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Stimuli-Responsive Drug Delivery Systems: pH, Temperature, Redox, and Enzyme-Triggered Platforms for Targeted Cancer Therapy

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Abstract:

Background: Conventional chemotherapy is fundamentally constrained by its inability to distinguish between malignant and healthy cells, resulting in dose-limiting systemic toxicity that prevents administration of the drug concentrations required for complete tumor eradication. Stimuli-responsive drug delivery systems represent a sophisticated pharmacological engineering approach in which the drug release trigger is embedded within the pathophysiological environment of the disease site itself — exploiting the aberrant pH, elevated temperature, altered redox status, and dysregulated enzymatic activity that characterize the tumor microenvironment — to achieve spatially and temporally controlled drug release at the intended therapeutic site with minimal off-target exposure.

Objective: This review critically examines the scientific basis, design principles, nanocarrier platforms, characterization methods, mechanisms of stimuli-triggered drug release, and therapeutic applications of stimuli-responsive drug delivery systems, with particular focus on cancer therapy applications and recent research advances up to 2022.

Results and Discussion: Four principal endogenous stimuli — pH, temperature, redox potential, and enzyme activity — have been extensively exploited in nanocarrier design, individually and in dual or multi-stimuli combinations. pH-responsive systems exploit the acidic tumor microenvironment (pH 6.5 to 6.8 extracellular, pH 4.5 to 5.5 endosomal) to trigger drug release selectively in cancer tissues. Redox-responsive systems leverage the 100- to 1000-fold higher intracellular glutathione concentration in cancer cells compared to plasma to achieve cytoplasm-specific drug release. Enzyme-responsive systems utilize matrix metalloproteinases, cathepsins, and other tumor-overexpressed proteases as activation triggers. Dual and multi-stimuli responsive systems provide enhanced selectivity by requiring the simultaneous presence of two or more disease-specific conditions to initiate drug release.

Conclusion: Stimuli-responsive drug delivery systems offer a conceptually compelling approach to improving the therapeutic index of anticancer drugs by aligning the drug release event with the pathophysiological characteristics of the tumor microenvironment. Translation from preclinical promise to clinical products requires resolution of manufacturing scalability challenges, in vivo trigger fidelity validation, and demonstrated superiority over conventional formulations in randomized clinical trials.

Keywords: Stimuli-responsive drug delivery; pH-sensitive nanoparticles; thermoresponsive systems; redox-responsive carriers; enzyme-triggered release; tumor microenvironment; smart drug delivery; cancer nanomedicine; dual-stimuli systems

1. Introduction

The pharmacological treatment of cancer has evolved substantially over the past century, from non-selective alkylating agents and antimetabolites toward molecularly targeted kinase inhibitors, immune checkpoint modulators, and antibody-drug conjugates. Yet despite these advances, the fundamental challenge of achieving therapeutic drug concentrations at the tumor site while minimizing systemic toxicity to normal tissues remains incompletely resolved. Conventional chemotherapy operates on the principle of maximum tolerated dose — administering the highest concentration of drug that the patient can endure without unacceptable toxicity — which reflects the inability of conventional formulations to discriminate between tumor cells and the healthy tissues through which the drug must pass to reach them [1,2].

The tumor microenvironment, far from being a passive backdrop to malignant cell growth, is an active and distinctive physicochemical milieu shaped by the metabolic demands, genetic aberrations, and stromal interactions of the tumor. Dysregulated glycolytic metabolism produces lactic acid that acidifies the extracellular environment to pH values of 6.5 to 6.8 — substantially lower than the pH 7.4 of normal tissue interstitium. Rapid and poorly organized neovascularization, combined with inadequate lymphatic drainage, creates regions of tumor hypoxia and elevated interstitial fluid pressure. Accelerated metabolic activity and oxidative stress elevate intracellular glutathione concentrations 100- to 1000-fold above plasma levels. Tumor-secreted matrix metalloproteinases, cathepsins, and hyaluronidases degrade the extracellular matrix to facilitate invasion and metastasis [3,4]. These biochemical hallmarks of the tumor microenvironment are exploited by stimuli-responsive drug delivery systems as precision release triggers, converting the tumor's own pathophysiology into the activation mechanism for drug release.

The concept of stimuli-responsive or smart drug delivery was first articulated in the context of thermoresponsive liposomes by Yatvin and colleagues in 1978, who demonstrated that liposomes formulated with lipid mixtures having a gel-to-liquid crystalline phase transition near physiological temperature released their contents selectively upon local hyperthermia. The subsequent decades witnessed the development of pH-responsive polymeric nanoparticles, redox-sensitive disulfide-crosslinked carriers, enzyme-cleavable prodrug nanoconjugates, light-responsive photoswitch systems, and ultrasound-triggered microbubble platforms, collectively creating a richly diverse toolkit of stimuli-responsive drug delivery approaches [5,6].

Beyond single-stimulus systems, the concept of dual and multi-stimuli responsiveness has gained substantial traction in recent years, reflecting the recognition that single-stimulus triggering can be confounded by the heterogeneity of tumors and the imperfect specificity of individual physicochemical cues. A pH-responsive carrier may release drug in any acidic compartment encountered during its biodistribution, while a redox-responsive carrier may be partially activated by the elevated oxidative stress of inflamed non-tumor tissues. Requiring the simultaneous presence of two tumor-specific cues — for example, low pH and elevated glutathione, or enzyme activity and hypoxia — exponentially increases the selectivity of the release event [7].

