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Review

Intelligent Polymeric Nanocarriers Responding to Physical or Biological Signals: A New Paradigm of Cytosolic Drug Delivery for Tumor Treatment

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Abstract: The physicochemical properties of stimuli-responsive polymers change with physical or biological signals, such as pH, enzyme concentrations, and temperature. These polymers have attracted considerable attention in the field of drug delivery. The drug carrier system, which was revolutionized by the introduction of these polymers, has recently provided a new paradigm of maximizing the therapeutic activity of drugs. This review highlights recent studies regarding stimuli-responsive drug carriers tailor-made for effective cytosolic drug delivery, with particular emphasis on tumor treatment.

Keywords: stimuli-responsive polymer; cytosolic drug delivery; tumor treatment

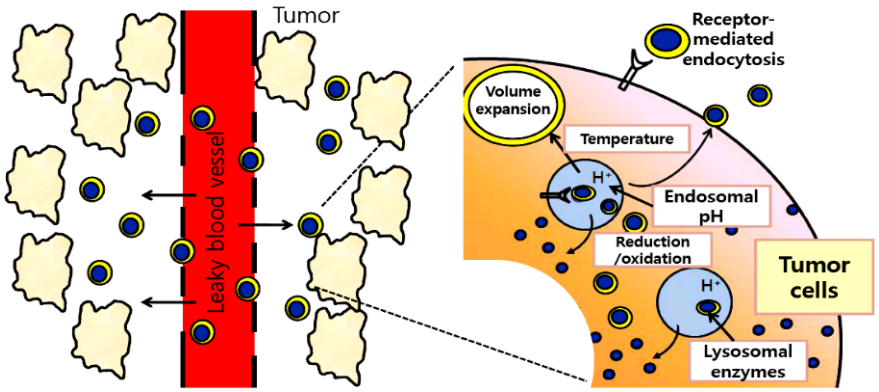
1. Introduction

In general, the targeting of a cytotoxic agent to solid tumors entails the passive accumulation of nano-sized drug carriers around solid tumor tissues, followed by active internalization into tumor cells [1-12]. The cellular internalization of either the drug alone, or with its carrier, is necessary, because the majority of cytotoxic drugs function intracellularly, whereas targeting agents for diagnostic purposes and imaging may not necessarily require internalization [13-15].

The passive accumulation of drugs or drug carriers relies heavily on the probability of the extravasation of nano-sized particles through the walls of tumor microvessels. Fortunately, the leaky characteristics of tumor blood vessels, which result from hypervasculature, defective vascular architecture, and a variety of permeability enhancing mediators secreted from tumor cells, are attributed to the enhanced extravasation of a given nano-sized drug carrier into a tumor site from circulation in the blood [12-14]. However, active internalization is generally thought to occur with drug carriers that are directed by a monoclonal antibody (mAb) binding to a tumor associated surface antigen, or by the binding of a ligand to its corresponding receptor on the surface of tumor cells [15-19]. The information in the relevant literature demonstrates that the active targeting of drug carriers with mAbs or ligands results in higher levels of drug accumulation in tumors [20-25]. This active process was expected to result in a remarkable cell killing activity against tumor cells. However, this process does not provide an actual driving force for the effective killing of tumors, because drug carriers localized in vesicles (*i.e.*, endosomes, lysosomes) after endocytosis do not demonstrate vesicular-escaping activity. This activity is needed to selectively elevate drug concentrations in the cytoplasm and nucleus (potential drug acting sites) [20-25].

Recently, nano-sized drug carriers that respond to physical or biological signals, such as pH, temperature, and enzyme expression, have been extensively evaluated for achieving spatial site-specific drug delivery [26-50]. These systems are designed to minimize the rates of drug release into the extracellular space, while maximizing drug concentration in their target site under a particular signal. For example, the acidic pH in endosomes and specific enzymes can constitute internal signals to facilitate drug transport from endosomes or lysosomes to the cytoplasm. Temperature may also be manipulated as an external signal for the modulation of drug release kinetics for the cytoplasm. It is worth noting that the use of these signals has promoted the creation of a new mode of cytosolic drug/gene delivery system (Figure 1), thus suggesting the potential for a new system of highly sophisticated drug release control [26-59].

