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Review

Graphene Oxide and Reduced Graphene Oxide: Efficient Cargo Platforms for Cancer Theranostics

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ABSTRACT

Any imbalance in body's pH or temperature may alter immune response and lead to conditions such as autoimmune disorders, infectious diseases, cancer, and diabetes. Dual pH and thermo-responsive carriers are being considered as next-generation drug delivery systems, which are capable of releasing drugs intelligently in response to external or internal stimuli. These systems are characterized by minimal toxicity, improved therapeutic efficacy and reduced exposure to normal cells. Besides polymeric nanoparticles and polymeric micelles, which are most studied, there has been research being carried on other drug delivery systems which can respond to changes in pH and temperature for targeted drug release. Among those, gels have been studied mostly for their dual responsiveness. This review is prepared with an intention to provide an update on the progress of gel-based dual pH and temperature responsive drug delivery systems and other less-studied systems such as dendrimers, membranes, liposomes, microcapsules, microspheres, polymeric films, hollow spheres and protein nanoparticles for dual responsiveness. Various systems reported so far under these categories for targeted and controlled delivery of different classes of drugs such as antidiabetic and antibiotic drugs with special emphasis on anticancer drugs are discussed in this review.

1. Introduction

In recent years of drug formulation research, one of the main focus or challenge has been as how to assure the safety of drug carriers to the human body with good biocompatibility and non-toxic side effects [1]. With recent advancements in drug delivery technology, several nanoparticulate drug delivery systems (NDDS) have been designed to deliver their payload specifically at the target site. Some of the nanoparticulate delivery systems/carriers include liposomes, polymeric micelles, polymeric nanoparticles, gold, silver, silica and other metal nanoparticles, niosomes, nanogels, membranes, and dendrimers. First generation NDDS were able to address challenges such as in vivo drug stability and specific tissue targeting. In this context, novel drug delivery systems have been developed which can simultaneously or sequentially confront challenges such as overcoming multiple physiological barriers in vivo, increased circulation time and specific tissue targeting. Stimuli-responsive novel drug delivery systems have been developed to specifically act at the target site with long circulation times and enhance intracellular drug delivery [2,3]. These drug delivery systems have the capability of responding to both internal and external stimuli. Internal stimuli to which drug delivery systems can respond include pH, temperature, and redox conditions, which are characteristics of the pathological site, and certain biologic molecules (enzymes) specific to tissue or disease. External stimuli can be a magnetic field, ultrasound field, and light. In stimuli-responsive drug delivery systems, the active drugs are encapsulated in nanocarriers such as liposomes or polymeric micelles (Fig. 1) [4,5].

Among various stimuli, pH and temperature are the primary choices, as they are simple to understand [6]. Any imbalance in body's pH or temperature may alter immune response and lead to autoimmune diseases, infectious diseases, cancer, diabetes, Parkinson's disease, etc. Also, changes in the pH of the body fluids may sometimes cause serious conditions such as metabolic acidosis or alkalosis where pH of body fluids is decreased or increased, respectively. Examples include conditions such as lactic acidosis and chlorine alkalosis where there is a change in pH of the body fluids from the normal physiological pH [7–10]. Also, certain autoimmune diseases are also associated with changes in the physiological pH; for example, in Hashimoto's thyroiditis, changes in iodine concentration alter the physiological pH and impacts the cellular pumps, channels, transporters, and isoenzymes.

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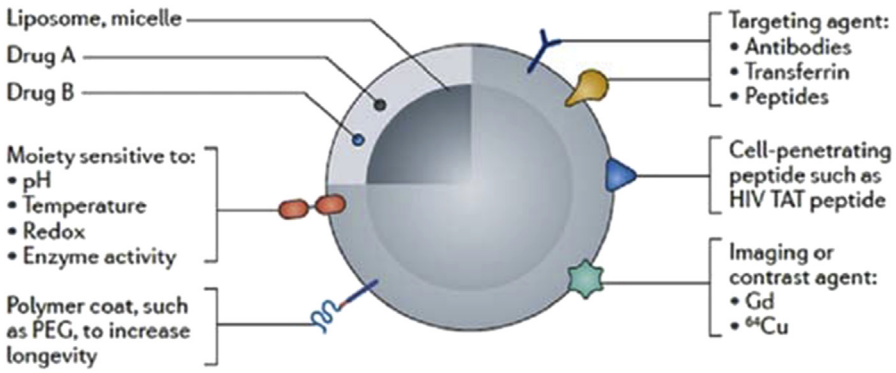

Fig. 1. Schematic of a drug-loaded multifunctional stimuli-sensitive NDDS. Drugs (Drug A and Drug B) can be loaded into a pharmaceutical nanocarrier, such as a liposome or polymeric micelle. Depending on the purpose of the NDDS, various agents can be added to the nanoparticle to target the NDDS to a particular tissue, to increase cell penetration, to enable imaging or to release the drugs in response to a given stimulus. PEG, poly (ethylene glycol). Reproduced with permission from Ref. [4].

Inflammatory conditions are associated with an increase in temperature and this, in turn, will stimulate the immune cells to release inflammatory markers such as cytokines, interleukins, interferons and tumor necrosis factor. For example, in rheumatoid arthritis, an autoimmune disease associated with inflammation, high or elevated temperatures are observed in joints [9]. Also, in cancer, as malignant cells proliferate, they exhibit higher temperatures and acidic microenvironment. Controlled release drug delivery systems which are capable of releasing the drugs in response to physiological changes such as elevated temperatures and reduced pH could result in targeted drug delivery and reduced toxicity to normal cells. This concept has encouraged researchers towards the development of thermo and pH dual stimuli-responsive polymeric nanomaterials for cancer treatment [11–13]. Dual pH and thermo-responsive carriers are being considered as next-generation drug delivery systems, which are capable of releasing drugs intelligently in response to external or internal stimuli. These systems are characterized by minimal toxicity, improved therapeutic efficacy and reduced exposure to normal cells [11,14–16]. Temperature and pH-responsive polymers undergo physicochemical changes upon changes in pH and temperature of surrounding medium. These polymers consist of pH and temperature responsive blocks in their structure. For example, poly-N-isopropylacrylamide (PNIPAAm) is used as a temperature responsive segment [17], and polymeric blocks containing chemical functionalities such as amines and acids, acetal, amino alkylmethacrylate, ortho ester, vinyl ester and hydrazone can be utilized as pH-responsive segments [18]. In certain pathological conditions such as cancer, temperature and pH at the tumor site is different compared to normal body tissues. With changes in temperature and pH, transitions occur in responsive polymers resulting in disruption of prepared formulation/nano-system leading to release of their payload. These transitions could be reversible changes in physical state, solvent interactions, hydrophilic and lipophilic balances (HLB), conductivity, shape, and solubility. These transitions can occur due to neutralization of charged groups by pH shift and change in HLB or hydrogen bonding due to increase or decrease in temperature. Polymers utilized for temperature response undergo a change in solubility properties where lowest critical solution temperature (LCST) govern the polymers conformation. The slight increase in temperature may cause the polymer to suffer a phase transition into being insoluble and releasing the encapsulated payload. Similarly, polyacids or polybases based polymers upon pH variations undergo conformational change due to gain or loss of protons causing rapid adjustment of the net charge to the polymer chain resulting in the release of encapsulated payloads [17].

Polymeric micelles and polymeric nanoparticles are extensively studied systems for pH and temperature responsiveness. This review aims to provide an update on the progress of gel-based dual pH and temperature responsive drug delivery systems and other less-studied systems such as dendrimers, membranes, liposomes, microcapsules, and microspheres, etc. There may be points of overlap within sections such as microcapsules and microspheres being similar to microgels. The major difference is with water and hydration content which constitutes a gel while the microcapsules and microspheres are composed of semisolid polymers [19]. Therefore, each section is focused on the description of a drug delivery vehicle with properties tuned for dual responsiveness. Overlap can also be felt because of preparation of multicomponent systems, for example, incorporation of microcapsules in hydrogels which could also be possibly regarded as microgel system. Therefore, based on physical appearance, the method of preparation and other features we have categorized these systems as separate delivery systems than microgels. This review manuscript discusses various systems reported so far under these categories for targeted and controlled delivery of various classes of drugs such as antidiabetic and antibiotic drugs with special emphasis on anticancer drugs.

2. Dual pH and temperature responsive drug delivery systems

2.1. Gels

There is no generally adopted definition of the term “gel,” but it could be defined based on any of the aspects of the problem dealt with in the published literature. Polymeric gels are usually composed of a polymer-solvent system. Although there is a possibility of presence of several solvents in the system, in most cases these systems are binary. In these polymer-solvent systems, there is an existence of a three-dimensional network comprising of macromolecules or their aggregates. The three-dimensional networks are capable of retaining a large amount of the solvent, usually tens to hundreds folds greater than the polymer [20]. From a drug delivery scientist's perspective, gels can be defined as semisolid dosage forms containing a liquid phase constrained in a polymeric network held together by physical or chemical interactions and capable of absorbing water or biological fluids to swell. Pharmaceutical gels contain medicinal, cosmetic or other agents with sizes varying from nanometers (nm) to micrometers (μm). Depending on their size, gels can be classified into macrogels, microgels or nanogels. Macrogels have large cross-linked structures with a size range > 1 μm compared to micro and nanogels [21]. Gels are considered as reliable drug delivery systems due to their high stability, biocompatibility, less/no irritation, and swelling ability [22]. They also exhibit sensitivity towards variables such as pH, temperature, ionic strength, electric potential, salt concentration, light, ultrasonic sound, electric current, electric magnetic field, and biomolecules, to control rate of drug release [22–24]. Intravenous gel formulations can form an in-situ gel to release the desired drug depending on the levels of pH and temperature [22]. Gels can be prepared from various natural polysaccharides which include heparin, alginic acid, hyaluronic acid (HA), chitosan (CS), and dextran. These polysaccharides can be chemically modified so that they can be responsive to external or internal stimuli. Due to their release characteristics, gels can encapsulate agents such as drugs and release at the desired target site. Some gels could have growth factors, fillers, and/or cells to provide repair on specific areas [2]. For instance, in one study, gels equipped with human growth hormone (hGH), existed in liquid state at pH 6.0, 23 °C however, they formed gels at physiological

