are analogous to the graphene in having layered structures.

Another recent group of materials with photothermal capability are semiconductors nanomaterials (Cu$_x$S, Cu$_x$Se, and Cu$_x$Te). Semiconductors have the advantages of high plasmonic absorption in a wide near-infrared (NIR) range. They have a relatively low cost, good biocompatibility, high photothermal efficiency, and high in vivo stability.

The majority of mentioned photothermal agents are nonbiodegradable and would retain in the body for a long time. This could result in a consequent long-term toxicity of these materials. Therefore, concerns about the potential long-term toxicity of the inorganic photothermal agents have caused a delay in their future clinical transition. Hence, to overcome this concern, scientists have tried to explore the possibility of using organic nanomaterials as substitution photothermal agents. Consequently, in the recent years, various nanomaterials complexes, conjugated polymers, or even organic/inorganic nanocomposites have been successfully synthesized and administrated employing their photothermal capability. Comprehensive reviews over these new promising group of biodegradable materials exist in the literature, which readers are encouraged to refer for more detailed information.

8.1. Applications of Photoinduced Thermoresponsive Nanocarriers

In photoinduced thermoresponsive nanocarriers, the released heat can be used to damage or destroy the nearby diseased cells, e.g., cancer cells. This medical treatment, in which cancerous tissue is ablated by being exposed to a higher temperature than normal condition is called as hyperthermia. Applying NIR light (wavelength = 700–1100) is the mostly preferred as the light source for PTT applications, because of noninvasive and relatively deep tissue penetration. Therefore, the main challenge in developing photothermal agents is to produce material with a high light absorbance and high photothermal conversion efficiency.

Among metal NPs, gold NPs are the basic photothermal materials for the initial development of PTT, and hence, they are the most studied photothermal agents. Gold NPs exhibit strong SPR and scatter the incident NIR light with a large extinction cross-section. The large absorption cross-section and high photothermal conversion efficiency of gold NPs allow for less invasive PTT treatment with higher NIR light wavelengths. There are good reviews in the literature, discussing the development and progress of gold NPs in PTT applications. In addition, PTT could be combined with chemotherapy, by means of
thermoresponsive polymers, as a highly effective cancer therapy. Combination of thermally responsive polymers with photothermal agents results in an intelligent NIR-controlled drug release. The produced heat by photothermal agents when stimulated by NIR light, can trigger the shrinkage of drug loaded thermoresponsive hydrogels and the consequent controlled drug release. This mechanism increases the efficiency of drug release at the cancer site in addition to hyperthermia of the cancerous cells. Ying Qu et al. developed a combined hydrogel system by doping gold nanorods (GNRs), as the photothermal agents, into the DOX embedded hydrogels. They demonstrated that efficient release of DOX from hydrogel matrix, by means of stimulating GNRs photothermal agents, resulted in a remarkable decrease of the tumor recurrence in mice 4T1 breast cancer models. In a similar study, a conjugate of GNR-poly(ethylene glycol)-block-poly(caprolactone) (PEG-b-PCL)-DOX was utilized as a switch for controlled release of DOX via a 808 nm NIR irradiation induced thermoresponsiveness. In other studies, photosensitizer dyes such as indocyanine green (ICG) and also photothermal semiconductor nanocrystals such as Cu1.75S281 have been reported to show NIR triggered thermoresponsive controlled release of anticancer drugs from polymeric-based nanocarriers (Figure 13a, b).

9. OTHER APPLICATIONS OF TEMPERATURE-RESPONSIVE NANOCARRIERS

Apart from the aforementioned applications, some other potential applications are also provided in the recent researches for the thermoresponsive DDSs. For instance, considering the flexibility of hydrogels, they can carry out a variety of applications. Protein delivery is the most frequently studied application. So far, various proteins, such as insulin, lysozyme, BSA, as well as FITC as a protein label have been studied and their release profiles have been monitored. Moreover, the delivery of human growth hormone (HGH) and DFS as an anti-inflammatory drug, have been the subject of other studies. Co-delivery systems also have been studied in order to achieve enhanced therapeutic activity. In this regard, a study on the combined delivery of both insulin and GSH was carried out. Boustta and coworkers focused on simultaneous codelivery of model neutral proteins and cobalt acetate, an ionic drug. Delivery of therapeutic enzymes is a rather less studied area, but as mentioned earlier, encapsulated hydrogels are novel versatile ways for site-specific drug delivery. In a study, calcium alginate (CaAlg) encapsulated hydrogels, were able to deliver not only hydrophilic and hydrophobic model drugs (FITC and piroxicam) but also could deliver horse radish peroxidase (HRP) as a model enzyme.