Figure 1. Schematic illustration of actions of the stimuli-responsive nanocarriers in the tumor site. Nano-sized drug carriers will be extravasated into tumors and internalized into tumor cells using pinocytosis, receptor-mediated endocytosis, and other internalized mechanisms. The drug release kinetics from nano-sized drug carriers internalized into the cells may be modulated by stimuli, such as pH, enzymes concentrations, and temperatures, to enable effective cytosolic drug delivery.



In this review, we summarize and discuss recent representative studies on polymeric drug carriers responding to physical or biological signals for effective cytosolic drug delivery, with a particular emphasis on tumor treatment. However, polymeric carriers for genetic materials, such as plasmid DNA, short-interfering RNA (siRNA), and oligonucleotides (ODN), are not covered here, because a number of review articles regarding cytosolic gene delivery have already been reported.

2. Stimuli-Responsive Delivery Systems

2.1. Acidic pH-Activating Systems

Cellular components, such as cytoplasm, mitochondria, endosomes, lysosomes, and Golgi bodies have their own characteristic pH values: cytoplasm (pH 7.2), mitochondria (pH 8.0), endosomes (pH 5.0–6.0), lysosomes (pH 4.0–5.0), and Golgi bodies (pH 6.4) [26]. In particular, the more acidic endosomal/lysosomal compartments function as important sites for the pharmaceutical application of pH-responsive polymers as drug carriers (Table 1). After endocytosis, the sequestration of drug carriers in these compartments is ultimately subject to extensive degradation or metabolism in the lysosome. Therefore, the development of drug carriers that respond to endosomal/lysosomal pH and escape from endosomal compartments will tend to increase the bioavailability of delivered drugs, resulting in avoidance of the sequestration and degradation pathways.

Table 1. Examples of pH-responsive polymers.

Polymer	Description	Drug	Ref.
PolyHis-*b*-PEG	Protonation of the imidazole group in His block at lower pH (≤ pH 7.2), resulting drug release	DOX	[20]
Blended polymers of polyHis-*b*-PEG-folate /PLLA-*b*-PEG-folate	Micelles were destabilized in the pH range of 7.2-6.6, resulting drug release	DOX	[28,29]
Pullulan acetate and oligo-sulfadimethoxine (PA-*g*-OSDM)	Acid pH induced internal structural change, resulting drug release	DOX	[21]
Poly[($_{L}$-histidine)-*co*-($_{L}$-phenyl alanine)]-*b*-PEG (HF-b-PEG)	Drug release by the micelle destabilization at tumor extracellular pH	DOX	[46]
Poly(*N*-isopropylacrylamide) (PNIPAM)	A coil-to-globule transition at acidic pH was utilized to destabilize intracellular vehicle membrane	DOX	[47]
Folate-PEG-*b*-poly(aspartate hydrazone doxorubicin) [Folate-PEG-*b*- poly(Asp-Hyd-DOX)]	Hydrazone bond between drug and polymer was cleaved intracellularly at low pH (5.5), resulting in drug release	DOX	[48]
Poly(ε-caprolactone-*co*-lactide)-*b*-PEG-*b*-poly(ε-caprolactone-*co*-lactide) with sulfamethazine oligomer (OSM-PCLA-*b*-PEG-*b*-PCLA-OSM)	Rapid sol-to-gel transition with change in pH, resulting in triggering drug release	PTX	[49]
Poly(*N*-isopropylacrylamide-cobutylmethacrylate-*co*-acrylic acid)	A pH-dependent drug release behavior was observed in the pH range of pH 5.0-6.0	Insulin	[50]

Table 2. Examples of enzymatically responsive polymers.