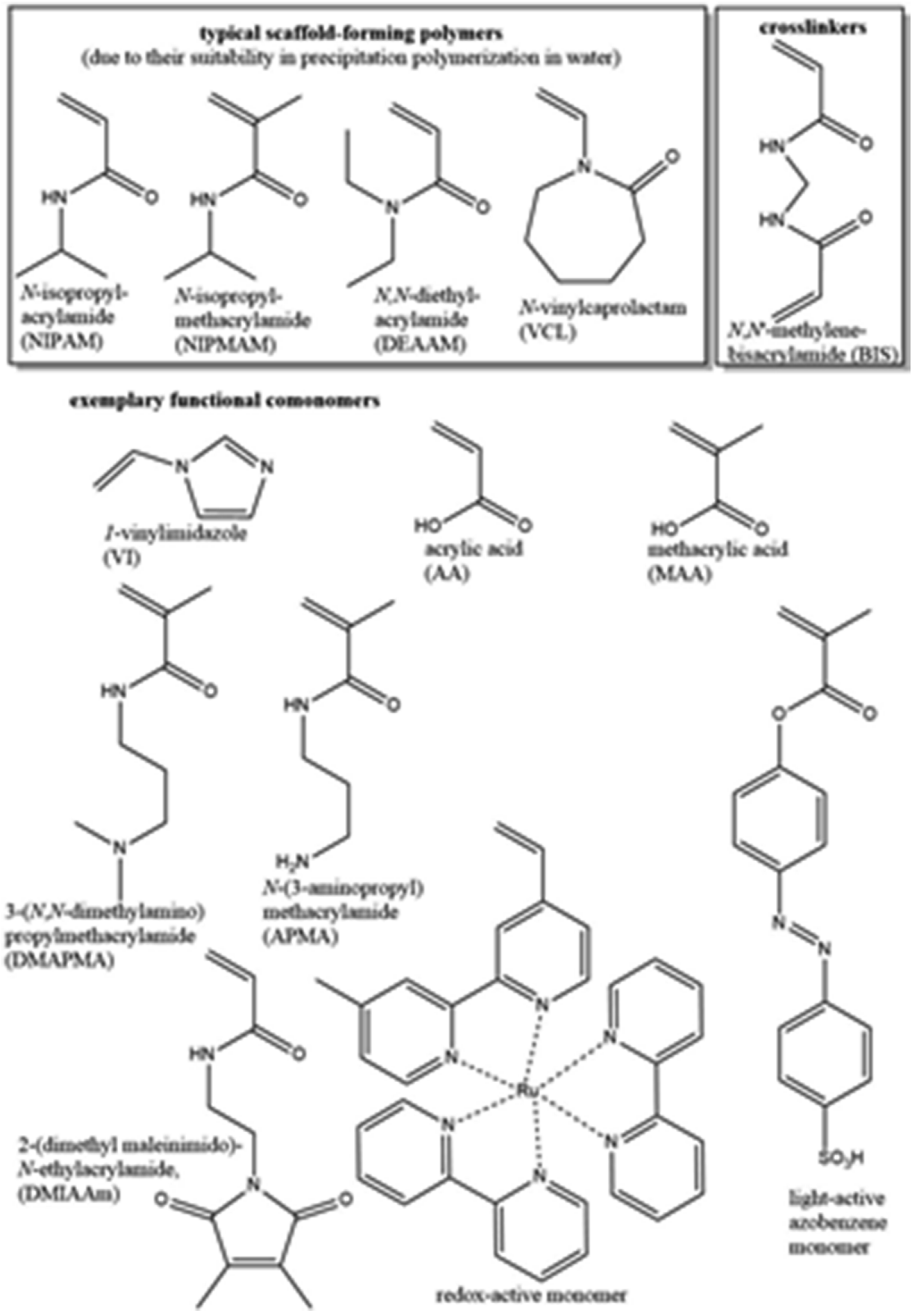

Fig. 2. Exemplary monomers used for functional microgels. Reproduced with permission from Ref. [34].

pH and temperature (7.4 and 37 °C) in vivo. It has been concluded that by configuring temperatures and pH of the polymers used in preparation of gels, gelation rates, mechanical strength and viscosity of gels can be regulated. Gels in this study were prepared from negatively charged hormones or medications that combine in an ionic form of polymers (poly (ethylene glycol)-poly (amino carbonate urethane) (PEG-PACU)) to form a positive complex, and thus can demonstrate a controlled delivery of medication. Pharmacokinetic studies in SD rats showed that absorption of hGH was slower with hydrogels compared to free hGH solution. In addition, bioavailability was higher with hydrogels compared to free hGH solution. Most of the gels prepared exhibited cell viability of more than 83% and had good biocompatibility and low toxicity. This study highlights that by varying concentrations of aqueous polymers such as PEG-PACU in hydrogels could result in sustained/controlled drug delivery with enhancement in bioavailability, stability and patient compliance [25]. Various types of gels formulated thus far are summarized in below sections.

2.1.1. Hydrogels

Hydrogels are cohesively held three-dimensional polymer webs which exhibit certain pH and temperature sensitive responses when prepared using stimuli-responsive polymers. Sensitivities to pH and temperature can be beneficial in drug delivery systems because they give hydrogels the ability to specifically release medication where appropriate range of pH or temperature is desired [15,26,27]. Till date, various polymers such as xylan, PNIPAAm/carboxymethyl CS, β-CD-conjugated poly(ε-lysine) (βCDPL) and 3-trimethylsilylpropionic acid, poly [*N,N*-dimethyl aminoethyl methacrylate-co-poly(poly(ethylene glycol) methyl ether methacrylate] [poly(DMAEMA-co-MPEGMA)], β-cyclodextrin, 2-methylacrylic acid and N,N′-methylene diacrylamide have been used for preparing hydrogels [1].

Scientists were successful in synthesizing dual responsive hydrogels

encapsulated with paclitaxel [28], proteins [29], and 5-aminosalycilic acid [30] using different polymers. However, they performed only in-vitro studies to prove pH and temperature sensitivity. In the recent times, multimembrane/multilayered hydrogels have been considered as promising drug carriers for biomedical applications. They are three dimensional spherical or tubular multi membrane structures with intermembrane spaces for drug linking. The intermembrane space can be loaded with drugs for achieving controlled delivery [31]. Nita et al., synthesized norfloxacin loaded multimembrane/multilayered poly (*N,N*-dimethylacrylamide-co-3,9-divinyl-2,4,8,10-tetraoxaspiro (5.5) undecane) (p(DMA-co-U)) hydrogel system using *N,N*-dimethylacrylamide (DMA) and 3,9-divinyl-2,4,8,10-tetraoxaspiro (5.5) undecane. Release studies revealed that multi-membrane hydrogels exhibited delayed release with primary effect during the first 300 min followed by a continuous release up to 1500 min. Also, hydrogels proved good in vivo biocompatibility with the decreased local inflammatory reaction in Wistar rats (n = 6) [32]. This study proved that p(DMA-co-U) based multi membrane hydrogels could be a potential pH and temperature responsive drug delivery system. However, this study lacks the evaluation of stability and release profile of norfloxacin from hydrogels at different pH and temperature conditions. This kind of study would have resulted in understanding whether the drug release pattern was sustained or burst depending on different pH and temperature conditions. Based on the particle size, hydrogels can further be classified into microgels and nanogels. These systems are described in detail under section 2.1.2 and 2.1.3.

2.1.2. Microgels

Microgels are macromolecular gels comprised of a three-dimensional polymeric network. The polymeric network may be formed by covalent cross-linking, physical interactions such as Van der Waals, hydrogen bonding, electrostatic and hydrophobic/hydrophilic interactions, glassy junctions by co-polymers, an interlamellar interaction of polymers [33]. They are polymeric gel particles in the size range of micrometers uniformly dispersed in a solvent medium and have swelling properties [34–36]. Various techniques used for the synthesis of microgels include precipitation polymerization, precipitation emulsion, radical polymerization and emulsion polymerization, where monomers are crosslinked to form polymeric structures which are held together by crosslinkers [34,36–38]. Many scientists were successful in preparing microgels encapsulated with therapeutic agents such as proteins and anti-cancer compounds. Polymers and co-monomers used for preparing microgels include PNIPAAm [39], hexafluorobutyl methacrylate (HFMA) [40], poly (*l*-glutamic acid-2-hydroxylethyl methacrylate) (PGH) and hydroxypropyl cellulose-acrylic acid (HPC-AA) [41], oligo (polyethylene glycol) fumarate and sodium methyl acrylate [42], poly (*N*-vinylcaprolactam) (PVCL), and poly(*N,N*-diethylacrylamide) (PDEAAM). Fig. 2 provides the list of monomers used for the synthesis of microgels [34]. Summary of dual responsive microgels is provided in Table 1. Although microgels share similar polymeric chemical properties with hydrogels, they differ in physical properties due to the difference in size of particles. For example, they exhibit lower viscosity, high surface area, fast thermal response and fast solution response as compared to hydrogels. Also, microgels can respond to external stimuli such as temperature, pH, pressure, electrochemical stimuli, light, and ionic strength to change their confirmation and swelling properties [34–36]. Initially, the major focus was on either pH or temperature responsive microgels. Dandsetan et al., successfully encapsulated doxorubicin into microgels prepared using oligo (polyethylene glycol) fumarate and sodium methyl acrylate. Microgels synthesized with varying concentrations of sodium methyl acrylate exhibited maximum loading efficiency of 96.6%. Release studies in phosphate buffered saline (PBS) revealed that at 37 °C, microgels exhibited slower release of doxorubicin at physiological pH compared to acidic pH (13.4% at pH 7.4 versus 27% at pH 5.0). In vitro antitumor activity in human chondroma cells had shown that cell death was up to 90% with doxorubicin-loaded microgels whereas microgels without doxorubicin were non-toxic. Authors concluded that microgels were sensitive to changes in pH and ionic strength with good in vitro drug release and anti-cancer activity [42].

The first report on the synthesis of pH and temperature dual responsive microgel particles was reported in 2007 [43]. Teng et al., prepared biocompatible glucosamine carrying dual responsive microgels for insulin delivery. Using radical polymerization technique, microgels were synthesized by incorporation of N-isopropylacrylamide (NIPAAm) and acrylic acid (AAc) co-polymerized with acrylamido-2-deoxyglucose (AADG). Results from the study showed that the microgels were responsive at different pH and temperatures with low cytotoxicity. In vitro insulin release was high at acidic pH (6.9) and elevated temperature (41 °C). Modification of the AADG concentrations in microgels altered the volume phase transition temperature (VPTT) of microgels and addition of glucosamine increased biocompatibility of microgels. Thus, changing concentration of co-polymers could alter the responsiveness of microgels to external stimuli [44]. Although these were preliminary studies reporting dual responsive microgels, transmittance studies [45] along with dynamic light scattering (DLS) measurements could have given strong confirmation of swelling behavior of gels. As glucosamine inherently exhibits antitumor activity, these microgels could be tailored by incorporating other anticancer agents to treat various tumors. Owing to the poor biocompatibility of PNIPAAm, investigators successfully synthesized insulin-loaded microgels sensitive to pH and temperature using more biocompatible PGH and HPC-AA polymers. Results have shown that the microgels were sensitive to changes in temperature as there was a decrease in size with increase in temperature irrespective of pH (Fig. 3). In vitro release studies showed that at acidic pH (1.2), insulin release was slow compared to release at pH 6.8 (Fig. 4) [41]. It would have been interesting if this study had also evaluated the pH-responsive release of insulin at body temperature. Nigro et al., characterized the local structure of dual responsive interpenetrated polymer network microgels at different pH and temperatures. These microgels were prepared via radical polymerization using NIPAAm, AAc and BIS. Results have shown that microgels exhibited structural changes with variations in pH and temperature. Increase in temperature resulted in a microgel with porous solid structure from a microgel with inhomogeneous interpenetrated polymer network [46]. Almeida et al., prepared curcumin loaded magnetic microgels using pectin maleate, NIPAAm, and iron oxide through inversion polymerization technique. The size of microgels ranged from 10.1 μm to 26.3 μm. Cumulative release studies were conducted in simulated intestinal fluid (pH = 6.8) and simulated gastric fluid (pH = 1.2) at different temperatures (25 °C and 37 °C) and with or without a magnetic field. Results showed that the encapsulation efficiency (%EE) was up to 60%. Also, slow and sustainable curcumin release was achieved under the influence of external magnetic field. At 25 °C and under a magnetic field, 90% sustained release was achieved in simulated intestinal fluid whereas, the release was limited to 10% in the simulated gastric fluid. At 37 °C, curcumin release was 80%–95% in simulated intestinal fluid and 6%–20% in the simulated gastric fluid. Loading of curcumin into microgels resulted in improved stability, bioavailability and solubility of curcumin [47] (Fig. 5). This study which could have been further strengthened in vivo pharmacokinetic studies. Zayed et al., synthesized silylated HPMC microgels at different temperatures and pH using emulsion templating process. Results demonstrated that the microgels were stable both physically and chemically and were capable of encapsulating hydrophilic as well as hydrophobic drugs [48].

2.1.3. Nanogels

Nanogels are three-dimensional hydrogel materials in the nanoscale size range formed by crosslinked swellable polymer networks with a high capacity to hold water and without actually dissolving into the aqueous medium [52]. They are prepared from polymers linked via physical/chemical network and are used for targeted drug delivery

Table 1
Summary of pH and temperature responsive microgels.