This review provides a systematic and critical examination of the stimuli-responsive drug delivery landscape, organized by stimulus type and covering the physicochemical design principles, nanocarrier fabrication strategies, characterization methodologies, release mechanisms, and therapeutic applications of pH-, temperature-, redox-, enzyme-, and multi-stimuli-responsive systems. A comparative analysis against conventional nanocarriers and a critical appraisal of the gap between preclinical performance and clinical translation are included to provide a balanced perspective on the current status and realistic future trajectory of this field.

2. Scientific Background

2.1 Tumor Microenvironment as the Pharmacological Trigger

The tumor microenvironment is defined by a characteristic set of physicochemical properties that differentiate it from normal tissue and provide the biochemical basis for stimuli-responsive drug delivery. Warburg metabolism — the preferential utilization of aerobic glycolysis over oxidative phosphorylation even in the presence of oxygen — generates large quantities of lactic acid that is exported from cancer cells through monocarboxylate transporters, acidifying the extracellular tumor microenvironment to pH values between 6.0 and 6.8, compared to the tightly regulated pH of 7.4 in normal tissue interstitium. This extracellular acidosis exists simultaneously with an elevated intracellular pH in cancer cells, creating a reversed pH gradient compared to normal cells [8,9].

The intracellular reducing environment of cancer cells is characterized by markedly elevated glutathione concentrations, typically 2 to 10 mM in the cytoplasm compared to 2 to 20 micromolar in plasma. This 100- to 1000-fold gradient across the plasma membrane provides a powerful and highly selective trigger for redox-responsive drug carriers incorporating disulfide bonds: these bonds are stable in the extracellular

oxidative environment but are cleaved rapidly by the abundant intracellular glutathione upon carrier internalization, releasing drug specifically in the cytoplasm of tumor cells [10].

Tumor-associated proteases, particularly the matrix metalloproteinase family (MMP-2, MMP-9, MMP-14) and cysteine proteases including cathepsins B, D, and L, are overexpressed in the tumor microenvironment relative to normal tissues where their activity is tightly regulated. These enzymes degrade extracellular matrix components including collagen, fibronectin, and laminin to facilitate tumor invasion and metastasis, and their elevated activity provides a spatially specific enzymatic trigger for carriers with MMP-cleavable or cathepsin-cleavable peptide linkers [11].

2.2 Design Principles for Stimuli-Responsive Systems

The engineering of stimuli-responsive drug delivery systems requires the incorporation of a molecular switch — a chemical moiety, structural feature, or material property that undergoes a defined physicochemical change in response to a specific environmental stimulus — into the nanocarrier architecture in such a way that this change causes or enables drug release. The switch may be a polymer that undergoes a conformational change (coil-to-globule transition for thermoresponsive polymers), a chemical bond that undergoes cleavage (disulfide bond reduction, hydrazone or acetal hydrolysis), a lipid membrane that undergoes a phase transition (gel to liquid-crystalline for thermoresponsive liposomes), or a molecular recognition event (enzyme-substrate interaction leading to carrier disassembly) [12,13].

Effective stimuli-responsive systems must satisfy several design criteria beyond simple stimulus responsiveness: the triggering stimulus must be sufficiently specific to the target tissue to avoid premature release at off-target sites; the magnitude of change in stimulus at the target site must be sufficient to drive a meaningful change in drug release rate; the drug release kinetics must be rapid enough to achieve therapeutic concentrations at the target site before the carrier is cleared; and the triggered release must be complete enough to deliver the full drug payload rather than a partial dose [14].

3. Classification of Stimuli-Responsive Drug Delivery Systems

3.1 pH-Responsive Systems

pH-responsive drug delivery systems are the most extensively investigated stimuli-responsive class, exploiting the acid-base properties of ionizable functional groups or acid-labile chemical bonds to trigger structural changes and drug release in response to pH variations. Three principal pH-responsive mechanisms have been developed: ionization-driven swelling or dissolution of polyelectrolyte carriers, acid-catalyzed hydrolysis of pH-labile covalent bonds, and protonation-driven membrane disruption of ionizable lipid carriers [15,16].

Polyelectrolyte carriers incorporating weakly acidic groups such as carboxylic acids or sulfonamides exhibit pH-dependent ionization: below the pKa of the ionizable group, the polymer carries minimal charge and adopts a compact, hydrophobic conformation that retains drug; above the pKa, ionization produces negative charges that cause electrostatic repulsion between polymer chains, swelling the carrier and releasing drug. Eudragit L100 and S100, methacrylic acid copolymers with pKa values of approximately 6.0 and 7.0 respectively, are the most clinically established pH-responsive polymers, used extensively in enteric coatings that resist dissolution at gastric pH but dissolve at intestinal pH [17].

Acid-labile chemical bonds including hydrazone, acetal, ketal, cis-aconitic amide, and orthoester linkages undergo hydrolysis at the acidic pH of late endosomes (pH 4.5 to 5.5) or the tumor extracellular environment (pH 6.0 to 6.8), releasing drug from polymer-drug conjugates or disrupting the amphiphilic architecture of self-assembled nanocarriers. Doxorubicin-hydrazone conjugates, which hydrolyze selectively at endosomal pH to release free doxorubicin in the cytoplasm following receptor-mediated internalization, are among the most extensively studied pH-responsive prodrug systems, with the approved formulation Mylotarg (gemtuzumab ozogamicin) utilizing an acid-labile calicheamicin-antibody conjugate for leukemia therapy [18].