Enzyme-activated polymer	Enzymatic site	Drug	Ref.
N-(2-hydroxypropy1) methacrylamide-GFLG-mesochlorin e6 (HPMA-GFLG-Mce 6)	GFLG	Mce6	[60]
N-(2-hydroxypropy1) methacrylamide-GFLG- Geldanamycin (HPMA-GFLG-GDM)	GFLG	GDM	[62]
Poly(ε-caprolactone)–*b*-poly(ethyl ethylene phosphate)	Disulfide bond	DOX	[84]
PEG-*b*-poly(amidoamine) dendrimer- succinic acid-PTX	Ester bond	PTX	[89]
Polyglycerol dendrimer- Ala-Phe-Lys-MTX	Ala-Phe-Lys	MTX	[90]
Trimeric pro-drug (DOX/CPT/etoposide)	Aldol, Retro-aldol	DOX, CPT, etoposide	[91]
Poly(γ-glutamic acid)-based nanoparticles (γ-PGA NPs)	Phenylalanine ethyl ester	OVA	[92]
N-(2-hydroxypropyl) methacrylamide with methacryloylglycylglycine 4-nitrophenyl ester (PHPMA)	Ester bond	CPT	[63]

2.1.1. Synthetic anticancer drug delivery

Our group has developed a poly(L-histidine) (polyHis)-based drug carrier system consisting of polyHis-*b*-poly(ethylene glycol)-folate (polyHis-*b*-PEG-folate) and poly(L-lactic acid) (PLLA)-*b*-PEG-folate [27-29], targeted toward effective cytosolic anticancer drug delivery by virtue of the fusogenic activity of polyHis. Herein, the incorporation of a non-ionizable block copolymer (PLLA-*b*-PEG-folate) within the micellar structure of polyHis-*b*-PEG-folate improved micelle stability at a pH of 7.4, and shifted micelle destabilization to a lower pH (*i.e.*, pH 6.5–6.8), similar to early endosomal pH. The folate, coupled with polyHis-*b*-PEG or PLLA-*b*-PEG, enabled the efficient cellular uptake of drug carriers for human breast carcinoma MCF-7 cells overexpressing folate receptor (FR). It was noted that drug carriers sequestrated into early endosomes after FR-mediated endocytosis responded to endosomal pH and released encapsulated anticancer drugs (e.g., doxorubicin: DOX) into the cytoplasm [27-29]. It appeared that the protonation of polyHis at endosomal pH resulted in the destabilization of the endosomal membrane, which is associated with the proton sponge effect [30]. It has been well established that the vacuolar ATPase-H^+ pumps in endosomal membranes modulate proton transfer from the cytoplasm to endosomal compartments [30]. If certain materials with protonatable groups are entrapped within these acidic compartments, the transferred protons will be captured by protonatable moieties. This proton buffering leads to a smaller increase in endosomal pH. This causes a simultaneous influx of both protons and their counterions (Cl^-) into the endosomes, thus creating osmotic imbalances between the cytosol and the endosomal compartments. This induces endosomal swelling, as a result of continuous water influx. This ultimately leads to endosomal rupture. Therefore, the polyHis-based drug carrier system effectively carries anticancer drugs to the cytosol of tumor cells, consequently resulting in high cytotoxicity to MCF-7 tumor cells (over 90%) [27-29].