Polymer used	Active ingredients	Main In vitro/in vivo Characterizations	Major Findings	Reference
Pectin, Poly(vinyl alcohol) (PVA), NIPAAm and iron oxide	Curcumin	• Characterization: Fourier transform infrared spectroscopy (FTIR), SEM, Flame atomic absorption spectroscopy • Release studies • Cytotoxicity assay in Caco2 colon cancer cells and healthy VERO cells	• Size of the microgels ranged from 10 μm to 20 μm • %EE was 60% • Curcumin release from microgel was dependent on pH, temperature and magnetic field o At 25 °C and under magnetic field, sustained release of about 90% was achieved in simulated intestinal fluid whereas, release was limited to 10% in simulated gastric fluid o At 37 °C, magnetic field did not have any impact on the release profile; curcumin release was 80%–95% in simulated intestinal fluid and 6%–20% in simulated gastric fluid • Microgels without curcumin showed high cytotoxicity against Caco2 cells	[47]
Hydroxypropyl cellulose (HPC), AAc, Hydroxyethyl methacrylate and γ-benzyl-L-glutamate	Insulin	• Characterization: FTIR, DLS, SEM • Phase transition measurements • Insulin loading and release studies	• Increase in pH increased the swelling properties of microgels • Microgels synthesized exhibited pH and temperature responsiveness o Irrespective of pH value, size of the microgels decreased with increase in temperature o At constant temperature, microgels exhibited smaller particle size at acidic pH • Insulin release from microgels was slow at low pH (1.2) compared to high pH (6.8)	[41]
N-vinylcaprolactam (VCL), acetoacetoxyethyl methacrylate (AAEM), vinylimidazole (VIm), BIS, Ferric chloride ($FeCl_3$), and Ferrous chloride ($FeCl_2$)	Not applicable	• Characterization: DLS, SEM, transmission electron microscopy (TEM) and x-ray diffraction (XRD)	• VPTT of microgels was 28 °C • Iron salts (magnetite) loaded microgels exhibited both pH and temperature responsiveness o Decrease in pH from 7 to 4 shifted the VPTT to a higher values • Microgels accommodated up to 15 wt% magnetite without any impact on stability	[49]
NIPAAm, BIS and 2-Vinyl pyridine	Sodium dodecylbenzene sulfonate	• Characterization: DLS, SEM • Uptake and release studies	• Size of the microgels increased with decrease in pH below 4.5 • At any temperature above VPTT of NIPAAm (32 °C), swelling ratio of the microgels is high at pH 3 compared to pH 7	[50]
NIPAAm, Allyl acetic acid (AAA) and BIS	Not applicable	• Characterization: DLS, TEM and zeta potential measurements	• pH and temperature responsive microgels were synthesized with various concentrations of allyl acetic acid (0%–15%). Size of the microgels ranged from 462 nm to 1018 nm. o Increase in the size of microgels was observed with increase in concentration of allyl acetic acid • Microgels with varying concentrations of allyl acetic acid showed pH and temperature responsiveness: the phase transition temperatures varied about 15 °C	[39]
NIPAAm, AAc, BIS	Not applicable	• Characterization: DLS, TEM, atomic force microscopy and FTIR • Measurement of LCST and critical aggregation concentration	• Microgels exhibited pH and temperature responsive behavior with varying concentrations of AAc at pH 4.0, 5.5 and 6.0	[43]
NIPAAm and BIS	Not applicable	• Characterization: DLS, thermogravimetric analysis (TGA) and small angle neutron scattering	• Microgels with interpenetrated polymer network were successfully synthesized and exhibited pH and temperature responsive behavior • Changes in the pH and temperature resulted in structural changes of the microgels	[46]

(*continued on next page*)

Table 1 (*continued*)

Polymer used	Active ingredients	Main In vitro/in vivo Characterizations	Major Findings	Reference
NIPAAm, AAc, 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and 1-hydroxybenzotrizole (HOBT)	Insulin	• Characterization: DLS, SEM, FTIR • Measurement of VPTT • Insulin loading and release studies • Cytotoxicity studies	• Microgels synthesized were spherical with a diameter of 50–60 nm • At higher temperature and pH values, microgels exhibited increase in the size • Incorporation of AAc and acrylamido-2-deoxyglucose increased the VPTTs of the microgels • Insulin release from the microgels was rapid at high temperatures and acidic pH • Incorporation of glucosamine increased the biocompatibility of microgels	[44]
HPMC and 3-glycidoxy-propyltrimethoxysilane	Nile red	• Interfacial tension and rheological properties • Epi-fluorescence microscopy and confocal microscopy • Determination of chemical and physical stability	• Silylated HPMC microgels were successfully synthesized and were capable of encapsulating both hydrophilic and hydrophobic drugs	[48]
VCL, 10-undecenoic acid and BIS	Doxorubicin	• Characterization: DLS, determination of LCST, SEM • In vitro drug loading and release studies • Cytotoxicity studies	• VCL based microgels exhibited pH and temperature responsive behavior • Maximum %EE of the microgels was 45.8% • Faster release of doxorubicin was observed at low pH (pH = 6.0) compared to physiological pH (pH = 7.4) • Doxorubicin release from microgels was also temperature dependent with faster release at 37 °C compared to 25 °C • Cytotoxicity studies in 3T3 murine fibroblast cells revealed that the doxorubicin loaded microgels were nontoxic with high cell viability	[51]

[53,54]. Active drugs are entrapped into nanogels by hydrogen/salt bonds and hydrophobic interactions. Nanogels are ideal for targeted drug delivery as they offer high drug loading capacity (%LC), high stability, and sensitivity to environmental factors such as ionic strength, pH, and temperature [55]. Various methods have been explored for preparing nanogels which include self-assembly of polymers via physical bonds, polymerization of monomers, cross-linking of polymers and nanofabrication. Nanogels are capable of carrying large-sized biomolecules to small molecules [56–58]. Summary of dual responsive nanogels is provided in Table 2. Wu et al., were successful in encapsulating temozolomide in hybrid nanogels prepared using CS and poly (methyl acrylic acid). These nanogels exhibited pH-responsive properties with controlled release behavior. Drug %LC and loading efficiency of nanogels was 48.9% and 46.4%, respectively. Release profiles at pH 7.38, 6.67, 6.17 and 5.03 had shown that a rapid release was observed at higher pH compared to lower pH values. Compared to blank nanogels, temozolomide loaded nanogels displayed high cytotoxicity (IC_{50}: 52.65 μg/mL for temozolomide loaded nanogels vs. 672.30 μg/mL for blank nanogels) [59]. Karnoosh-Yamchi and colleagues prepared insulin-loaded pH-responsive nanogels using NIPAAm-methacrylic acid (MAA)-HEM polymers via radical polymerization technique and were tested at two different pHs i.e., 1.2 and 6.8. At pH of 1.2, nanogels were able to stay afloat in 100 mL of PBS, whereas at pH of 6.8, nanogels were able to stay afloat in 100 mL of HCl. Samples analyzed from the nanogels in both PBS, and HCl solutions showed that insulin release from nanogels was high in pH 6.8, and low in acidic environments. Thus, pH responsive insulin loaded nanogels can be considered as a potential candidate for oral insulin therapy [60]. Interpenetrating pH-responsive polymeric nanogels were prepared using biocompatible gelatin macromolecules and poly(acrylamidoglycolic) acid using free radical emulsion polymerization technique. These nanogels were further loaded with curcumin to evaluate anticancer activity. The aqueous solubility of curcumin loaded pH-sensitive nanogels was higher compared to free curcumin resulting in enhanced bioavailability and superior anticancer activity. The %EE of the curcumin-loaded nanogels ranged from 42% to 48%. Cytotoxicity studies performed in human dermal fibroblast cells have shown that nanogels were highly biocompatible with cell viability ranging from 97% to 100%. In addition, curcumin loaded nanogels showed superior anticancer activity against colorectal cancer cell line compared to free curcumin. This study has concluded that curcumin loaded interpenetrating polymer network nanogels may be used for colorectal cancer treatment [61].

Xiong et al., prepared pH and temperature sensitive poly (NIPAAm-co-acrylic acid) nanogels loaded with doxorubicin. Nanogels were spherical (380 nm) at 20° C and when the temperature was increased to 37 °C, nanogels collapsed to irregular shapes with a diameter of 60 nm (Fig. 6). Change in the shape of nanogels was due to transition from hydrophilicity to hydrophobicity. Transition to hydrophobicity at higher temperatures was due to the weakening of hydrogen bonds between water and polymer system. Moreover, nanogels also appeared to be pH sensitive; as the LCST increased to 50 °C, 43 °C, and 41 °C with an increase in pH (7.4, 6.8 and 5.3 respectively). Rapid release of doxorubicin was observed at low pH compared to high pH (70% at 150 h for pH 5.3 versus < 20% at 150 h for pH 6.8 and 7.4). Blank nanogels were non-toxic as cell viability was more than 90%. Anticancer activity of doxorubicin-loaded nanogels was slightly higher compared to free doxorubicin without any impact of changes in temperature or pH [45]. Although this study proved the dual responsiveness of nanogels at different pH and temperatures, it lacked in evaluating the effect of higher temperatures (> 37 °C, usually observed during infections) on the release of doxorubicin. Peng and colleagues prepared pH and temperature sensitive nanogels (130 nm–250 nm) loaded with cisplatin using NIPAAm and methylether methacrylate via emulsion polymerization. Nanogels displayed slow release of cisplatin at 37 °C versus 25 °C (room temperature). Furthermore, cisplatin release from the

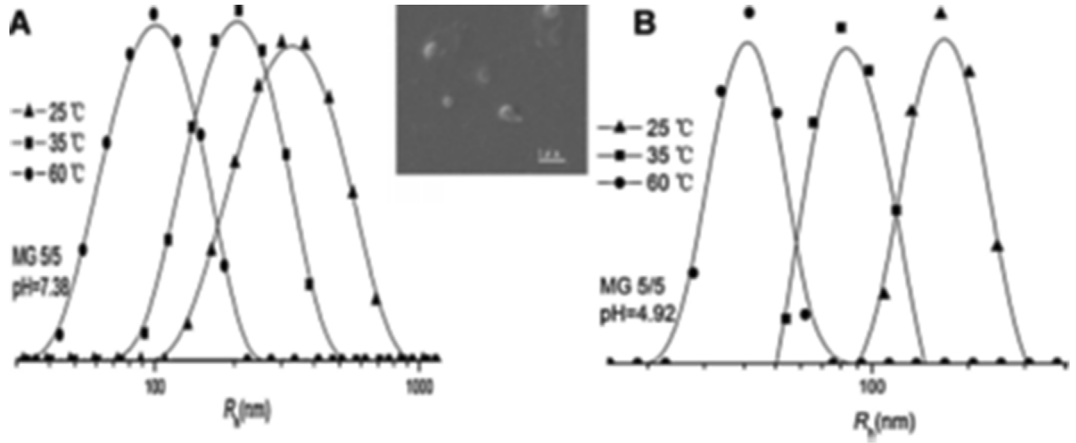

Fig. 3. Hydrodynamic radius distributions for MG5/5 in aqueous solution of pH 7.38 and pH 4.92 at 25, 35 and 60 °C, respectively. Scanning electron microscopy (SEM) image of MG5/5 is in the inset. Reproduced with permission from Ref. [41].

nanogels was 50%, 65% and 80% at pH 7.38, 6.0 and 5.0, respectively. Cytotoxicity of cisplatin-loaded nanogels was low compared to free cisplatin against MCF-7 and Hela cells due to controlled release with nanogels. Whereas, in A549 cells, cisplatin loaded nanogels were highly toxic compared to free cisplatin. In vivo, pharmacokinetic studies in mice showed longer circulation time with nanogels compared to free doxorubicin. The peak plasma concentration was 26.10 ± 10.98 mg/mL and 41.07 ± 12.20 mg/mL, with free cisplatin and cisplatin-loaded nanogels respectively. The area under the curve ($AUC_{0\sim\alpha}$) was 44.23 ± 18.67 mgh/mL and 121.31 ± 32.33 mgh/mL for free cisplatin and cisplatin-loaded nanogels, respectively. The plasma concentration profiles are shown in Fig. 7. In vivo anticancer activity in mice was also better with nanogels compared to free cisplatin with reduced adverse effects associated with cisplatin therapy [62]. Results suggest that doxorubicin loaded nanogels could be a potential drug delivery system in the treatment of cancer in vivo. It would be great to understand the purpose of carrying drug release study at room temperature and its clinical significance. Preparation of smart nanogels has also been reported with other polymers such as VCL, acrylamidoglycolic acid (AGA) [63] and poly(vinylcaprolactam-co-2-dimethylaminoethyl methacrylate) [P(VCL-co-DMAEMA)] [64]. Nanogel developed demonstrated responsiveness to changes in pH and temperature for drug release.