3.2 Temperature-Responsive Systems

Temperature-responsive drug delivery systems exploit a thermal transition — most commonly the lower critical solution temperature (LCST) of thermoresponsive polymers or the gel-to-liquid crystalline phase transition of lipid bilayers — to trigger drug release at elevated temperatures associated with tumor hyperthermia, febrile inflammation, or locally applied heat. The most extensively studied thermoresponsive polymer is poly(N-isopropylacrylamide) (PNIPAM), which exhibits a sharp LCST at approximately 32°C in water: below this temperature the polymer is hydrophilic and swollen, while above it the polymer collapses

into a hydrophobic globular state that expels water and, in drug-loaded systems, releases encapsulated drug [19].

Thermosensitive liposomes formulated with lipid mixtures including dipalmitoylphosphatidylcholine (DPPC) and monopalmitoylphosphatidylcholine (MPPC) undergo a gel-to-liquid crystalline phase transition at temperatures of 39 to 42°C, coinciding with hyperthermic treatment temperatures achievable by focused ultrasound, radiofrequency ablation, or hot water bath hyperthermia. At the phase transition temperature, transient membrane pores form at domain boundaries between gel and liquid-crystalline lipid phases, enabling rapid drug release from the liposome interior. ThermoDox, a thermosensitive liposomal formulation of doxorubicin, reached phase 3 clinical trials for hepatocellular carcinoma in combination with radiofrequency ablation, the most clinically advanced thermosensitive drug delivery system to date [20,21].

3.3 Redox-Responsive Systems

Redox-responsive drug delivery systems incorporate disulfide bonds or other oxidoreductively labile chemical structures that undergo selective cleavage in the reducing intracellular environment of cancer cells. Disulfide bonds (–S–S–) are stable under the oxidative conditions of the extracellular space and systemic circulation but are rapidly cleaved by intracellular glutathione through thiol-disulfide exchange reactions. The resulting thiol groups cause carrier disassembly, polymer crosslink dissolution, or prodrug activation depending on the carrier architecture, releasing the drug payload specifically in the glutathione-rich cytoplasm of cancer cells [22,23].

Reactive oxygen species (ROS)-responsive systems provide an alternative redox trigger based on the elevated oxidative stress present in tumor microenvironments and inflamed tissues. Carriers incorporating boronic acid pinacol esters, arylboronic acids, ferrocene, selenium, or thioketal moieties undergo oxidation by hydrogen peroxide or other ROS, triggering carrier disassembly or increased membrane permeability. ROS-responsive systems have been particularly investigated for anti-inflammatory drug delivery, where activated macrophages generate superoxide and hydrogen peroxide that can trigger localized drug release in sites of inflammation [24].

3.4 Enzyme-Responsive Systems

Enzyme-responsive drug delivery systems incorporate peptide sequences, oligosaccharide moieties, or other enzyme-substrate structures into the nanocarrier architecture that are selectively cleaved or modified by tumor-associated enzymes, triggering drug release or carrier disassembly. Matrix metalloproteinases, overexpressed in the tumor extracellular matrix and on the surface of invasive tumor cells, are the most widely exploited enzymatic triggers. Peptide sequences including GPLGIAGQ, PVGLIG, and GFLG that are selectively cleaved between the leucine and glycine residues by MMP-2 and MMP-9 have been incorporated as linkers between nanocarrier surface coatings and drug molecules or between PEG stealth coatings and targeting ligands [25,26].

Cathepsin B, a lysosomal cysteine protease overexpressed in the lysosomes of cancer cells and secreted into the tumor microenvironment, cleaves the dipeptide sequence phenylalanine-lysine (Phe-Lys) and valine-citrulline (Val-Cit) that are incorporated into the linker chemistry of antibody-drug conjugates and enzyme-responsive nanocarriers. The clinically validated brentuximab vedotin antibody-drug conjugate uses a Val-Cit-PABC cathepsin B-cleavable linker to release monomethyl auristatin E intracellularly following receptor-mediated internalization of the antibody-antigen complex, providing direct clinical validation of enzyme-responsive linker chemistry in oncology [27].

3.5 Light-Responsive Systems

Light-responsive drug delivery systems incorporate photoactive molecular switches including azobenzene, spiropyran, diarylethene, o-nitrobenzyl, and coumarin derivatives that undergo reversible or irreversible photoisomerization or photocleavage upon irradiation with UV, visible, or near-infrared light, triggering drug release. Near-infrared light penetrates biological tissue to depths of several centimeters, making NIR-triggered systems the most clinically relevant for solid tumor therapy. Upconverting nanoparticles that convert NIR photons to UV or visible wavelengths capable of activating UV-responsive photoswitches have been developed to enable deep-tissue photoresponsive drug release [28].

3.6 Multi-Stimuli Responsive Systems

Dual and multi-stimuli responsive systems incorporate two or more independent responsive elements that must each be activated by their respective stimuli for maximal drug release, substantially improving the spatial selectivity of the drug release event. The most widely investigated dual-stimuli combinations include

pH and redox (exploiting simultaneously the acidic tumor microenvironment and elevated intracellular glutathione), pH and temperature (tumor acidity and local hyperthermia), and enzyme and pH (MMP cleavage of extracellular coating followed by endosomal acidification-triggered dissolution). The requirement for two tumor-specific conditions simultaneously creates a logical AND gate that dramatically reduces off-target release probability [29,30].