In a study, PNIPAAm hydrogels were used in order to deliver colloidal drug carriers (CDCs). This route was investigated as a treatment for myocardial infarction (heart attack). Core–shell structures that are temperature-responsive can release their loaded drug with a programmed delay. Some common diseases like diabetes and hypercholesterolemia need regular doses of drugs delivered over a long time. Therefore, an application that was proposed for these temperature-responsive NPs was to carry drugs for these chronic diseases, because of their delayed drug release. Therefore, such NPs could be used for
carrying common drugs like the cholesterol-lowering Atorvastatin or antiinflammatory drugs.300

Another group of temperature-responsive systems are microgel particles which have various applications as active carrier systems for the immobilization of catalytically active enzymes onto metal NPs. Manipulation of the properties of the embedded NPs depends on the volume transition of the microgel particles.119

Antimicrobial peptides (AMPs) are excellent nominees as antimicrobial agents rather than antibiotics that are less effective for treatment of bacterial infections because of their extensive use. Ping Dong et al.301 innovated a drug delivery system against the main mankind pathogen of Gram positive bacteria – *Staphylococcus aureus* – by assembly of alamethicin, an AMP, to a thermosensitive polymeric carrier (PSBMA (poly(2-(adenine-9-y1) ethanol methacrylate-cosulfobetaine methacrylate) (poly(AEM-co-SBMA))). The copolymer had UCST in water that provided controlled release of alamethicin. This idea can be generalized for treatment of microbial strains by temperature-responsive delivery of AMPs.

It is demonstrated that, by incorporating magnetic resonance imaging (MRI) contrast agents into the structural design of thermoresponsive micellar copolymers with tumor targeting and controlled release abilities loaded with anticancer therapeutic agents, it is possible to acquire efficient theranostic systems, provided that (1) the unimolecular micelles have size range of 20–100 nm to minimize recognition by reticuloendothelial systems; (2) the hydrophilic corona is biologically inert and noncytotoxic; (3) hydrophobic core is capable of sufficient loading capacity for hydrophobic therapeutic drugs; (4) targeting moieties and T1 contrast agents (DOTAeGd) were covalently attached to the hydrophilic periphery to achieve cancer-targeted delivery of chemotherapeutic drugs and contrast agents.302

10. CONCLUSION AND FUTURE PERSPECTIVE

In the present review paper, we have summarized the use of temperature-responsive nanocarriers for drug and gene delivery. The LCST and the UCST temperature transitions, which are defined by the properties of particular polymer functional groups are the defining feature of these smart delivery vehicles. Thermally responsive nanocarriers such as hydrogels, micelles, core–shells, etc., can be used for delivery of therapeutic drugs (particularly anticancer drugs) and for gene delivery purposes.

In recent years, targeted smart DDSs due to using low doses of drugs, local targeting, decreasing side-effect and high efficiency for disease treatment are intensively taken into consideration by researchers. Temperature sensitive nano-carriers as one of the most important smart nanocarriers for drug or gene delivery for treatment of various diseases and cancer therapy were highlighted in this study. Solubility, phase transition behavior, release of drug or gene, synthesis, and characterizations methods of thermo-responsive NPs, membranes, in core–shell structures, micelles, etc., were discussed. Furthermore, the capability of thermo-responsive structures in combination with other stimuli including pH, light, redox, magnetic field, etc., forming dual/multi-responsive systems was discussed.

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Apart from drug/gene delivery applications of thermoresponsive NPs, their other innovative applications for delivery of antimicrobial agents, anti-inflammatory drugs, enzymes as well as codelivery systems and theranostic nanosystems were mentioned.