Gui et al., successfully synthesized fluorescent dual sensitive (pH and temperature) biocompatible nanogels with mesoporous silica nanoparticles. These doxorubicin loaded hybrid nanospheres (150 nm at 25 °C) contained PNIPAAm and CS shell (nanogel) embedded with the mesoporous silica nanoparticles. In vitro characterization revealed that the hybrid nanospheres were pH and temperature sensitive with an LCST of ~33 °C. Maximum %LC and loading efficiency of doxorubicin into gels was 45.2% and 61.1%, respectively. In vitro release studies at different pH's and temperatures showed that release of doxorubicin was higher at temperatures above LCST (> 42 °C) and acidic pH (5.0) compared to physiological temperature (37 °C) and pH (7.4). Moreover, the blank nanospheres were endocytosed into HepG2 cells and showed cell viability of 83% indicating low toxicity and high biocompatibility. Doxorubicin-loaded nanospheres showed a cell viability of 21.9% indicating high anticancer activity [65]. These nanospheres could be an appropriate anticancer drug delivery system in future if in vitro results are translated to in vivo.

Proteins such as bovine serum albumin (BSA) [66] and insulin [67] were encapsulated into nanogels sensitive to pH and temperature. Leon et al., were successful in encapsulating nanogels with BSA. These nanogels were synthesized using PNIPAAm, cross-linked with dendritic polyglycerol (dPG) and semi-interpenetrated with either 2-(dimethylamino)ethyl methacrylate (DMAEMA) or 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) [66]. Whereas, Zhao et al., encapsulated insulin in nanogels prepared using hydroxypropyl methyl cellulose (HPMC) [67]. These nanogels were characterized for in vitro controlled release of proteins at different pH and temperatures and showed promising results as an efficient drug delivery system.

Very recently, Salep modified graphene oxide was used as a capping

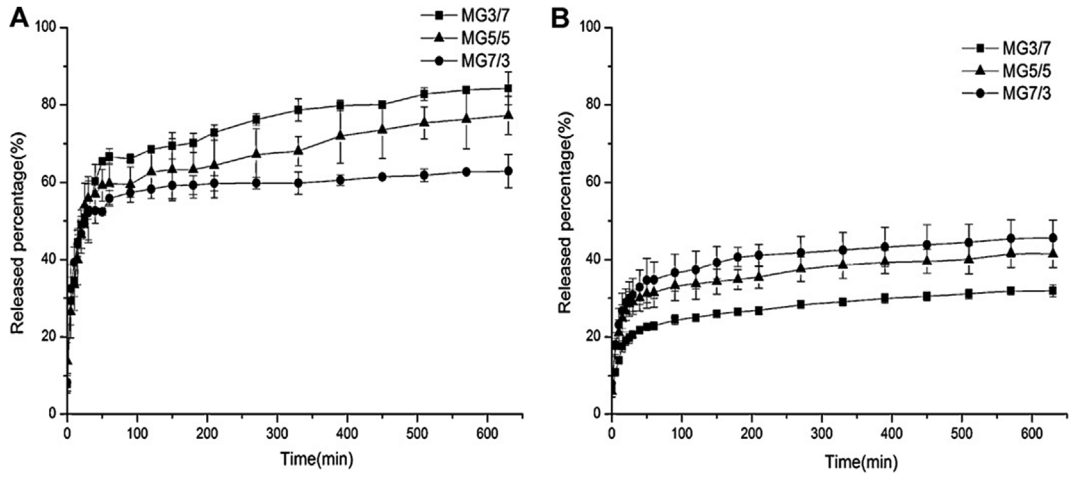

Fig. 4. Cumulative release of insulin from the microgels at pH 6.8 (A) and pH 1.2 (B) as a function of time at 37 °C. Reproduced with permission from Ref. [41].

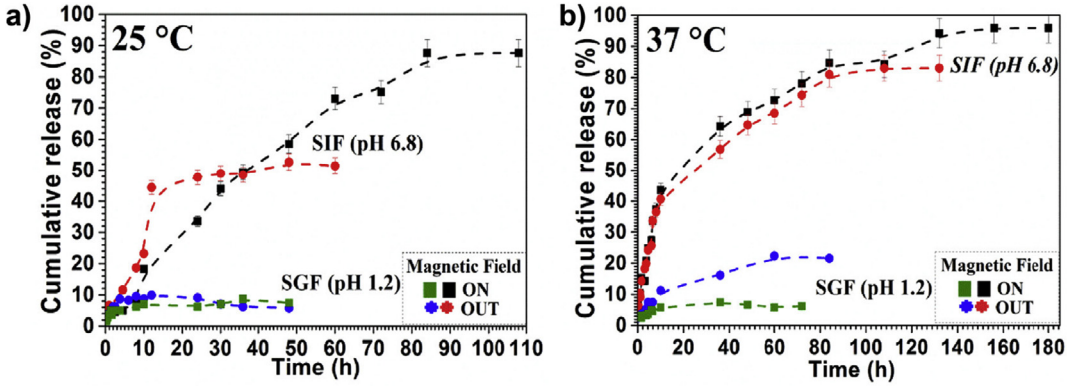

Fig. 5. Fraction of released curcumin (%) from the AP-MA/PNIPAAm(10)-Fe3O4(1) at simulated intestinal fluid and simulated gastric fluid under the influence of a magnetic field or absence of it: (a) at 25 and (b) at 37 °C. Reproduced with permission from Ref. [47].

agent in preparation of nanogels from PNIPAAm and AAc. Salep, a polysaccharide obtained from tubers was used as a reducing agent for modification of graphene oxide. Nanogels were spherical and uniformly distributed with an average size of 82 nm. In vitro, drug release studies showed that the nanogels exhibited sustained and faster release of doxorubicin in acidic conditions (pH = 5.0) and high temperature (42 °C) compared with physiologic conditions. Cytotoxicity studies in HeLa cells revealed that doxorubicin loaded nanogels showed superior cytotoxicity compared with free doxorubicin [68]. Zhou et al., synthesized a novel crosslinker containing three vinyl groups and co-polymerized with NIPAAm to prepare novel nanogel sensitive to pH and temperature. Prepared nanogels with different concentrations of the crosslinker have shown shrinking properties at low pH (1–7) and swelling properties at increasing temperatures (25 °C-37 °C) [69]. Although nanogels reported in this section exhibited dual responsiveness to pH and temperature, the majority of the studies were limited to in vitro characterization. It would be interesting to evaluate the efficacy and toxicity of these promising drug delivery systems in animal models.

2.2. Dendrimers

Dendrimers are three-dimensional synthetic amphiphilic macromolecules with various terminal functional groups (multivalency) and inner space to accommodate small molecules. Multivalency of dendrimers could be advantageous in attaching various therapeutic agents and diagnostic agents. In recent times, the main focus of research has been the development of smart dendrimers which are responsive to various stimuli such as temperature, pH, redox conditions, etc. Development of smart dendrimers can be helpful in effective drug delivery at the desired site of action with minimal side effects [71,72]. Scientists were successful in synthesizing various stimuli-responsive dendrimers which were responsive to temperature [73,74], light [72], redox/pH conditions [75–77]. In addition, protein sensitive dendrimers have also been reported [71].

Very few attempts have been made to synthesize pH and temperature dual responsive dendrimers. A summary of dual responsive dendrimers is provided in Table 3. Hui et al., synthesized Polyamidoamine (PAMAM) based pH and temperature dual responsive dendrimers using atom transfer radical polymerization. These dendrimers were loaded with chlorambucil, an anticancer drug. Results revealed that at low temperatures, the dendrimers demonstrated chain-like configuration, however, when the temperature was increased up to LCST, the dendrimers showed globular configuration. Temperature responsiveness of the dendrimers can be attributed to the hydrophobic interaction between *N, N*-dimethyl aminoethyl groups in PAMAM. Dendrimers also demonstrated pH responsiveness. Drug release studies have shown rapid release of chlorambucil at acidic pH (pH = 1.4) compared to basic pH (pH = 10) at 37° C [78]. Shen et al., synthesized doxorubicin loaded poly(β-aminoester) based dual responsive (pH and temperature) dendrimers using sequential sticking method. At room temperature and pH < 8, the dendrimers were readily dissolved in water; however, when the pH was raised to 8.3, the dendrimers precipitated. Also, LCST of the dendrimers increased with a decrease in the pH. Temperature and pH responsiveness could be attributed to change in hydrophilicity/hydrophobicity of polymer chains in dendrimers. Moreover, dendrimers without any chromophores showed concentration-dependent fluorescent behavior in response to ultraviolet (UV) light. Drug release studies have proved that doxorubicin release improved at low pH (95% release at pH = 4 at 36 h) relative to neutral pH (32% release at pH = 7.4 at 24 h). pH-responsive drug release could be attributed to protonation of interior tertiary amine groups in dendrimers, facilitating the drug release [79]. Synthesis of pH and temperature responsive dendrimers has also been reported using polymers such as poly(benzyl ether) and poly(2-isopropyl-2-oxazoline) (PiPrOx) [80] and poly-oligomeric silsesquioxane (POSS), PNIPAAm and poly(2-hydroxyethyl methacrylate) (PHEMA) [81]. In 2014, Liu and colleagues encapsulated vitamin E acetate in PAMAM based dendrimers synthesized via surface engineering. These dendrimers were both pH and temperature responsive with higher drug loading at higher pH compared to lower pH (22 mol/mol at pH 10 vs. 10 mol/mol at pH 5). Release studies demonstrated that the drug release was higher at acidic pH compared to physiologic pH [82].

In summary, scientists were successful in synthesis and characterization of pH and temperature dual responsive dendrimers using various polymers.

2.3. Membranes

Polymeric membranes are novel drug delivery systems similar to hydrogels. They can be incorporated into gel-based depot formulations and can be delivered via various routes such as intravenous or subcutaneous using core membrane nanoparticles [83,84], transdermal [85,86] and buccal [87,88]. In the recent times, the main focus has been on the development of stimuli-responsive membrane drug delivery systems sensitive to stimuli such as pH, temperature, ionic strengths, electric and magnetic fields, and chemicals [89]. Various polymers and co-polymers have been used for synthesizing stimuli-responsive membranes. Examples of polymers and co-polymers include PNIPAAm, poly [(2-(diethylamino)ethyl methacrylate] (PDEAEMA), PNIPAAm-vblock-poly(2-(*N,N*-dimethylamino) ethyl methacrylate) (PNIPAAm-b-PDMAEMA), poly(acrylic acid)-block-poly(NIPAAm) (PAAc-b-PNIPAAm), poly- (2-hydroxyethyl-methacrylate)-block-PNIPAAm (PHEMA-b-PNIPAAm), and PNIPAAm-block-poly(methacrylic acid) (PNIPAAm-b-PMAAc), polysulfone-graft-(poly(isopropylacrylamide-co-acrylic acid)-random-PMAAc)) [90–93]. Lee et al., synthesized *N*-isopropylacrylamide (NIPAAm) and AAc-based pH and temperature

Table 2
Summary of pH and temperature responsive nanogels.