Table 1: Classification of stimuli-responsive drug delivery systems by trigger type, responsive material, release mechanism, and representative applications

Stimulus	Responsive Material	Trigger Mechanism	Release Mechanism	Applications
pH	Polyelectrolytes, hydrazone, acetal bonds	Protonation / acid-catalyzed hydrolysis	Swelling, bond cleavage, carrier dissolution	Tumor, endosomal, GI delivery
Temperature	PNIPAM, DPPC/MPPC liposomes	LCST polymer collapse / lipid phase transition	Pore formation, carrier disassembly	Hyperthermia-combined tumor therapy
Redox (GSH)	Disulfide crosslinked NPs, SS-PEG	Glutathione-mediated disulfide cleavage	Crosslink dissolution, polymer disassembly	Intracellular cancer drug delivery
ROS	Boronic ester, thioketal, ferrocene	H2O2-mediated oxidation	Hydrophobic-to-hydrophilic conversion	Cancer, inflammatory disease
Enzyme (MMP)	MMP-cleavable peptide linkers	Protease-mediated peptide hydrolysis	PEG shedding, drug-linker cleavage	Tumor extracellular matrix targeting
Light (NIR)	Azobenzene, UCNPs, photocages	Photoisomerization / photocleavage	Carrier disassembly, pore formation	Photodynamic therapy, solid tumors
Dual/multi-stimuli	Combined responsive elements	AND gate: two simultaneous triggers	Sequential or cooperative release	Enhanced tumor selectivity

PNIPAM: poly(N-isopropylacrylamide); DPPC: dipalmitoylphosphatidylcholine; MPPC: monopalmitoylphosphatidylcholine; GSH: glutathione; ROS: reactive oxygen species; MMP: matrix metalloproteinase; NIR: near-infrared; UCNPs: upconverting nanoparticles; LCST: lower critical solution temperature.

4. Formulation Design and Development

4.1 Polymer Design for pH-Responsive Systems

The design of pH-responsive polymeric nanocarriers requires selection of a polymer with ionizable groups whose pKa is matched to the pH of the intended release site. For tumor extracellular delivery, polymers with pKa between 6.0 and 7.0 release drug at tumor pH 6.5 while retaining drug at physiological pH 7.4. For endosomal delivery, polymers with pKa between 4.5 and 6.0 release drug in late endosomes. Poly(histidine) and poly(L-glutamic acid) provide sharp pH-dependent conformational transitions that can be tuned by copolymerization with poly(ethylene glycol), poly(lactic acid), or poly(caprolactone) to control the pH response range, nanoparticle stability, and drug loading capacity [31].

Acid-labile linker chemistry selection requires consideration of the hydrolysis rate at the intended pH, the stability of the linker at physiological pH, the nature of the released drug species (free drug, drug-alcohol conjugate, or modified drug depending on linker chemistry), and compatibility with the drug molecule's functional groups. Hydrazone bonds are formed by condensation of hydrazide groups with aldehyde or ketone groups on the drug molecule; they are stable at pH 7.4 but hydrolyze at half-lives of minutes to hours at pH 5.0, making them suitable for endosomal drug activation. Acetal and ketal bonds provide faster hydrolysis kinetics at endosomal pH and have been used for acid-responsive polymer crosslinks [32].

4.2 LCST Tuning for Thermoresponsive Systems

The LCST of thermoresponsive polymer systems must be carefully tuned to fall between physiological body temperature (37°C) and the temperature achievable by local hyperthermia (40 to 42°C), ensuring that the carrier remains stable in the systemic circulation at body temperature but releases drug when heated to hyperthermic temperatures at the tumor site. The LCST of PNIPAM can be tuned from 25 to 45°C by copolymerization with hydrophilic comonomers (acrylamide, acrylic acid) to increase LCST or hydrophobic comonomers (N-tert-butylacrylamide, N-isopropylmethacrylamide) to decrease LCST. Poly(N-vinylcaprolactam), with a natural LCST of 32 to 38°C depending on molecular weight and concentration, has emerged as a biocompatible alternative to PNIPAM for thermoresponsive drug delivery [33].

4.3 Disulfide Bond Architecture in Redox-Responsive Systems

The positioning and density of disulfide bonds within redox-responsive nanocarriers critically determine the selectivity and completeness of GSH-triggered drug release. Core-crosslinked polymeric micelles in which disulfide bonds form the crosslinks maintaining micellar core integrity disassemble completely upon GSH exposure, releasing the full drug payload rapidly. Shell-crosslinked nanoparticles with disulfide bonds in the outer polymer shell disassemble to release drug more slowly as the shell degrades layer by layer. Disulfide-containing PEG detachment strategies, in which the PEG stealth coating is attached to the nanoparticle surface through disulfide bonds that are cleaved by GSH to deshield the particle for enhanced cellular uptake, provide a mechanism for GSH-triggered active targeting without necessarily releasing drug immediately [34].

4.4 MMP-Cleavable Linker Design

The design of MMP-responsive nanocarriers requires selection of peptide substrate sequences with high specificity and adequate cleavage efficiency for the target MMP. PVGLIG is cleaved by MMP-2 and MMP-9 at the leucine-isoleucine bond with kinetic constants (kcat/Km) of approximately 10^3 to 10^4 $M^{-1}s^{-1}$ in vitro, sufficient for clinically relevant substrate turnover at tumor MMP concentrations. The peptide linker must be incorporated into the carrier architecture such that MMP cleavage at the tumor extracellular space produces the desired carrier response — dePEGylation to unmask targeting ligands, release of drug-peptide conjugates, or initiation of carrier disassembly [35].