Bae *et al*. previously developed a novel intracellular pH-sensitive polymeric micelle with a pH-labile bond (e.g., hydrazone bond) [31-33]. The self-assembly micelles were prepared from PEG-*b*-poly(aspartate-hydrazone-DOX) copolymers obtained after the chemical conjugation of DOX to the side chain of PEG-*b*-poly(aspartate) copolymers via an acid-labile hydrazone linker. Such pH-sensitive

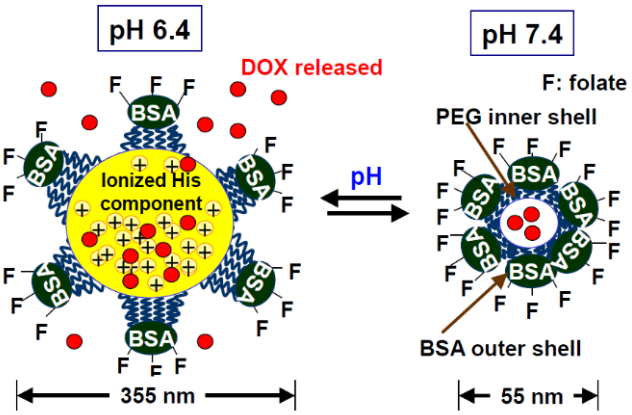
polymeric micelles hold the drugs at physiological conditions (pH 7.4), but release active DOX at a low pH (pH 5.0). Similarly, Hruby *et al*. reported a pH-sensitive polymeric micelle with a hydrazone bond between the polymer and DOX [34]. After the incubation of micelles in buffers at 37 °C, DOX was released more rapidly at pH 5.0 (43% DOX released within 24 hours) than at pH 7.4 (16% DOX released within 24 hours). Despite this pH-selectivity, the pharmaceutical uses of hydrazone bonds may prove somewhat limited, as the chemical cleavage of the hydrazone bond can also release inactive DOX fragments.

Recently, our group designed a novel mode of drug delivery using polyHis (Mw 5K)-*b*-PEG (Mw 2 K) and PLLA (Mw 3K)-*b*-PEG (Mw 2K)-*b*-polyHis (Mw 2K)-TAT peptide [TAT: transactivator of transcription, Gly-Cys-(Gly)3-Tyr-Gly-Arg-(Lys)2-(Arg)2-Gln-(Arg)3]] [35-36]. It is known that the TAT peptide, a non-specific cell penetrating peptide, is derived from human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2), which have been reported to facilitate the rapid translocation of various macromolecules into mammalian cells [37]. In this study, we utilized both copolymers for the preparation of a self-assembled mixed micelle consisting of a hydrophobic core [PLLA and polyHis (Mw 5 K)] and a hydrophilic shell (PEG and TAT peptide) at physiological pH. Here, the shorter polyHis block (Mw 2 K) within the PLLA-*b*-PEG-*b*-polyHis-TAT is self-assembled at the interface between the hydrophobic core and the hydrophilic PEG shell, owing to the presence of the hydrophilic PEG and TAT peptides on both sides of polyHis (Mw 2 K). In particular, the interfacial polyHis (Mw 2 K) induced the bending of the PEG chain within the PLLA-*b*-PEG-*b*-polyHis-TAT, resulting in the burial of the TAT peptide coupled with polyHis (Mw 2 K) within the stretched PEG shell derived from the polyHis-*b*-PEG block copolymer. However, as the pH is lowered below pH 7.0, the degree of ionization of the interfacial polyHis (Mw 2 K) block increases. This weakens the hydrophobic interaction between polyHis (Mw 2 K) and the micellar core, resulting in the stretching of the bended PEG chain and exposing the TAT peptide from the PEG shell via this pop-up mechanism. This process may enhance the cellular uptake of drug carriers via TAT peptide-mediated endocytosis. Furthermore, when the pH is lowered further (pH < 6.5; close to endosomal pH), the micellar core consisting of polyHis (Mw 5 K) and PLLA blocks is destabilized in the endosomal compartments, resulting in the disruption of the endosomal membrane as a result of the proton sponge effect of polyHis (Mw 5 K) [35,36]. This process was demonstrated to increase drug concentration in tumor cells and improve drug therapeutics.