Formulation	Polymer used	Preparation method	Active ingredients	Main In vitro/in vivo Characterizations	Major Findings	Reference
Nanohybrid hydrogels	PNIPAAm /carboxymethyl chitosan (CMCS)/multi walled carbon nanotubes	In situ free radical cross-linking polymerization	Doxorubicin	• Characterization and size measurement of hydrogels • Equilibrium swelling and swelling kinetics • Cytotoxicity assay • Drug loading and in vitro release	• Hydrogels exhibited three-dimensional network morphologies with neat porous structure • Hydrogels with critical pH of 6.8–7.4 and phase transition temperature of 35 °C–45 °C. • Successful entrapment of doxorubicin in the hydrogel with high anti-tumor activity.	[1]
Polymeric Nanogels	PNIPAAm and BIS	Free radical emulsion polymerization	Not applicable	• SEM, TEM and DLS	• Carboxyl rich allyl amides have been successfully used as crosslinkers in nanogels based on PNIPAAm • Size of the nanogels decreased with increase in pH from 1 to 7 at 37 °C • Size of the nanogels increased with increase in temperature from 25 °C to 37 °C at pH 1.	[69]
Nanogels	HPMC, MAA and poly(ethylene glycol) diacrylate (PEGDA)	Surfactant free polymerization	Insulin	• DLS, TEM • Insulin loading and entrapment efficiency • Insulin release studies	• Increase in concentration of crosslinker (PEGDA) up to 6% decreased the size and polydispersity index (PDI) of nanogels • Insulin release was 49% at intestinal pH (pH = 7.4) and 8% at gastric pH (pH = 1.2) • Increase in temperature increased the release of insulin from nanogels (32% at 32 °C and 48% at 37 °C) • Maximum drug loading and entrapment efficiency was 21.3% and 95.7%, respectively	[67]
Nanogels	NIPAAm and BIS	Not available	Doxorubicin	• TEM, photon correlation spectroscopy for size analysis • In vitro drug release • Flow cytometry • Cytotoxicity	• Increased in temperature resulted in transition from hydrophilicity to hydrophobicity in nanogels • Size of the nanogels decreased with increase in temperature • Increase in LCST was increased with increase in the pH (41 °C, 43 °C and 50 °C at pH 5.3, 6.8 and 7.4, respectively • Rapid release of doxorubicin was observed at low pH (5.3) compared to higher pH (7.4 and 6.8) (70% vs. < 20% at 150 h) • At incubation pH and temperature, cell viability in HepG2 cells was 80%–90% • Decrease in cell survival ratio was observed with increase in temperature and decrease in pH (79.8 ± 5.4% at pH 7.4 and 37 °C vs. 66.3 ± 1.4% at pH 6.8 under 43 °C)	[45]
CS based hybrid nanogels	CS and BIS	In-situ immobilization	Temozolomide			[59]

(*continued on next page*)

Table 2 (*continued*)

Formulation	Polymer used	Preparation method	Active ingredients	Main In vitro/in vivo Characterizations	Major Findings	Reference
				• Characterization using TEM, confocal microscopy and FTIR • Stability studies • Drug loading and in vitro release studies • In vitro cytotoxicity	• At both low and high pH, size of the nanogels increased. Minimum size was achieved at pH 5.0 to 5.5 • Nanogels exhibited swelling properties when temperature was increased from 22.1 °C to 37.2 °C • Nanogels were readily phagocytosed by mouse melanoma cells (B16F10) • Maximum drug %LC and loading efficiency of the nanogels was 48.9% and 46.4%, respectively • Sustained release of temozolomide was observed with hybrid nanogels at different pH values. Rapid release was observed at pH 7.38 • Compared to blank nanogels, temozolomide loaded nanogels displayed high cytotoxicity (IC50: 52.65 μg/mL for temozolomide loaded nanogels vs. 672.30 μg/mL for blank nanogels)	
Semi-interpenetrating cationic hydrogels	Monomers: (3-Acrylamidopropyl)-trimethylammonium chloride (APTMACl), acrylamide (AAm) and 2-hydroxyethyl methacrylate (HEMA) Crosslinker: BIS	Inverse emulsion and redox polymerization	Not applicable	• Characterization: SEM and TEM • Swelling properties • Gel electrophoresis	• Cationic gels with varied sizes were successfully synthesized • Swelling properties of the gels were more noticeable at pH values between 2 and 5	[70]
Nanogels	NIPAAm, poly(ethylene glycol) methyl ether methacrylate (mPEGMA), BIS and MAA	Emulsion polymerization	Cisplatin	• Characterization: SEM, TEM and DLS • Cisplatin loading and release studies • In vivo pharmacokinetics and anti-cancer activity • Cytotoxicity and cellular uptake	• Size of cisplatin loaded nanogels was 250 ± 5.8 nm at 25 °C and 214.6 ± 3.1 nm at 45 °C • Maximum cisplatin %LC and loading efficiency was 25% and 95%, respectively • Nanogels showed pH dependent cisplatin release with 50%, 65% and 80% at pH 7.38, 6.0 and 5.0, respectively after 168 h • Rapid release of cisplatin was observed at low pH and low temperatures (25 °C and pH = 5.0) compared to high pH and high temperatures (37 °C and pH = 7.38) • Cytotoxicity of cisplatin loaded nanogels was low compared to free cisplatin against MCF-7 and Hela cells due to controlled release with nanogels. Whereas, in A549 cells, cisplatin loaded nanogels were highly toxic compared to free cisplatin	[62]
Nanogels	VCL, AGA and BIS	Free radical emulsion polymerization	5-flouro uracil	• Characterization: DLS, differential scanning calorimetry and TEM • Drug loading and %EE • In vitro release studies	• Size of the nanogels ranged from 51 nm to 100 nm • Cumulative release of 5-flouro uracil was low at higher temperatures and pH (37 °C and pH 7.4) compared to lower temperatures and pH (25 °C and pH 1.2)	[63]
Nanogels immobilized on to honey comb porous surfaces	NIPAAm, 2-(dimethylamino) ethyl methacrylate (DMAEMA), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and poly(styrene)	Not reported	Proteins (rhodamine labelled BSA)	• Characterization: SEM, DLS • Encapsulation and release of BSA	• Size of nanogels ranged from 160 nm to 220 nm • Nanogels were successfully immobilized on to amino group containing honey comb porous films to form semi-interpenetrated networks • Protein release from the nanogels was temperature dependent with thermosensitive release at temperatures above cloud point	[66]
	PNIPAAm, CS and BIS (99%),		Doxorubicin			[65]

(*continued on next page*)

Table 2 (*continued*)

Formulation	Polymer used	Preparation method	Active ingredients	Main In vitro/in vivo Characterizations	Major Findings	Reference
Biocompatible fluorescent nanospheres coated with quantum dots-embedded mesoporous silica nanoparticles		Temperature regulated one-pot co-polymerization		• Characterization: DLS, TEM and confocal microscopy • Cellular uptake in HepG2 cells • Drug loading and release studies • In vitro cytotoxicity	• Size of nanospheres was 125 nm at 25 °C with PDI of 0.075 • Nanospheres with LCST of 33 °C exhibited swelling and shrinking properties at 25 °C and 42 °C, respectively • Decrease in the size of nanospheres was observed with increase in pH • Endocytosis of nanospheres by HepG2 cells indicated cellular uptake • %LC and loading efficiency of nanospheres was up to 45.2% and 61.1%, respectively • Rapid release of doxorubicin was achieved at low pH (5.0) and high temperatures (> 42 °C) • Doxorubicin loaded nanospheres showed cell viability up to 21.9% indicating high anticancer activity compared to blank nanospheres	
pH and temperature responsive smart nanogels	VCL and DMAEMA	Surfactant free emulsion polymerization	Rhodamine B	• Characterization: TEM, DLS • In vitro drug loading and drug release studies	• Size of the nanogels ranged from 81 nm to 368 nm • At 25 °C, size of the nanogels decreased with increase in the pH from 5 to 10 • Equilibrium swelling ratio of the nanogels showed a temperature dependent effect with notable decrease in size at 45–48 °C • %EE and drug %LC of the nanogels was 43% and 22%, respectively • Drug release from the nanogels was rapid at high temperatures (47 °C) and acidic pH • Nanogels also released rhodamine in response to ultrasound waves	[64]
Salep modified graphene oxide nanogels	NIPAAm, AAc, BIS and Salep	Not reported	Doxorubicin	• Characterization: SEM, TEM, DLS • In vitro doxorubicin release • In vitro cytotoxicity in human cervical cancer cells (HeLa)	• Average size of nanogels synthesized was 83 nm • Nanogels exhibited faster release of doxorubicin at acidic pH (5.0) and high temperatures (40 °C) compared to physiological pH (7.4) and low temperatures (40 °C) • Cytotoxicity studies in HeLa cells revealed that doxorubicin loaded nanogels showed superior cytotoxicity compared with free doxorubicin	[68]

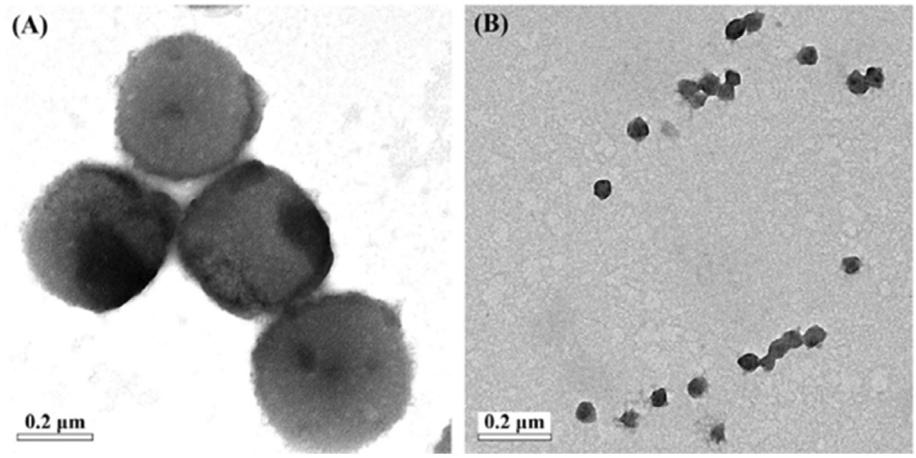

Fig. 6. Micro-morphology of doxorubicin-poly (NIPAAm-co-AAc) nanogels at (A) 20 °C and (B) 37 °C respectively. Reproduced with permission from Ref. [45].

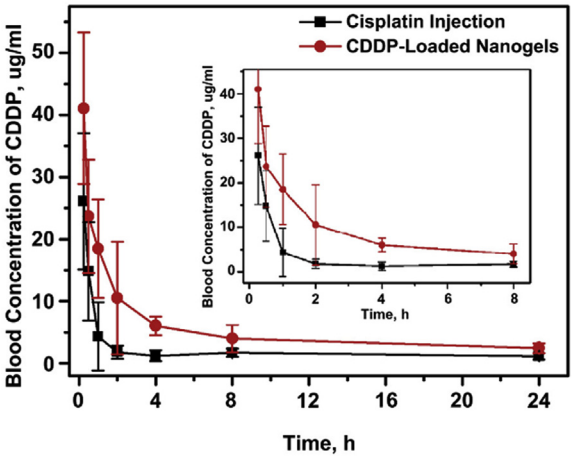

Fig. 7. Blood concentration time curve in mice plasma after intravenous administration of equal dose of 5 mg cisplatin/kg body weight of free Cisplatin injection and cisplatin-loaded nanogels. Inset: the magnification of blood concentration ranged from 0 to 8 h. Reproduced with permission from Ref. [62].

responsive membranes via plasma polymerization technique. Riboflavin was used as the model drug to evaluate the permeability and release properties of the membrane. Permeability studies have shown that decrease in pH and increase in temperature caused a rapid increase in the release of riboflavin from membranes. Permeability of riboflavin decreased with increase in pH (5.7×10^{-6} cm^3 cm cm^{-2} s^{-1} at pH 7 vs. 6.3×10^{-6} cm^3 cm cm^2 s^{-1} at pH 4). Authors concluded that the poly (amide-g-(AAc-NIPAAm)) based membranes could be a potential pH and temperature responsive drug delivery system for controlled drug delivery of riboflavin [94]. In 2012, Shi et al., were successful in using a one-step method to synthesize biocompatible/biomineralized alginate membranes with pH and temperature responsiveness. Schematic representation of hydrophobically modified alginate membranes is provided in Fig. 8. The membranes consisted of layers of polysaccharides (alginate and CS) with hydrophobic components and coupled with controlled precipitation of calcium phosphate. PNIPAAm and sodium palmitate constituted the hydrophobic component in the membrane. Indomethacin was used as a model drug to study pH and temperature responsiveness of hybrid composite membranes. The swelling study revealed that the swelling ratio was less at low temperatures (25 °C) and acidic pH (pH = 2.1) compared to high temperatures (37 °C) and neutral pH (pH = 7.4). These differences in the swelling ratios could be attributed to high water penetration at higher temperatures and ionization of carboxylic groups in alginate at low pH. Corresponding to the swelling ratios of membranes at different pH and temperatures, indomethacin release was high at higher temperatures and pH values (Fig. 9) [95]. Results suggest that at physiological pH, higher drug release was observed with higher temperatures up to 37 °C. However, the effect of higher physiological temperature (> 37 °C) which is normally observed during infection has not been studied.