5. Preparation Methods

5.1 Synthesis of pH-Responsive Polymers

pH-Responsive polymers for drug delivery are synthesized by controlled radical polymerization methods including atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT) polymerization, and nitroxide-mediated polymerization, which provide precise control over molecular weight, dispersity, block sequence, and end-group functionality. RAFT polymerization of methacrylic acid with hydrophobic comonomers produces amphiphilic block copolymers that self-assemble into pH-responsive micelles with tunable pH response ranges. Ring-opening polymerization of cyclic carbonate and lactide monomers initiates from PEG macroinitiators to produce PEG-polyester block copolymers with pendant hydrazone or acetal groups [36].

5.2 Synthesis of Disulfide-Crosslinked Nanoparticles

Redox-responsive nanoparticles incorporating disulfide crosslinks are prepared by emulsion polymerization or nanoprecipitation of thiol-bearing monomers that spontaneously form disulfide crosslinks under mild oxidizing conditions during or after assembly. Alternatively, preformed thiol-bearing polymers are assembled into nanoparticles under reducing conditions and subsequently crosslinked by controlled oxidation with potassium iodate or dilute hydrogen peroxide. The degree of crosslinking, controlled by the thiol monomer feed ratio and oxidation conditions, determines the GSH concentration threshold required for carrier dissolution and the drug release kinetics upon triggering [37].

5.3 Preparation of Thermosensitive Liposomes

Thermosensitive liposomes are prepared by the thin-film hydration method, in which a lipid mixture of DPPC, MPPC or DSPE-PEG, and optionally cholesterol is dissolved in chloroform, evaporated to a dry film on a rotary evaporator, and hydrated above the gel-to-liquid crystalline phase transition temperature with a drug-containing aqueous buffer. The resulting multilamellar vesicles are extruded through polycarbonate membranes of defined pore size to produce monodisperse large unilamellar vesicles of 100 to 200

nanometers. The phase transition temperature of the resulting liposomes is measured by differential scanning calorimetry and must fall between 39 and 42°C for hyperthermia-triggered cancer therapy applications [38].

5.4 Fabrication of Multi-Stimuli Responsive Systems

Multi-stimuli responsive nanocarriers are typically fabricated by sequential assembly strategies that incorporate each responsive element in a defined spatial arrangement. A common approach for pH and redox dual-responsive systems involves preparation of a disulfide-crosslinked polymeric nanoparticle core using a pH-responsive polymer shell, such that MMP-mediated or pH-driven dissolution of the outer shell triggers cellular uptake, followed by GSH-mediated disulfide cleavage in the cytoplasm releasing the drug payload. Layer-by-layer assembly of alternating responsive polymer layers enables precise spatial positioning of multiple stimuli-responsive elements in a single nanocarrier architecture [39].

6. Characterization and Evaluation

6.1 pH-Dependent Size and Drug Release

pH-responsive nanocarriers are characterized by measuring particle size, zeta potential, and morphology at multiple pH values spanning the physiological range from pH 7.4 to pH 4.5. A sharp increase in particle size at the critical pH transition point indicates polymer swelling or aggregation-driven disassembly, while a change in zeta potential confirms ionization of the responsive groups. Drug release studies are performed using dialysis bags or membrane-free release methods in buffers of pH 7.4 and 5.0 (simulating physiological and endosomal conditions respectively), with drug quantification by HPLC or UV spectrophotometry at defined time points [40].

6.2 Thermal Characterization of Thermoresponsive Systems

Differential scanning calorimetry is the primary technique for characterizing the phase transition temperature and enthalpy of thermoresponsive liposomes and LCST polymers. The endothermic peak in the DSC heating thermogram provides the main phase transition temperature (Tm) and the cooperativity of the transition. For thermosensitive liposomes, a Tm between 39 and 42°C confirms suitability for hyperthermia-triggered release. Turbidimetry at increasing temperatures provides a rapid and accessible measurement of LCST for thermoresponsive polymer solutions, defined as the temperature at which solution transmittance decreases to 50% of its baseline value [41].

6.3 GSH-Triggered Drug Release Assay

Redox-responsive nanocarriers are evaluated by drug release studies in the presence of physiologically relevant concentrations of GSH. Standard assay conditions include 10 mM GSH in phosphate buffer pH 7.4 to simulate intracellular cytoplasmic conditions, 2 micromolar GSH in plasma-simulating buffer to confirm extracellular stability, and a GSH-free control to confirm that disulfide cleavage rather than passive diffusion drives the observed release. The half-time of GSH-triggered release and the percent of total drug released after 24 hours in each condition are reported to characterize GSH responsiveness and extracellular stability [42].

6.4 In Vitro Cellular Evaluation

Cellular evaluation of stimuli-responsive nanocarriers includes confocal microscopy studies to confirm intracellular drug delivery and colocalization with organelles (endosomes, lysosomes, cytoplasm), flow cytometry to quantify cellular uptake efficiency and the stimuli-dependent enhancement of uptake or drug accumulation, and cytotoxicity assays (MTT, MTS, or resazurin) to compare the IC50 of stimuli-responsive formulations against free drug and non-responsive carrier controls. Importantly, cellular studies should be performed in both stimuli-permissive conditions (low pH, high GSH, high MMP) and stimuli-absent conditions to confirm stimulus-specific activation [43].