On the other hand, a virus-mimetic nanogel (VM nanogel) was developed for the modulation of cytosolic drug concentration in a controlled manner. VM nanogel consists of a hydrophobic core [poly(His-*co*-Phenylalanine)] and two layers of hydrophilic shells [PEG and bovine serum albumin (BSA) (Figure 2) [38]. One PEG end is multi-linked to BSA, and the other end is linked to the core polymer, which forms a capsid-like outer shell. At low pH, the core of this system is swelled by the ionization of polyHis, whereas at high pH, the core shrinks. In this regard, the DOX release rate can be accelerated from swollen nanogels at endosomal pH (e.g., pH 6.4), whereas the DOX release rate was reduced from the shrunk nanogels at cytosolic pH (e.g., pH 7.4–6.8). Moreover, the expansion of the volume of VM nanogels and the proton sponge effect of polyHis at endosomal pH are assumed to provide profound endosomal escape properties. These functionalities of VM nanogel allow for the translocation of drugs or nanogels from the endosomes to the cytoplasm, where the VM nanogels

rapidly shrink back to their original size under a more neutral local pH. This shrinkage is followed by the minimization of the drug release rate. It is important that anticancer drugs released under the stimulus of endosomal pH diffuse into the nucleus, ultimately leading to cell apoptosis. Furthermore, apoptosized cell bodies will release the shrunk nanogels (still active) for subsequent infection and action in other neighboring cells. This VM nanogel system was identified as a 'serial killer' of tumor cells [38].

Figure 2. Virus-mimetic nanogel system. The virus-like infectious nanogel consists of a hydrophobic core [poly(His-*co*-Phe)] and two layers of hydrophilic shells [(PEG and BSA)]. Reproduced with permission from [38].