Recently Kamoun et al., synthesized pH and temperature sensitive interpenetrating hydrogel membranes loaded with ampicillin via redox polymerization. HA and PNIPAAm contributed to pH and temperature responsiveness, respectively. Hydrogel membranes were stabilized by BIS and epichlorohydrin (EPI) as crosslinkers. Membranes were synthesized with varying concentrations of HA. Swelling studies revealed that increase in HA content improved the swelling ability of the gel, however, increase in swelling ratio was limited. Moreover, HA grafted on to PNIPAAm increased the LCST from 32 °C to 37 °C and increased swelling ability at alkaline pH (pH = 8). In vitro biocompatibility studies in Caco2 and MDCK, human cell lines revealed 100% cell viability with membranes without HA. However, increase in the HA content in membranes reduced the cell viability. In vitro ampicillin release studies demonstrated rapid initial release followed by sustained release for 6 h; proportional increase in the release was observed with increase in HA content of membranes (Fig. 10). Interestingly, ampicillin loaded membranes did not show any activity against bacteria and yeast [91].

Scientists were also successful in synthesizing pH and temperature dual responsive polymers without loading any therapeutic agent. Examples include cellulose membrane grafted simultaneously with PNIPAAm and PDEAEMA [92], biocompatible saline-soluble zein electrospun fibrous membranes [96], 3 dimensional membranes of NIPAAm and MAA polymerized within colloidal crystals [97], and composite membranes of poly(styrene)-block-poly(4-vinylpyridine) (PS-b-P4VP) and PNIPAAm [90].

2.4. Liposomes

Liposomes as carriers have been of growing research interests in the scope of modifying their properties to produce an enhanced delivery system. Liposomes exhibit potential as viable drug delivery vehicles as they provide a naturally occurring architectural design as a vesicle with biocompatible and biodegradable properties. Development of liposomal drug delivering systems with optimized properties linked to pH- and temperature-sensitivity could prove to be beneficial in that drug release would only be initiated under certain environmental conditions [98].

Ta et al., formulated polymer-modified thermosensitive liposomes (pTSL) that were developed through conjugation of pH- and temperature-sensitive polymers propylacrylic acid (PAA) and NIPAAm, respectively, via reversible addition-fragmentation chain transfer polymerization (RAFT) yielding: p(NIPAAm-co-PAA). NIPAAm

Table 3
Summary of pH and temperature dual responsive dendrimers.

Polymers used	Preparation Method	Active ingredient	Main In vitro/in vivo Characterizations	Main Findings	Reference
●PAMAM Core ● Poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMA) shell	Atom transfer radical polymerization (ATRP)	Chlorambucil	● Instrument analyses including H1 nuclear magnetic resonance spectroscopy (NMR), FTIR, UV spectroscopy and particle size analyses ● Transmittance measurement to determine LCST ● Chlorambucil loading and release studies	● Synthesized dendrimers showed dual responsiveness to pH and temperature ● Rapid release of chlorambucil was observed at pH 1.4 compared to pH 10 suggesting pH responsiveness of dendrimers	[78]
●Poly(β-aminoester) ● 2-methacryloyl-oxyethyl acrylate and ● Cysteamine	Sequential sticking method	Doxorubicin	● Determination of LCST and pKa ● pH dependent solubility ● Measurement of fluorescence and photoluminescence ● Doxorubicin loading and release ● Cell uptake by confocal microscopy	● Poly(β-aminoester) based dendrimers exhibited photoluminescence along with pH and temperature responsiveness ● LCST decreased wit increase in pH of the solution ● Rapid release was observed at acidic/lysosomal pH (pH 4–5) which facilitates intracellular release ● Poly(β-aminoester) based dendrimers could be ideal parenteral drug delivery system due to low toxicity and biodegradability	[79]
●Poly(benzyl ether) ● Poly(2-isopropyl-2-oxazoline) (PiPrOx)	Copper-mediated click chemistry	Not applicable	● Size and zeta potential using DLS ● Morphology using TEM ● Determination of LCST using spectrophotometer	● Dual responsive dendrimers showed broad variations in LCST with changes in pH ● Impact of temperature and pH was observed in size and morphology of dendrimers	[80]
●Polyoligomeric silsesquioxane (POSS) ● PNIPAAm ● PHEMA	ATRP	Not applicable	● Measurement of cloud point temperature ● Release profile of dendrimers loaded with DN1H and calcein blue	● Dendrimers synthesized showed both pH and temperature responsiveness ● Release studies demonstrated controlled release of dye with pH and temperature responsiveness	[81]

demonstrated a LCST ranging from 38 °C - 40 °C which proved to be appropriate for physiological conditions (~37 °C) and diseased states, which operate at elevated temperatures. PAA exhibited pH sensitive characteristics in the modified pTSL system tuned appropriately for tumorigenic environments at pH 4.9 - pH 5.5 (endosomal) and pH 6.5 - pH 7.5 (extracellular). With the conjugated pTSL system, it had been observed that LCST of NIPAAm constituent had lowered to ~28 °C at pH 5.0 which proved viable for controlled release within the endosomal region of the tumor. pTSLs were loaded with doxorubicin for %EE and release profiling studies. Doxorubicin's self-quenching fluorescent properties were utilized to investigate the %EE's and release profiles via spectrofluorometry. The pTSLs exhibited 89.5 ± 9.0 %EE which indicated appropriate levels of encapsulation capabilities similar to traditional thermosensitive liposomes (TSLs). Release profiles exhibited 40.8% of doxorubicin release at pH 5.0 at 37 °C (Fig. 11). These results indicated that the pTSL system did exhibit sensitivity to tumorigenic conditions and successful dual-response characteristics with conjugated liposome-copolymer were instilled [98]. While the data provided by Ta et al. is suggesting a promising mode of anticancer drug delivery, further investigation such as determining target cancers to be treated and mode of delivery to enhance bioavailability would have added more insights.

Similarly, Kaiden et al., reported the development of a dual-responsive copolymer conjugated to liposomal membranes. The copolymer was formulated using hyperbranched poly(glycidol) (HPG) with NIPAAm and succinylate (Suc) groups yielding NIPAAm-Suc-HPG via step-wise polymerization technique. NIPAAm-Suc-HPG exhibited volume shrinkage in weak acidic environments indicating that pH sensitivity existed. The reported LCST of NIPAAm (~38 °C - 42 °C), when conjugated to Suc-groups, had altered to physiological conditions, 37 °C. This determined that the pH- and temperature-sensitive properties could be tuned by certain NIPAAm/Suc molar ratios during polymerization. NIPAAm-Suc-HPG was conjugated onto egg yolk phosphatidylcholine liposomes (EYPC) loaded with pyranine, the model drug. Release profiles of pyranine were determined via spectrofluorometry. With a 56:33 ratio (NIPAAm/Suc) there was an enhanced release profile of pyranine from the EYPCs at neutral pH 7.0 and temperatures of 37 °C and 45 °C which confirmed that EYPC- NIPAAm-Suc-HPG was able to respond to varying temperature and neutral pH conditions. Incubation of liposomes with HeLa cells at 45 °C and neutral pH, the released pyranine fluoresced near cytosol and increased intensity with increase in temperature (> 35 °C). This indicated that the polymer chains anchored onto liposomal membrane destabilized surrounding lipids allowing a controlled release of pyranine when activated by designated conditions and deep pyranine penetration towards the cell cytosol [99]. This study lacks investigation on morphology. Providing morphological and characterization data of the delivery vehicle is equally important.

Liang et al., reported liposomes coated with various peptide chains to exhibit pH- and temperature-sensitivity. Liposomes consisted of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) conjugated with 1,2-dipalmitoyl-sn-glycero-3-phosphocholinglycerol (DPPG) and two key peptide chains, W2K3 and W2R3. Peptide chains were formulated and investigated for pH and temperature response when coated on the liposomes. Tryptophan (W) was used for its fluorescent properties and anchoring capabilities deep into liposomal membranes with high affinity for membrane-water interaction. Whereas, lysine (K) or arginine (R) exhibited positive charges that influenced pH sensitivity to initiate liposomal membrane disruption. Identical liposomal melting temperatures (~41 °C), when coated with either of the peptide chains, were observed and exhibited phase transitions. When induced to a weak acidic environment (pH 5.0) and melting temperature (41 °C), W2R3 showed enhanced calcein release (48.3%) which could be associated with deep R residues anchored into the membrane of liposome causing the membrane to be hydrophobic for the enhanced release of calcein within disease-like conditions [100].

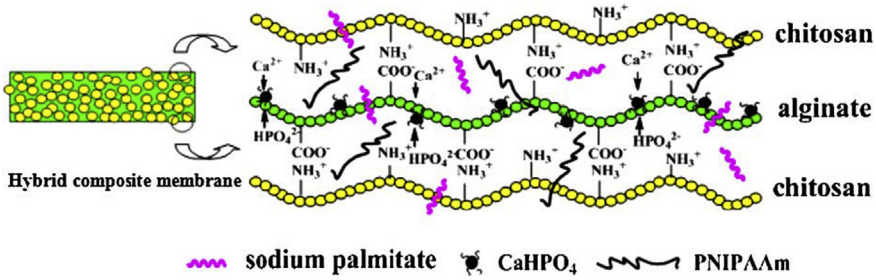

Fig. 8. Schematic illustration of hydrophobically modified alginate membrane. Reproduced with permission from Ref. [95].

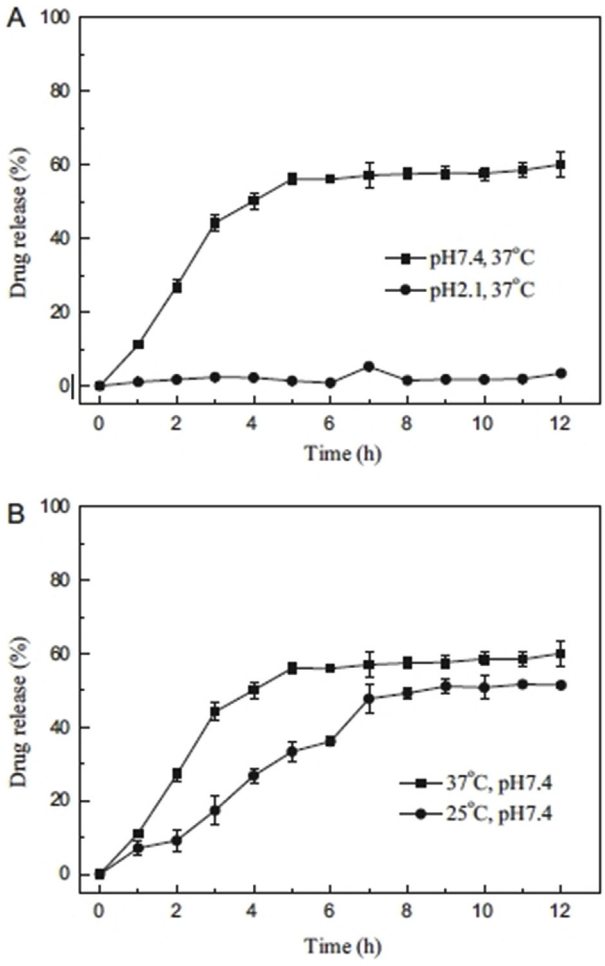

Fig. 9. pH-dependent release profiles of indomethacin at 37 °C measured at pH 2.1 and 7.4 (A) and temperature-dependent release profiles of indomethacin at pH 7.4 measured at 25 °C and 37 °C (B) from alginate/sodium palmitate/BP membranes. Reproduced with permission from Ref. [95].

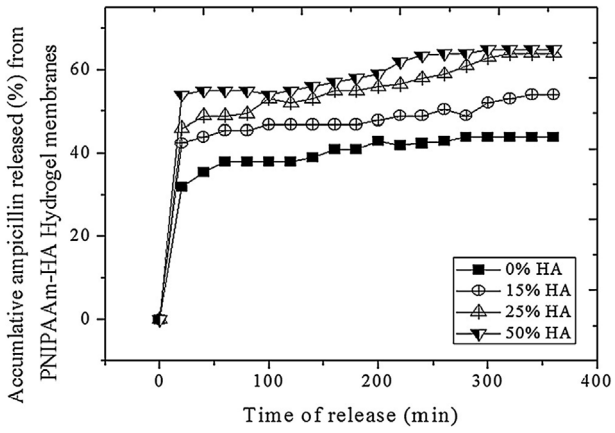

Fig. 10. In vitro cumulative release profile of ampicillin from PNIPAAm-HA hydrogel membranes as a function of different HA contents (the release profile was carried out in distilled water of pH ~ 7.5 at 37 °C). Reproduced with permission from Ref. [91].