6.5 In Vivo Tumor Model Evaluation

In vivo evaluation of stimuli-responsive nanocarriers is typically performed in subcutaneous or orthotopic tumor xenograft models in immunodeficient mice. Fluorescently labeled or radiolabeled nanocarriers are administered intravenously, and intratumoral drug accumulation and distribution are assessed by fluorescence imaging, gamma scintigraphy, or by direct tissue extraction and quantification at defined time points. Efficacy studies measure tumor growth inhibition, tumor volume regression, and survival compared to free drug and non-responsive nanocarrier controls at equivalent doses. Key pharmacokinetic parameters

including area under the curve in plasma and tumor tissue, half-life, and tumor-to-plasma drug ratio are calculated to quantify the targeting benefit of stimuli-responsive release [44].

7. Mechanism of Drug Delivery

7.1 pH-Triggered Release Mechanisms

pH-responsive drug release operates through two principal mechanisms depending on carrier design. In ionization-driven swelling systems, decreasing pH below the polymer pKa reduces ionization of carboxylate or sulfonamide groups, increasing polymer hydrophobicity and causing micelle formation or gel contraction that releases encapsulated drug by a squeeze-out mechanism. Conversely, in polycationic systems such as poly(L-histidine), decreasing pH protonates the imidazole groups (pKa approximately 6.0), converts the polymer from neutral to cationic, and causes carrier swelling and drug release through electrostatic repulsion. In acid-labile linker systems, covalent bond hydrolysis at tumor or endosomal pH liberates free drug from polymer-drug conjugates or crosslink-stabilized carriers through a chemical rather than physical mechanism [45].

7.2 Thermoresponsive Phase Transition Mechanism

The thermally triggered drug release mechanism in PNIPAM-based systems involves the entropy-driven dehydration of isopropyl side chains above the LCST. Below the LCST, water molecules form ordered hydrogen-bonded solvation shells around the hydrophobic isopropyl groups, maintaining polymer solubility at an entropic cost. Above the LCST, the entropic gain from releasing these ordered water molecules overcomes the enthalpic cost of polymer-polymer contacts, driving a sharp hydrophilic-to-hydrophobic phase transition that expels water and drug from the polymer network. In thermosensitive liposomes, heating to the lipid phase transition temperature creates transient grain boundary defects at gel-liquid crystalline lipid domain interfaces that transiently increase bilayer permeability by orders of magnitude, enabling rapid drug efflux before the boundary defects anneal [46].

7.3 Glutathione-Mediated Disulfide Cleavage

Disulfide bond cleavage by glutathione proceeds through a nucleophilic thiol-disulfide exchange reaction in which the thiolate anion (GS^-) attacks the electrophilic sulfur atom of the disulfide bond, forming a mixed disulfide intermediate that is subsequently displaced by a second thiolate to regenerate oxidized glutathione (GSSG) and the free thiol. The reaction follows second-order kinetics with rate constants for glutathione-disulfide exchange typically in the range of 10 to 100 $M^{-1}s^{-1}$ for small molecule disulfides, sufficient to achieve complete nanoparticle disassembly within 30 to 60 minutes at 10 mM intracellular GSH concentrations [47].

7.4 Enzyme-Catalyzed Linker Cleavage

MMP-mediated cleavage of peptide linker sequences in enzyme-responsive nanocarriers follows Michaelis-Menten kinetics, with the catalytic efficiency (kcat/Km) of the enzyme-substrate pair determining the rate of carrier activation in the tumor microenvironment. Following MMP cleavage of the peptide linker, the structural consequence for the carrier depends on its architecture: in dePEGylation strategies, removal of the PEG stealth coating exposes charged or hydrophobic carrier surfaces that increase non-specific cell uptake or activate targeting ligands; in prodrug nanoconjugates, peptide linker cleavage releases the drug-amino acid fragment that undergoes rapid intramolecular cyclization to release free drug [48].

8. Therapeutic Applications

8.1 Cancer Chemotherapy

pH-responsive doxorubicin nanocarriers are the most extensively studied stimuli-responsive cancer drug delivery systems. Doxorubicin conjugated to polymer nanoparticles through hydrazone bonds releases 10 to 30% of total drug at pH 7.4 over 24 hours but 60 to 90% at pH 5.0 in the same time period, providing a 3- to 9-fold selectivity ratio that translates to dramatically improved cytotoxicity against cancer cell lines with acidic endosomal compartments compared to non-cancerous cell lines. In vivo studies in MCF-7 breast cancer xenograft models consistently demonstrate superior tumor growth inhibition and reduced cardiotoxicity compared to free doxorubicin at equivalent doses [49].

Disulfide-crosslinked polymeric micelles for paclitaxel and docetaxel delivery have demonstrated 40 to 100-fold reduction in IC50 values compared to free drug in GSH-supplemented cancer cell cultures, attributed to rapid intracellular drug release from collapsed micelles following GSH-mediated disulfide

cleavage. In vivo efficacy studies in paclitaxel-resistant cancer models showed significant tumor growth inhibition by GSH-responsive paclitaxel micelles at doses below the maximum tolerated dose of free paclitaxel, suggesting that intracellular drug release enhances efficacy in resistant tumors where lysosomal drug sequestration limits free drug activity [50].

8.2 Combination Cancer Therapy

Co-delivery of two or more anticancer agents within a single stimuli-responsive nanocarrier enables spatiotemporally controlled combination therapy with a defined drug ratio that cannot be achieved by separate administration of conventional formulations. pH-responsive nanoparticles co-loading doxorubicin and siRNA targeting MDR1 (multidrug resistance gene) release both payloads simultaneously in the endosomal compartment, with siRNA silencing of P-glycoprotein preventing doxorubicin efflux while the locally released doxorubicin exerts its DNA-damaging effect. Dual pH/redox responsive carriers enable sequential release — pH-triggered release of an immunostimulatory molecule in the tumor microenvironment followed by GSH-triggered intracellular release of chemotherapy — to combine local immune activation with direct cytotoxicity [51].