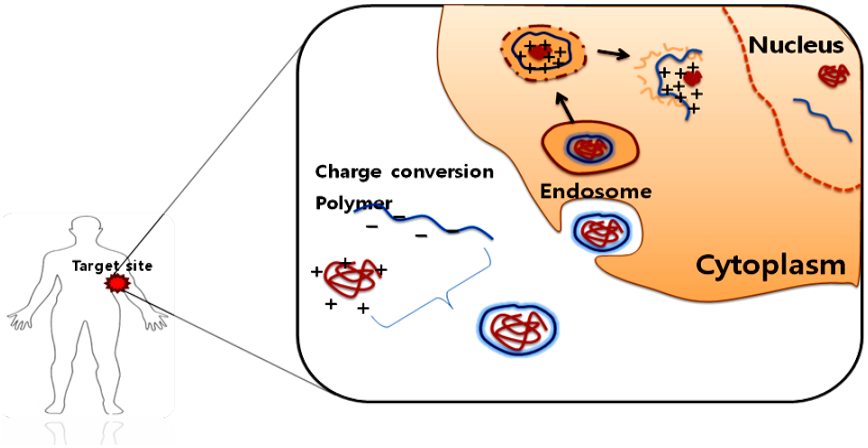


2.1.2. Protein drug delivery

Kataoka's group introduced a charge conversion polymer, specifically PEG-*b*-poly(N′-citraconyl-2-aminoethly-aspartamide) [PEG-*b*-pAsp(EDA-Cit)], in order to fabricate a novel protein drug carrier for cytosolic delivery [39,40]. The polyionic complex (PIC) was prepared in an aqueous solution via the electrostatic interaction between a charge conversion polymer and a cationic protein (lysozyme). Interestingly, PEG-*b*-pAsp(EDA-Cit) has a pH-labile component (*i.e.*, citraconic amide) that is degradable under endosomal acidic pH conditions. At a pH of 5.5, the citraconyl-2-aminoethyl group is transferred to the free 2-aminoethyl group, presenting the charge switching from negative (citraconyl-2-aminoethyl group) to positive (2-aminoethyl group). This change in electrical charge accelerates the protein release rate at pH 5.5 by virtue of the electro-repulsive force between the positively charged 2-aminoethyl group and the lysozyme (Figure 3). Furthermore, PIC prepared from PEG-*b*-poly(N,N′-2-aminoethyl-2-aminoethyl aspartamide) [PEG-*b*-pAsp(DET)] and cytocrome c-citraconic acid (Cyt-Cit) or cytocrome c-cis-aconitic (Cyt-Aco) demonstrated pH-stimulated protein (Cyt) release, depending on the chemical cleavage of Cyt-Cit or Cyt-Aco to Cyt at pH 5.5 (close to endosomal pH). In particular, Cyt that is chemically free from Cit or Aco absorbs protons, which may cause swelling or burst the endosomes, as a result of the proton sponge effect. This system consequently resulted in high Cyt concentration in the cytoplasm, as evaluated from the *in vitro* cell test. Similarly, Liu *et al*. synthesized poly(allylamine)-citraconic acid for the preparation of a polyelectrolyte multi-layer that responds to endosomal pH (pH 5.0) [41]. The multi-layer film system allowed for the triggering of protein release at pH 5.0. These systems are indeed comparable with that

of a charge conversion polymer modified with 2,3-dimethylmaleic acid (DMA). Lee *et al.* suggested that other polymeric nanocomplexes, consisting of cationic protein (lysozyme) and glycol chitosan (GC) grafted with DMA (GC-*g*-DMA), might exist. Unlike the work of Kataoka's group, the GC-*g*-DMA/lysozyme complex disintegrated rapidly at pH 6.8 (close to tumor extracellular pH), owing to the chemical separation of DMA from GC-*g*-DMA [47,48]. This complex inhibits protein release at pH 7.4 and promotes protein release at pH 6.8.

Figure 3. Schematic representation of the actions of the charge-converting nanocomplex at endosomal pH.



On the other hand, hydrophobically-modified poly(γ-glutamic acid) (γ-PGA) nanoparticles have been previously utilized for cytosolic protein delivery [44]. The formation of the helix structure of γ-PGA at pH 5.5 serves as a hemolytic activator, providing the ability to escape from the endosomes and to deliver protein drugs to the cytoplasm.

Tada *et al*. utilized carbonate apatite nanoparticles for the transport of proteins to the cytoplasm [45]. This nanoparticle was prepared via the electrostatic interaction of BSA with the positively charged surfaces of carbonate apatite particles at a pH of 7.4. Interestingly, the high water solubility of carbonate apatite particles at endosomal pH values (pH 5.0) induced the accelerated release of the electrostatically associated proteins into the cytoplasm. Actually, the fluorescence-labeled BSA incorporated into BSA/carbonate apatite complexes was observed, via confocal microscopy, to be effectively delivered into the cytoplasm of HeLa cells.

2.2. Enzyme-Activating Systems

Cells harbor a variety of enzymes in the intracellular compartment, including endosomes, lysosomes, and the cytoplasm. These enzymes have recently been utilized as specific signals for triggered drug release from drug carriers [55-75]. For example, the labile linkers (such as disulfide bonds, ester bonds, and certain peptide spacers) in drug carriers are readily cleaved via certain enzymatic reactions. These linkers cleaved by specific enzymes in the target sites (e.g., lysosome, endosome, cytoplasm) signaled stimulated drug release by employing the strategy of destabilizing the drug carriers.

Kopecek's group synthesized a non-toxic and non-immunogenic *N*-(2-hydroxypropy1) methacrylamide (HPMA) copolymer grafted with anticancer drugs (e.g., DOX, mesochlorin e6

monoethylenediamine, geldanamycin (GDM), gemcitabine) via a lysosomally-degradable tetrapeptide linker (Gly-Phe-leu-Gly, GFLG) [60-72]. Here, GFLG is a specific area that can be cleaved by a lysosomal enzymatic system, such as cathepsin B and cysteine proteinase. It has been determined that lysosomes are the cells' garbage disposal system and harbor a variety of enzymes, including protease, nuclease, *etc.* [83]. The activities of these lysosomal enzymes have been previously employed in the development of a novel enzyme-degradable pro-drug (polymer-drug conjugate) with reduced toxic effects for normal tissue and with increased therapeutic efficacy for tumor sites. However, enzymatic metabolism may also prove effective with the free drug, which will significantly attenuate the therapeutic activity of the drug.