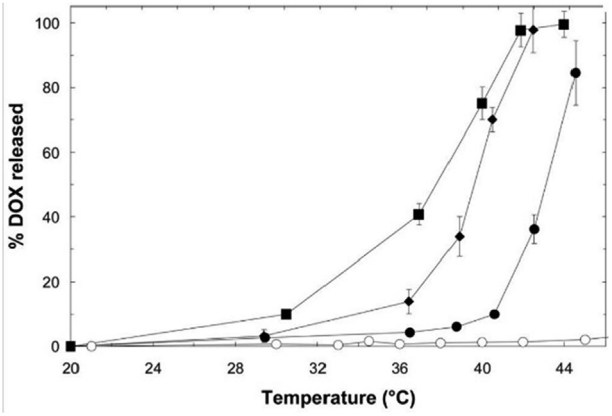

Fig. 11. Doxorubicin release in 20 mM HEPES as a function of temperature (5 min incubations), for NTSL (○, n = 4), traditional TSL (●, n = 4), pTSL at pH 7.5 (◆, n = 4), and pTSL at pH 5 (■, n = 4). Reproduced from Reproduced with permission from Ref. [98].

2.5. Microcapsules

A field with growing interest and research for possibilities for a drug delivery vehicle is the design and fabrication of microcapsules for the transport of compounds or drugs that exhibit poor water solubility [101]. With the designed microcapsule also being non-toxic, non-immunogenic, biocompatible, and biodegradable [102]. One such study by Yun et al., investigated the potential therapeutic effects of temperature responsive microcapsules loaded within a hydrogel that was pH responsive. The combined system (hydrogel + microcapsules) was called MCHs. The microcapsules were composed of copolymer PVA and PNIPAAm that was formulated via three-step interfacial emulsion polymerization technique. With SEM, it was observed that the microcapsules within the MCH at a swollen state were within a range of 10–50 μm with uniform distribution and weak interactions between each microcapsule. SEM results also reported that the microcapsules were spherical with a thin shell; the shell was the product of PVA-PNIPAAm polymerization. Yun et al., tested formulated microcapsules release profile, biocompatibility, and capability to enhance mechanical performance of the pH-sensitive hydrogel. Vitamin B12 (VB12) loaded PVA-PNIPAAm microcapsules were seeded into PVA-PAAc hydrogel creating MCH system. The release of VB12 from PVA-PNIPAAm microcapsules was determined at various pH and temperature conditions induced by the MCH system. It was observed that there was an

accelerated release of VB12 from PVA-PNIPAAm microcapsules at a temperature below LCST (32 °C) and higher pH (pH 10.0) due to swelling of PVA-PNIPAAm. Cell viability of mouse fibroblast cells revealed MCH biocompatibility as cell survival was ~85%. Yun et al., also observed that the PVA-PNIPAAm microcapsules seeded into MCH increased hydrogels compressive properties due to a higher intrinsic compressive modulus which allowed for higher rates of compression before fragmenting thus increasing MCHs mechanical strength [103]. While cell viability was high and release rates increased with higher pH and lower temperature; determining which treatment would these MCHs be most effective is crucial. Also, it would be interesting to see which active ingredients can be loaded into MCHs and respective release profiles, %EE, cell viability, cytotoxicity, bioavailability, site specificity.

2.6. Microspheres

Microspheres are attractive potential drug delivery vehicles as their size is in the range of particles that are commonly found in biological systems used to initiate various response processes and signaling pathways for cellular functions. Microspheres also exhibit capabilities to be tuned to specific environmental cues for site-specific and controlled drug release limiting exposure of payload to nearby healthy tissue. Particularly, pH- and temperature-responsive behavior has been an increasingly desired characteristic for controlled drug release to differentiate between normal healthy and diseased tissue for site-specific drug release [104]. Apart from the site-specific responsiveness to be attributed to the microspheres, they are also by material properties set to be biocompatible, biodegradable, and with an enhanced mechanical stability making the system highly desired for therapeutic applications [105].

Spizzirri et al., reported the development of dual-responsive copolymer microspheres that were synthesized with temperature- and pH-sensitive polymers that had been grafted into a single copolymeric system via reversible phase suspension polymerization. NIPAAm, HEMA, and MAA were copolymerized with *N-N′*-ethylenebisacrylamide (EBA) as the crosslinking agent yielding NIPAAm-co-HEMA or NIPAAm-co-MA. LCST for each conjugate was determined via FTIR spectroscopy analysis. It was observed that conjugates with higher EBA crosslinking ratios had an LCST of ~34 °C indicating phase transitions near physiological conditions. Using diclofenac diethylammonium salt (DDA) as the model drug, loading efficiencies (LE%) and release profiles of the microspheres under various induced conditions, temperature/pH were analyzed. High performance liquid chromatograpy analysis showed that the microspheres had a LE% > 90% with controlled gradual release profiles at 25 °C. At pH 7.0, DDA release was enhanced due to swelling of a pH-sensitive monomer of microspheres. Microspheres also exhibited enhanced DDA release under 45 °C conditions. Results confirmed that a stable structural configuration of copolymer microspheres for site-specific controlled release was achieved [105]. With the release profiles and LE% of the model drug at appropriate levels for the formulated microspheres, biological investigations concerning cell viability and cytotoxicity are required to determine that biocompatibility rates are appropriate for use to a host and ensure that diseased cells could be eliminated.

Liu et al., reported the development of copolymeric hollow microspheres with dual-responsive capabilities by conjugating folic acid (FA) to PNIPAAm and poly (methacrylic acid-co-ethyleneglycol dimethacrylate) (P(MAA-co-EGDMA)) with a silicone (SiO2) intermediate layer via two-stage facile distillation precipitation polymerization technique yielding: FA-P(MAA-co-EGDMA)-SiO2- PNIPAAm (Fig. 12). TEM analysis confirmed the spherical structure of the conjugate. Microspheres were loaded with doxorubicin, and it was observed that loading capabilities were enhanced with increasing pH from pH 5.0 to pH 10.0. The loaded doxorubicin content increased from 12.47 wt% (0.215 mmol g^{-1}) to 28.0 wt% (0.484 mmol g^{-1}). At 25 °C and pH 7.4, it was observed that FA-P(MAA-co-EGDMA)-SiO2- PNIPAAm exhibited 14.5% doxorubicin release within 8 h and 37.6% release within 54 h. When induced to pH 5.0, 60.3% doxorubicin was released from the system (Fig. 13). Slow controlled release at 25 °C was caused by a swelling state of PNIPAAm which restricted doxorubicin escape from the core of microsphere. Cell viability analysis indicated that 93.2%–100% cell viability of HepG2 cells, indicating enhanced biocompatibility [104]. This controlled release rate of doxorubicin and high rate of cell viability with the FA-P(MAA-co-EGDMA)-SiO2- PNIPAAm microspheres suggest that drug delivery vehicle shows promise as an effective anticancer drug delivery system.

2.7. Polymeric films

Polymeric films as a dosage form have been utilized as an alternative method for pediatric, geriatric and psychiatric patients with difficulty in swallowing conventional oral formulations such as capsules and tablets. These dosage forms are generally applied on body surfaces, where they release the payload at the site of action or into the systemic circulation via diffusion [106]. Polymeric films also exhibit potential for bypassing hepatic first-pass metabolism as the film readily dissolves and releases the payload at the desired site of action. Avoidance of first pass metabolism increases the bioavailability of the drug for enhanced effects [106]. Commercially available polymeric films include Listerine PocketPaks®, TheraFlu Thin Strips®, Sudafed PE Quick Dissolving Strips®, Gas-X Strips® and others. The presently available polymeric films are non-stimuli responsive and are intended for instantaneous effects, making them inefficient for sustained or controlled drug delivery. The immediate release is due to the low thickness of the film which mostly attributes to the rapid disintegrating property of buccal or sublingually administered films [107]. These limitations with currently available non-stimuli responsive films have, therefore, in recent years, brought focus to formulate stimuli responsive polymeric films to improve the drug efficacy. Specifically, pH- and temperature-sensitive polymeric films have been of interest and show various advantages over the currently available films with sustained and controlled drug release. One advantage associated with polymeric films is the possibility of multiple routes of drug administration such as oral, buccal, sublingual, ocular, and transdermal [106]. With the possibility of multiple routes of administration, the loaded drug can be delivered for systemic or local action. These films can be prepared using numerous techniques with a selection of various polymeric materials. Materials used in polymeric films must hold properties such as biocompatibility, biodegradability, flexibility, durability, toughness, adhesivity, and stress/strain parameters. Commonly used polymeric materials with pH and temperature sensitivity include HPMC, carboxymethyl cellulose (CMC), HPC, pullulan, pectin, CS, ethyl cellulose (EC), polyvinyl pyrrolidine (PVP), PVA, and gelatin [106,108]. Among these, pH-responsive polymers include CMC, HPC, and CS, while temperature responsive polymers include PVA, Pullulan, and Pectin [106]. Techniques used for synthesizing polymeric films include solvent casting, hot melt extrusion and most recently inkjet printing [106,108].

An example of a polymeric film with pH and temperature dual-responsiveness was reported by Montes et al. They synthesized a copolymeric film composed of VCL, and CS grafted via gamma (γ) radiation, yielding CS-γ-VCL copolymer with pH- and thermo-sensitivity. Differential scanning calorimetry thermograms indicated that CS-γ-VCL phase transitions at 125 °C, which may be due to the higher affinity bonds between the polymers, because of the radiation grafting technique. Thermal stability studies performed via TGA showed that CS-γ-VCL had an initial weight reduction associated with evaporation of water at the induced high temperature (125 °C) and followed with two-stages of decomposition at 235 °C and 393 °C. This decomposition was associated with the polymeric properties CS and PVCL, respectively. CS-γ-VCL at higher temperatures exhibited inherently increased thermal resistive properties due to its constituents grafted via γ-radiation. The

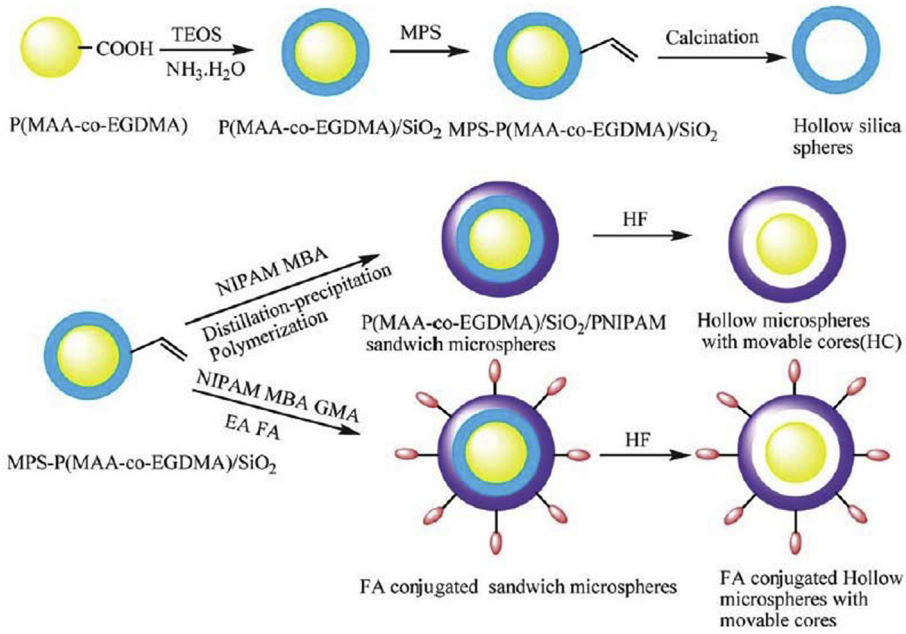

Fig. 12. Schematic illustration of the fabrication process of the hollow silica microsphere, the hollow microspheres with movable cores (HC) and FA-conjugated targeting temperature-sensitive hollow microspheres with movable pH-responsive cores. Reproduced with permission from Ref. [104].