8.3 Photodynamic and Photothermal Therapy

Light-responsive nanocarriers for photodynamic therapy combine photosensitizer delivery with stimuli-responsive drug release, enabling spatially precise activation by focused light irradiation at the tumor site. Near-infrared-absorbing gold nanorods and copper sulfide nanoparticles generate local heat upon NIR irradiation sufficient to trigger drug release from thermoresponsive polymer coatings while simultaneously inducing photothermal ablation of tumor cells, creating a synergistic chemo-photothermal therapy effect. The spatial precision of light as an exogenous trigger provides tumor selectivity that supplements or replaces reliance on tumor microenvironment-specific endogenous triggers [52].

8.4 Anti-Inflammatory Drug Delivery

ROS-responsive nanoparticles incorporating thioketal or boronic ester linkages have been investigated for anti-inflammatory drug delivery in conditions characterized by elevated reactive oxygen species production, including inflammatory bowel disease, atherosclerosis, and articular inflammation. The rationale is that activated macrophages at sites of inflammation produce superoxide and hydrogen peroxide at concentrations sufficient to trigger thioketal or boronic ester oxidation and carrier disassembly, releasing anti-inflammatory drug specifically at sites of macrophage-mediated inflammation without systemic immunosuppression. Oral ROS-responsive nanoparticles of dexamethasone demonstrated superior mucosal drug delivery and reduced systemic corticosteroid exposure compared to conventional oral dexamethasone in a dextran sulfate-induced colitis mouse model [53].

8.5 Diabetes and Metabolic Disease

Glucose-responsive insulin delivery systems represent a specialized class of metabolic disease-targeted stimuli-responsive carriers in which elevated glucose concentration serves as the physiological trigger for insulin release. Phenylboronic acid-functionalized nanoparticles or hydrogels undergo structural changes at elevated glucose concentrations through competitive displacement of the boronic acid-diol complex by glucose, triggering insulin release proportional to glucose concentration. Glucose oxidase-containing nanoparticles convert glucose to gluconic acid, locally lowering pH within the carrier microenvironment and triggering pH-responsive insulin release, while simultaneously consuming oxygen to generate a hypoxic microenvironment that can activate hypoxia-responsive release elements [54].

9. Recent Advances

9.1 Hypoxia-Responsive Drug Delivery

Tumor hypoxia, arising from inadequate oxygen delivery by poorly organized tumor vasculature, affects 50 to 60% of solid tumors and is strongly associated with poor prognosis, radiation resistance, and chemotherapy resistance. Hypoxia-responsive nanocarriers incorporating nitroimidazole, azo linkages, or quinone moieties that are selectively reduced under hypoxic conditions (oxygen partial pressure below 10 mmHg) have been developed to exploit tumor hypoxia as a drug release trigger. Nitroimidazole-functionalized nanoparticles are reduced by intracellular nitroreductase enzymes in hypoxic cells, converting the hydrophobic nitroimidazole group to a hydrophilic aminoimidazole, triggering micelle dissolution and drug release selectively in hypoxic tumor regions [55].

9.2 Tumor Microenvironment-Cascade Responsive Systems

Cascade-responsive systems exploit sequential physicochemical events in the tumor microenvironment to achieve multi-stage drug release with increasingly precise targeting at each stage. MMP-responsive PEG deshielding of nanoparticles at the tumor extracellular matrix exposes pH-sensitive cell-penetrating peptides that become protonated and positively charged at tumor extracellular pH 6.5, enhancing cellular uptake through charge reversal. Following endocytosis, endosomal acidification triggers acid-labile bond hydrolysis and drug release in the cytoplasm. This three-stage cascade — MMP activation, pH-responsive uptake enhancement, endosomal drug release — substantially improves tumor-selective drug delivery compared to any single-stage responsive system [56].

9.3 Ferroptosis-Inducing Redox-Responsive Systems

Ferroptosis, a form of regulated cell death driven by iron-dependent lipid peroxidation, has emerged as an attractive cancer therapeutic target owing to its activation in cancer cells with high iron content and elevated lipid peroxidation susceptibility. ROS-generating nanoparticles incorporating iron oxide cores or ferrocene moieties have been designed to simultaneously deliver anticancer drugs and induce ferroptosis through Fenton reaction-mediated hydroxyl radical generation in the iron-rich tumor microenvironment. Tandem chemo-ferroptosis responsive nanoparticles demonstrated synergistic cancer cell killing in cancer cell lines resistant to conventional chemotherapy, with cancer selectivity attributed to the substantially higher labile iron pool and lipid peroxide susceptibility in cancer cells [57].

9.4 Immunotherapy-Synergizing Stimuli-Responsive Nanocarriers

The combination of stimuli-responsive drug release with cancer immunotherapy represents one of the most promising recent developments in the field. pH-responsive nanoparticles releasing STING agonists in the acidic tumor microenvironment activate innate immune sensing pathways in tumor-infiltrating dendritic cells, enhancing antitumor immune responses that are further augmented by simultaneously released chemotherapy-induced immunogenic cell death. ROS-responsive nanoparticles co-delivering IDO1 inhibitors and anti-PD-L1 antibodies demonstrated synergistic antitumor immune activation in poorly immunogenic murine tumor models through combined metabolic immune checkpoint blockade and PD-L1 inhibition [58].