Wang *et al.* synthesized poly(ε-caprolactone) (PCL)-*b*-poly(ethyl ethylene phosphate) (PEET) with a disulfide-linker (-SS-) for intracellular drug delivery [84]. An anticancer drug (DOX) and PCL-*b*-PEET were dialyzed in order to construct the self-assembled polymeric micelles. The resultant micelles include a disulfide linker sensitive to the reduction reaction in cells. Reduction and oxidation (redox reactions) are chemical phenomena in which certain molecules gain or lose electrons [85-88]. In the majority of mammalian cells, intracellular compartments, such as cytoplasm and nuclei, favor more reduced forms of certain molecules, unlike the extracellular milieu, in which the oxidized counterpart forms are favored [85-88]. For example, reduced glutathione (GSH) was detected at ~20 μM in the plasma and ~10 mM in the cytoplasm [87]. Furthermore, the following preference order of GSH over oxidized glutathione (GSSG) in cells was observed: mitochondria > nuclei > cytoplasm > extracellular milieu [88]. This selectivity for each organelle may prove useful in the design of a site-specific drug delivery system sensitive to the redox reaction. As a consequence, DOX-loaded micelles with disulfide-linkers were destabilized in the cytoplasm as a result of the reduction reaction for the disulfide-linker, thus allowing for the release of DOX from the micelles [84].

Minko *et al.* previously prepared a poly(amidoamine) (PAMAM) dendrimer conjugated with anticancer drug (paclitaxel, PTX) via a succinic acid (SA) spacer [89]. Both sides of the SA spacer coupled with PAMAM or PTX include two ester bonds that can be cleaved by esterase. Thus, active drug or metabolized drugs (by lysosomal enzymes) may be released simultaneously from the dendrimer, resulting from the enzymatic degradation of the SA spacer. Similarly, Haag *et al*. designed a non-toxic, water-soluble, non-immunogenic polyglycerol (PG) dendrimer conjugated with DOX and methotrexate (MTX) via the dipeptide Phe-Lys or the tripeptide Ala-Phe-Lys linker [90]. These peptide linkers were employed as the substrates for lysosomal cathepsin B. They facilitated the release of DOX or MTX from the dendrimers enzymatically degraded in tumor cells. In addition, Shabat *et al*. demonstrated the synthesis of a trimeric pro-drug, which contains three anticancer drugs (such as DOX, etoposide, and camptothecin) linked to certain enzymatic substrates [91]. After the enzymatic degradation of substrates by the catalytic antibody 38C2 in human MOLT-3 leukemia cells, three anticancer drugs were simultaneously released.

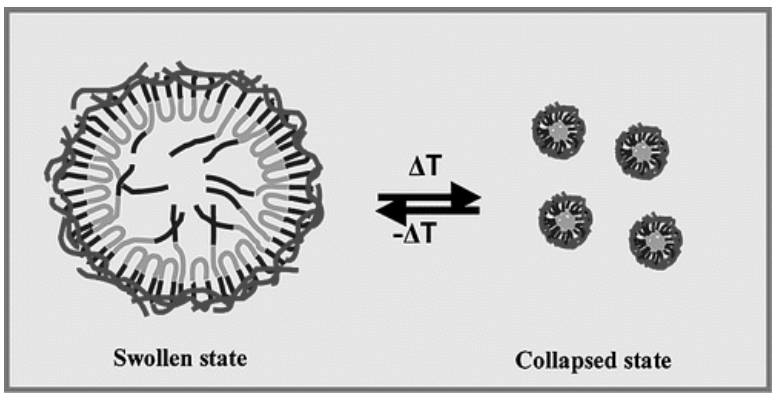
Nakagawa *et al*. employed γ-PGA-based nanoparticles (γ-PGA NPs) with L-phenylalanine ethyl ester (L-PAE) for cytosolic protein delivery [92]. However, the γ-PGA NPs encapsulating ovalbumin (OVA) require the enzymatic degradation of L-PAE for the release of OVA. It appeared that the protein drugs released from this carrier may also be degraded by a variety of enzymatic metabolisms.