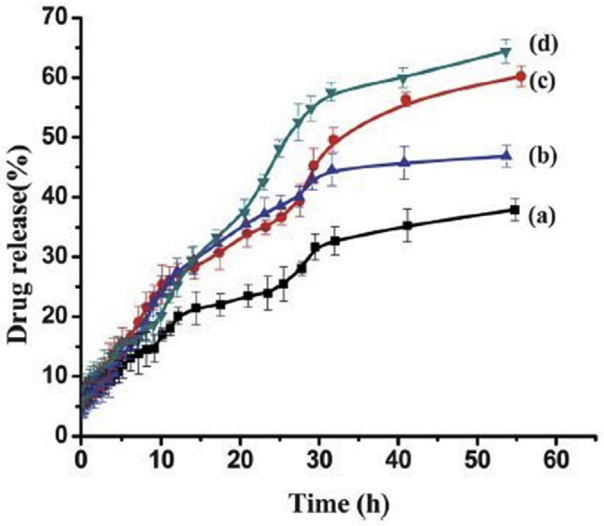

Fig. 13. In vitro drug release from the doxorubicin-loaded folic acid-conjugated temperature-sensitive hollow microspheres with movable pH-responsive cores at (a) pH 7.4 at 25 °C (b) pH 7.4 at 37 °C (c) pH 5.0 at 25 °C and (d) pH 5.0 at 37 °C, respectively. Reproduced with permission from Ref. [104].

swelling behavior of the CS-γ-VCL system was investigated, and an equilibrium swelling state was reached within 5 h. Increased hydrophilicity with the copolymer system was observed leading to larger maximum swelling rates. CS-γ-VCL exhibited higher swelling percentages in an acidic environment which are expected as the CS constituent is pH sensitive with a critical pH value of 3.75. However, when CS is grafted with VCL, the critical pH value shifted from 4.75 for CS-γ-VCL. This shift indicated protonation of CS's deacetylated amino groups, limiting the materials ability to uptake water. Thus, the swelling behaviors of CS-γ-VCL were suitable for controlled and efficient drug release. CS-γ-VCL proved to be a viable template for drug delivery systems with scopes of pH and temperature dual-responsive characteristics [109]. However, this study requires further evaluations such as in vitro release, film and drug encapsulation stability, %EE, and in vitro/vivo biological studies to establish that dual-responsive polymeric films could improve drug delivery and efficacy.

2.8. Polymeric hollow spheres (PHSs)

PHSs have been used for decades as a viable drug delivery vehicles due to their micro- and nano-encapsulation methods, well-defined morphology, uniform size, low density and large surface area [110,111]. Interestingly hollow spheres and microcapsules are interchangeably used terms, as both systems are formulated with the same techniques and materials. Both have a result of a drug delivery vehicle with thick walls and a hollow core. Some of the formulation techniques used to produce PHSs include liquid droplet method, dried-gel droplet method, self-assembly method, microencapsulation method, emulsion polymerization method, and template method [112]. PHSs are inherently attributed with sustained and controlled release patterns but limited to a single response characteristic as these systems are normally formulated with a single polymer [111]. The use of a single polymer classifies the current PHSs as single-layered systems. Although they prove to be effective drug delivery systems, improvement can be made for the enhancement of drug delivery. One such approach is to formulate double layered PHSs. Double-layered PHSs offer a possible solution to the limitations with current single polymer PHSs. Double-layered PHSs can further increase the encapsulation and release capabilities that are currently lacked with other forms of hollow spheres. Structurally, double-layered PHSs differ from single-layered micro- and nano-capsules in that dual layers rather than a single layer existing with double layered PHSs. The double layers of the PHSs are arranged as an inner sphere acting as the innermost layer which is then encapsulated within another sphere acting as the most outermost layer of the double-layered PHS. This type of drug delivery system is prepared using a two-step formulation process, typically with a silica-template polymerization technique or other similar techniques. The first step is the formulation of the innermost PHS using any one of the previously mentioned techniques. The second step includes the encapsulation of the first PHS within a second PHS of larger size utilizing a template type technique. Engulfment of the first PHS creates a double-layered PHS system with inner/outer layers. Material selection for the PHSs to exhibit pH and temperature sensitivity with either the inner and outer

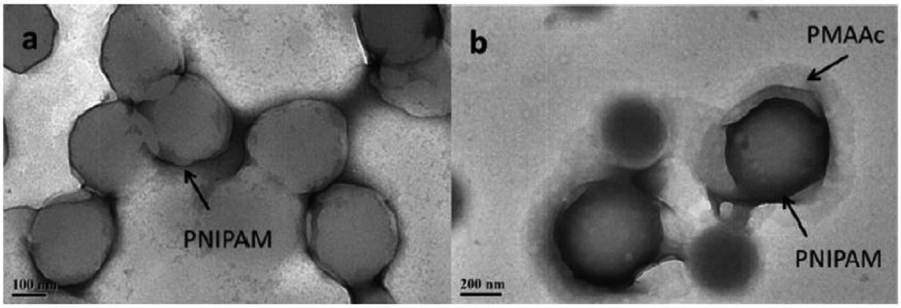

Fig. 14. TEM analysis of developed a) the single walled PNIPAAm PHS and b) double-walled PNNIPAM/PMAAc PHS. Reproduced with permission from Ref. [113].

layer creates a dual-responsive drug delivery system. A region between the inner surface of the external layer and the outer surface of the inner layer acts as a hollow space between the two polymeric layers. This space allows for the encapsulation of two drugs and potentially work with dual effects or stepwise drug release corresponding to pH- and temperature-sensitive materials. Double-layered PHSs offer enhanced site-specific payload delivery, biocompatibility, biodegradability and prolonged therapeutic effects. Current PHSs provide room for modification in which drug release may be triggered by not one but two cues for further enhancement of controlled release properties [113].

Cheng et al., reported dual pH and thermo-responsive double-walled PHSs prepared with PNIPAAm and poly (methacrylic acid) (PMAAc) respectively. TEM studies indicated that the developed PHSs were spherical and structurally double-walled (Fig. 14). The double-walled PHSs were structurally in the order of PNIPAAm as the innermost shell while PMAAc acted as the outermost shell. Confirmation that PMAAc successfully encapsulated PNIPAAm was observed via attenuated total reflection (ATR)-FTIR and ^{1}H NMR spectroscopy. DLS analysis showed that the entire PHSs size ratio increased from 156 nm to 369 nm when pH was increased from 4.1 to 9.0. The increased size also suggested that the drug loading capabilities enhance with respect to the increase in pH. Each PHS layer had been designed to operate from one another independently. Rhodamine B (RhB) was loaded into the core-shell (PNIPAAm) whereas BSA-fluorescein isothiocyanate conjugate (FITC-BSA) was loaded into the hollow space layer (PMAAc) of the double-walled PHS. %EE and %LC for RhB was 76.3 ± 5.8% and 29.2 ± 2.2%, respectively while for FITC-BSA these values were 9.3 ± 2.9% and 4.3 ± 1.3%, respectively. RhB exhibited efficient release from the PHS system when induced to 25 °C and pH 8.5 conditions with a burst release-like behavior. FITC-BSA was easily released from the shell in response to surrounding pH conditions and exhibited a pulse release-like behavior. It was found that both FITC-BSA and RhB release profiles were independent of one another. Independent release was based on the specific shell they were each respectively encapsulated. The release was due to each shell's response to the temperature and pH of the surrounding environment [113].

2.9. Protein nanoparticles

Protein nanoparticles are specialized drug delivery systems composed of biological materials exhibiting biocompatibility, biodegradability, and non-toxicity. These systems are metabolizable and can be manipulated with surface modifications [114]. Although polymeric nanoparticles have proven to be effective drug delivery systems, they possess disadvantages for applications with biological environments which include use of toxic solvents in formulation process, polymer degradation, drug leakage, and polymer cytotoxicity [115]. Interestingly, protein nanoparticles have an advantage in being effective in the targeted delivery of biologicals such as antibodies, vaccines, enzymes, hormones [116] or other forms of treatment such as gene therapy, chemotherapy, and viral vectors [117]. However, protein selection, for bioactivity is determined on the three-dimensional conformation of the protein itself [116]. Proteins can be selected from two sources; animal-based proteins such as gelatin, collagen, albumin, casein, silk protein and plant-based proteins such as zein, gliadin and soy protein [116]. Although protein nanoparticles are advantageous with certain treatments, they fall short in being effective drug delivery systems. Specifically, these systems cannot currently release the encapsulated payload with high specificity. These systems also tend to have single-responsive release conditions based on the type of protein used and drug encapsulated [114]. Denaturation of the protein also poses as a disadvantage for these systems, which can occur due to changes in environmental conditions such as pH, ionic strength, enzyme activity, denaturants, and temperature [114]. Using temperature and pH- sensitive proteins in the preparation of protein nanoparticles can improve the drug delivery efficacy by imparting pH and temperature responsiveness.

Matsumoto et al., reported the development of dual-responsive vault protein nanoparticles by conjugating poly(*N*-isopropylacrylamide-co-acrylic acid) (PNIPAAm-co-AAc) with human recombinant vaults (hMVP vaults) via RAFT polymerization yielding hMVP-PNIPAAm-co-AA. UV–visible turbidity studies revealed an increasing LCST with increasing pH (31.9 °C at pH 5.0, 44.0 °C at pH 6.0, ~60 °C at pH 7.0) for the conjugated system. DLS studies at pH 6.0 and pH 7.0 revealed at pH 7.0 no changes in size when induced with temperature changes 25 °C to 45 °C, 39.7 ± 13.7 nm and 40.4 ± 12.7 nm, respectively. However, when the conjugate was induced to pH 6.0 and induced temperature change, 25 °C to 45 °C, the conjugate increased in size and formed large aggregates, 41.3 ± 11.6 nm–~300 nm respectively. But the increase in size was found to be reversible when the system cooled back to equilibrium (25 °C, 39.3 ± 13.0 nm). Together the results from UV–visible turbidity and DLS both indicated that the conjugated system exhibited both pH and temperature sensitivity with hMVP-PNIPAAm-co-AA system [118]. They also confirmed the structural integrity of the conjugated system by observing architecture before and after heating the system above 45 °C and inducing pH conditions via electron microscopy (EM). At pH 6.0, it was observed that hMVP-PNIPAAm-co-AA remained structurally intact throughout the thermally triggered aggregation process and when induced to pH 6.5 the conjugate continued to remain intact with no damage to its structural composition. This indicated that the developed hMVP-PNIPAAm-co-AA system exhibited stable structural integrity to high temperature and weak acidic conditions suggesting that the system could be an ideal delivery system for hydrophobic molecules, chemotherapeutics or other forms of sensitive therapeutics. LCST values could be improved by altering the amount of AAc monomer units incorporated with the copolymer as PNIPAAM-co-AA LCST was reported at 31.9 °C at pH 5, 44.0 °C at pH 6 and above 60 °C at pH 7. These tunable properties suggest that the conjugated vault hMVP-PNIPAAm-co-AA system is ideal for application in treating diseased conditions [118]. Investigations to evaluate the structural integrity of the drug delivery system in response to diseased states is essential to confirm the efficacy of the hMVP-PNIPAAm-

co-AA protein nanoparticles. Also ensuring the biocompatibility, biodegradability, site specificity, bioavailability, cell viability and cytotoxicity rates are appropriate to advance with the above reported dual responsive drug delivery system.

3. Conclusion and future perspectives

Drug delivery systems implemented with pH and temperature sensitivity have been developed for enhanced site specificity and controlled drug release profiles. These systems also exhibited enhanced mechanical properties that were attributed with conjugated polymeric materials ensuring that payload delivery even when induced to high stress or strain conditions. Biocompatibility of developed drug delivery systems proved to be viable for biological systems exhibiting good levels of cell viability. Thus, these novel drug delivery systems with dual responsiveness capabilities have proved to be viable systems for enhanced drug delivery and candidates for further research. Scientists have been successful in synthesizing/formulating different drug delivery systems with sensitivity to both pH and temperature with promising in vitro results for high efficacy. However, very few studies have been conducted in animal models to confirm the results in vivo. For effective translation into clinical practice, these systems further need to be studied in depth using in vivo techniques. Moreover, the majority of the formulations were mainly limited to exploring loading of anticancer drugs such as doxorubicin and paclitaxel. Therefore, there is a need to explore the novel dual responsive systems with in vivo and in vitro studies that are not only limited to cancer but other diseases such as diabetes, infectious diseases, and autoimmune diseases etc., to ensure the efficacy of the drug delivery system with various disease treatments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jddst.2018.05.037.

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