Regarding the question, why temperature sensitive systems are considered significantly important besides other stimulus sensitive such as pH, light, enzyme, etc. One answer refers to the abnormal temperature of inflamed tissue (tumor), which has higher temperature in comparison with body temperature and can acts as an endogenous stimuli in order to nanocarrier activation, thus the therapeutic cargo can be released. Another reason is that external heating as an exogenous temperature can activate nanocarrier operation. Also drugs can be loaded in the nanovehicles in liquid state and without any surgery injected to the body. But despite many advantages, some challenges still exist. Although PNIPAAm due to the LCST about round 32 °C is very appealing for DGDSs, but its nonbiodegradable properties in body is a disadvantage; hence to overcome this disadvantage, PNIPAAm can copolymerized with PEG or polysaccharides. Encapsulating drugs and using membranes are some other appropriate solutions to decrease the initial burst release and have a continuous release without overdose. Toxicity of some drugs such as anticancer drugs limits their application, but temperature-sensitive nanocarriers have been presented to overcome this challenge. For reducing the problems with some hydrophobic drugs, core–shell structure and interpenetrating network are alternative strategies. Hydrophilic or hydrophobic polymers or photoresponsive groups such as azobenzene can conjugated to nanocarriers therefore inducing changes in the LCST or UCST for specific applications and increasing or decreasing the drug release rate. However, significant progresses that have been made in thermoresponsive DDSs suggest a hopeful future for exploring more practical targeted methods for cancer treatment. Another future direction for smart thermoresponsive stimuli-responsive NPs could be their application for treatment of chronic diseases, such as diabetes and hypercholesterolemia, which require regular doses of the drug to be administered. These nanocarriers by providing continuous and delayed release extend the specific period of drug effectiveness, so patients could have an injection only every 1 or 2 weeks, instead of each and every day. In addition to the temperature, other stimulus such as pH, enzyme, light irradiation, electricity and magnetic field, ultrasound, mechanical forces (e.g., shear stresses, etc.) can be added to temperature stimuli system through a logic gate algorithm and form the dual/triple and even multi-responsive nanocarriers for better efficiency. Also in the near future, codelivery systems for simultaneous gene and drug delivery, or dual therapy (chemotherapy and hyperthermia) plus MRI can be accomplished. Novel smart nanocarriers such as nanobombs, which can be swelled 800-fold by changing the temperature, because of inducing physical/mechanical forces can breakdown the diseased tissue, in addition to targeted delivery and release of drugs. Anyway, in answer to “How is our position for smart delivery of therapeutic agents, especially through administrating thermoresponsive nanosystems in the next ten years?”, it must be said that the future is bright.

References


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Figure 1.
Shrinkage of a loaded hydrogel and release of drug during LCST behavior. Reprinted with permission from ref 88. Copyright 2015 Royal Society of Chemistry (RSC).
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Figure 10.
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Figure 12.
In situ fabrication of core cross-linked (CCL) drug-loaded micelles. Reprinted with permission from ref 253. Copyright 2014 Elsevier.
Figure 13.
Schematic of (a) NIR triggered drug release of encapsulated DOX and ICG from polymeric nanogels via photoinduced thermoresponsive relaxation of β-CD and AD-based host–guest interactions. Reprinted with permission from ref 295. Copyright 2015 Royal Society of Chemistry (RSC). (b) NIR-triggered thermoresponsive drug release from Cu_{1.75}S@p(NIPAM-MAA) NPs. Reprinted with permission from ref 281. Copyright 2015 Springer.
### Table 1

Recent Reports on Thermoresponsive Hydrogels

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### Table 2

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<td>LMMNA</td>
<td>thermo-and pH-responsive</td>
<td>DPPC, Chol, DSPE-PEG$_{2000}$, MMNA</td>
<td>DOX TLX</td>
<td>solvothermal method, thin film hydration method</td>
<td>XRD, ZPA, TEM, AFM, VSM</td>
<td>205</td>
</tr>
<tr>
<td>stimuli-responsive nano-carrier</td>
<td>nanocarrier type</td>
<td>materials</td>
<td>drug</td>
<td>synthesis</td>
<td>characterization</td>
<td>refs</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>core–shell NPs (mPEG–PPAd NPs)</td>
<td>thermoresponsive</td>
<td>PPAd, mPEG</td>
<td></td>
<td>two-stage melt poly condensation method, oil-in-water emulsification and solvent evaporation technique</td>
<td>Ubbelohde capillary viscometer, GPC, DSC, WAXD, 1 H NMR, SEM, TEM</td>
<td>206</td>
</tr>
<tr>
<td>Hydrogel film</td>
<td>thermoresponsive</td>
<td>NIPAAm, EBA</td>
<td>DC, NPX</td>
<td>UV-initiated radical polymerization</td>
<td>FTIR, SEM, DSC</td>
<td>207</td>
</tr>
<tr>
<td>IPN hydrogels</td>
<td>thermo-and pH-responsive</td>
<td>PASP hydrogel pH-sensitive, PNIPAAm</td>
<td></td>
<td>redox radical polymerization in the presence of cross-linkers</td>
<td>SEM, DSC</td>
<td>208</td>
</tr>
<tr>
<td>injectable IPN</td>
<td>thermo-and pH-responsive</td>
<td>PEGMA, NIPAm, ALG, MA, BIS</td>
<td>DCS, BSA</td>
<td>esterification of hydroxyl groups, copolymerizing PEGMA and NIPAm</td>
<td>FTIR, rheometer</td>
<td>209</td>
</tr>
<tr>
<td>hollow polymer micro-capsules</td>
<td>thermoresponsive</td>
<td>DVB, KPS, NIPAm,</td>
<td></td>
<td>emulsion polymerization</td>
<td>TEM, DLS</td>
<td>210</td>
</tr>
</tbody>
</table>
### Table 3
Comparison of Viral and Nonviral Vectors for Gene Delivery