2.3. Temperature-Activating Systems

Thermo-responsive polymeric nanocarriers for use in anticancer drug delivery have been previously fabricated from a variety of polymer architectures consisting of temperature-sensitive blocks, hydrophilic blocks, and hydrophobic blocks [93-108]. Here, poly(N-isopropylacrylamide) (PNIPAAm), Pluronic® F-127 and poly(organophophazenes) have been used, principally as temperature-sensitive polymers. Recently, drug release kinetics have been controlled effectively with respect to the use of these polymers, even within the cytoplasm [93-97]. Unlike the heat shock (hyperthermia) applied to tumor cells for anticancer therapy [105-108], cold-shock using cooling processes resulted in an expansion of the volume of thermo-responsive drug carriers [93-97], thereby enabling the cytosolic translocation of drug or drug carriers from physically burst endosomes. Park's study group prepared Pluronic® F-127 nanocapsules via the cross-linking reaction of poly(ethylene imine) (PEI) (or heparin) and Pluronic® F-127 [93-95]. These nanocapsules evidenced a volume expansion over a temperature range of 24–33 °C: ~100 nm size at 37 °C and ~350 nm size at 25 °C. In particular, the reversible volume transition of these nanocapsules, in response to temperature, is attributable to the micellization or demicellization of Pluronic® F-127 within the nanocapsules, depending on the critical micelle temperature (CMT) (Figure 4) of Pluronic® F-127 [93-95]. Furthermore, they designed super-expandable nanogels upon cooling for thermally triggered tumor cell death [96]. The nanogels, consisting of oligo(L-lactic acid) (OLA)-*b*-PEG-*b*-poly(propylene oxide) (PPO)-*b*-PEG-*b*-OLA and PEG-grafted poly(L-lysine), evidenced a reversible volume transition from ~150 nm at body temperature to ~1.4 μm at 15 °C. Under cold shock conditions, HeLa cells treated with the nanogels evidenced a cell viability of 54.0 ± 22.1%, whereas those maintained at 37 °C evidenced a cell viability of 92.2 ± 7.2%. The nanogel undergoing a nano- to micro-scale volume transition in response to temperature seemed to evoke necrotic cell death under cold shock conditions. Overall, these carriers are expected to prove beneficial in the future, with respect to achieving effective therapeutic results.

Additionally, Okano's study group has reported that PNIPAAm conjugated to dimethylaminoethyl methacrylate (DMAEMA) and butyl methacrylate (BMA) was insoluble at temperatures above 21 °C and was soluble at temperatures below 21 °C [97]. The nanoparticles prepared using this polymer conjugate triggered drug release at temperatures below 21 °C, similar to what was observed with the cold shock effect.

Figure 4. Schematic diagram of Pluronic® F-127 nanocapsules. Reproduced with permission from reference [94].



3. Conclusions

Nanoscale drug delivery systems have been extensively developed over the past few years. The evolution of nanotechnology has created significant changes in drug delivery systems. A great deal of attention has been focused on the development of smart drug carrier systems that recognize the physicochemical or biological differences between non-targeted sites and targeted sites. Acidic pH in cells, specific enzymes in cells, and temperature effects for cells, have been investigated intensively and employed as crucial signals for the delivery of drugs to the cytoplasm. These recent studies are expected to usher in a new paradigm of improved drug therapeutics.

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