<table>
<thead>
<tr>
<th>parameter</th>
<th>viral</th>
<th>nonviral</th>
</tr>
</thead>
<tbody>
<tr>
<td>production</td>
<td>limited capacity for DNA delivery; small production scale; difficult production and expensive</td>
<td>easier and cheaper production</td>
</tr>
<tr>
<td>safety</td>
<td>On the basis of their residual viral elements, safety problems raised by: potential toxicity; immune response and inflammatory reactions; insertional mutagenesis</td>
<td>ability to safely transport gene cargo in vivo (even into specific subcellular ingredients; limited toxicological implications; randomly integrate DNA into the host cells</td>
</tr>
<tr>
<td>efficiency</td>
<td>high gene transfection efficiency</td>
<td>transfection efficiencies are not as high as viral</td>
</tr>
</tbody>
</table>
**Table 4**
Examples of Temperature-Responsive Polymers Utilized for Nonviral Gene Delivery

<table>
<thead>
<tr>
<th>polymer</th>
<th>advantages/properties</th>
<th>application</th>
<th>refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAAm-co-PEI</td>
<td>LCST increases up to 37 °C; good gene expression; low toxicity</td>
<td>DNA complexation; preventing the adsorption of serum proteins</td>
<td>227</td>
</tr>
<tr>
<td>P(NIPAAm-co MMA)</td>
<td>switch from hydrophilic surface (below 29.18°C as a transition temperature) to hydrophobic surface (above this temperature) occurs in this polymer</td>
<td>modulate cell adhesion and cell sheet retrieval</td>
<td>228</td>
</tr>
<tr>
<td>P(NIPAM-co-DMAEMA-co-BMA)</td>
<td>temperature changes modulate polyplex formation/dissociation</td>
<td>delivery of therapeutic agents and nucleic acids</td>
<td>229</td>
</tr>
<tr>
<td>P(NIPAAm-co-DMAAm)-b-PLA</td>
<td>interaction between the hydrated NIPAAm and the cells; leads to internalization above the LCST</td>
<td>delivery of nucleic acids and peptides/protein</td>
<td>230</td>
</tr>
<tr>
<td>PDMAEMA–PPO-PDMAEMA</td>
<td>forms rodlike polyplexes; DNA is encapsulated into biodegradable polymer shell; effective transfection</td>
<td>DNA delivery to HEK 293 cells</td>
<td>231</td>
</tr>
<tr>
<td>PMEO2MA-b-PHEMA diblock copolymers</td>
<td>condensation of DNA more efficiently by PEIMH. More exposure of surface positive charges of PEIMH/pDNA complexes due to collapse of PMEO2MA chains at temperature above LCST</td>
<td>DNA complexation. Gene expression level in HEK293 cells</td>
<td>232</td>
</tr>
<tr>
<td>PDMAPAAm-PNIPAAm</td>
<td>high transgene expression and good cell viability</td>
<td>plasmid DNA complexation</td>
<td>233</td>
</tr>
<tr>
<td>PEG-b-PNIPAAm</td>
<td>encapsulation of both hydrophilic drugs within the interior and hydrophobic molecules in the membrane</td>
<td>tissues such as tumors</td>
<td>234</td>
</tr>
<tr>
<td>PNIPAAm-b-PLLys</td>
<td>DNA is encapsulated into biodegradable polymer shell decorated with targeting functions; effective transfection</td>
<td>DNA delivery into cells</td>
<td>235</td>
</tr>
<tr>
<td>(PNIPAm -g-PEG)-b-PLLys</td>
<td>high potential for condensing DNA; low cytotoxicity; effective transfection</td>
<td>DNA delivery into cells</td>
<td>235</td>
</tr>
<tr>
<td>PNIPAAm-b-PLLys</td>
<td>DNA is encapsulated into biodegradable polymer shell decorated with targeting function; effective transfection</td>
<td>DNA delivery into cells</td>
<td>235</td>
</tr>
<tr>
<td>P(NIPAAm-co-HEMA-co-DMAEMA)</td>
<td>positively charged copolymers as vectors for gene delivery</td>
<td>deliver green fluorescent protein (GFP) gene into HEK293T cells</td>
<td>236</td>
</tr>
<tr>
<td>PNIPAAm -co-Am</td>
<td>by increasing from room to body temperature, the size of PCANs decreases because of phase transition from hydrophilic to hydrophobic; this enhances cellular uptake</td>
<td>RA delivery to direct hiPSC</td>
<td>237</td>
</tr>
<tr>
<td>PEG-b-poly-L-EG2Glu</td>
<td>this diblock copolymer displayed two levels of self-assembly behavior, which caused thermoinduced solubility and conformation transition</td>
<td></td>
<td>238</td>
</tr>
</tbody>
</table>