10. Comparative Analysis

Stimuli-responsive nanocarriers are best compared with passive EPR-targeting nanocarriers (non-responsive), conventional free drug formulations, and antibody-drug conjugates to contextualize their relative merits and limitations. Conventional free drug formulations distribute non-selectively throughout the body according to the drug's intrinsic pharmacokinetic properties, with tumor accumulation governed by passive diffusion and limited by rapid systemic clearance. Passive nanocarriers exploit the enhanced permeability and retention (EPR) effect to achieve greater tumor accumulation than free drug but do not discriminate between tumor tissue and normal tissue once accumulated [59].

Stimuli-responsive nanocarriers add a second layer of selectivity by ensuring that the drug release event, even for nanocarrier that has reached the tumor via EPR, occurs preferentially in response to the tumor-specific trigger rather than throughout the body. Antibody-drug conjugates achieve highly specific cellular targeting through antigen recognition but are limited by target antigen expression heterogeneity and carry risks of premature linker hydrolysis in systemic circulation causing off-target toxicity. Stimuli-responsive systems are antigen-agnostic and exploit physicochemical rather than biological recognition, enabling broader applicability but with lower absolute selectivity than antibody-mediated targeting [60].

Table 2: Comparative analysis of stimuli-responsive versus conventional drug delivery approaches in cancer therapy

Parameter	Free Drug	Passive NPs (EPR)	Stimuli-Responsive NPs	ADCs	Liposomes
Tumor selectivity	Poor	Moderate (EPR)	High (triggered release)	Very high (antigen)	Moderate
Drug release control	None	Passive diffusion	Stimulus-triggered	Enzymatic linker	pH / temperature

Systemic toxicity	High	Reduced	Substantially reduced	Reduced	Reduced
Applicability to biologics	Limited	Yes	Yes	Yes (antibody)	Yes
Manufacturing complexity	Low	Moderate	High	Very high	Moderate
Clinical approval status	Numerous	Several (Doxil, Abraxane)	Limited (ThermoDox Phase 3)	Several (Kadcyla, Enhertu)	Several

NPs: nanoparticles; EPR: enhanced permeability and retention; ADCs: antibody-drug conjugates.

11. Advantages and Limitations

11.1 Advantages

- Spatially controlled drug release aligned with the pathophysiological characteristics of the target disease site, improving the therapeutic index by reducing drug exposure at healthy tissues
- Temporal control over drug release enabling on-demand activation that can be synchronized with physiological events or exogenous stimuli application for maximum therapeutic effect
- Versatility of stimulus options — endogenous (pH, redox, enzyme, temperature) and exogenous (light, ultrasound, magnetic field) — enabling selection of the most appropriate trigger for each disease context
- Dual and multi-stimuli responsive systems provide logical AND gate selectivity that substantially reduces off-target activation probability compared to single-stimulus systems
- Compatibility with diverse drug payloads including small molecule chemotherapeutics, proteins, siRNA, and CRISPR components within stimuli-responsive nanocarrier architectures
- Ability to co-deliver synergistic drug combinations at controlled molar ratios within a single responsive carrier, enabling combination therapy optimization inaccessible to conventional formulations
- Stimuli-responsive release can overcome multidrug resistance by delivering drug directly to the cytoplasm via intracellular triggers, bypassing P-glycoprotein-mediated efflux at the plasma membrane

11.2 Limitations

- Manufacturing complexity of multi-component stimuli-responsive nanocarriers with precise molecular architecture presents significant scale-up and quality control challenges for pharmaceutical-grade production
- Tumor microenvironment heterogeneity within individual tumors and between patients means that the triggering stimulus may not be uniformly present throughout the tumor, resulting in spatially inhomogeneous drug release
- Premature drug release in systemic circulation, despite responsive design, occurs at a finite rate due to non-specific bond hydrolysis, enzyme activity in blood, and temperature variations, contributing to off-target toxicity
- The EPR effect — the passive tumor accumulation mechanism upon which most stimuli-responsive intravenous nanocarriers rely for tumor delivery — is heterogeneous and often inadequate in desmoplastic or well-perfused tumors including pancreatic and colorectal cancers
- In vitro stimuli conditions (10 mM GSH, pH 5.0, exogenous enzyme addition) do not fully recapitulate the spatial, temporal, and concentration heterogeneity of stimuli in the tumor microenvironment in vivo, reducing the predictive value of in vitro release studies
- Clinical translation has been extremely limited relative to the large preclinical literature, with no pH, redox, or enzyme-responsive nanocarrier achieving clinical approval as of 2022

12. Clinical Translation and Marketed Products

The clinical translation of stimuli-responsive drug delivery systems has proceeded considerably more slowly than the pace of preclinical publication activity might suggest. Among all classes of stimuli-responsive

nanocarriers, thermosensitive liposomes have achieved the most advanced clinical development, with ThermoDox (lyso-thermosensitive liposomal doxorubicin, Celsion Corporation) reaching a phase 3 clinical trial (HEAT study) in combination with radiofrequency ablation for hepatocellular carcinoma. The HEAT study did not meet its primary endpoint of progression-free survival improvement in the overall population, though a pre-specified subgroup analysis suggested benefit in patients receiving radiofrequency ablation treatment duration exceeding 45 minutes [61].

Antibody-drug conjugates using enzyme-responsive linker chemistry represent the most clinically successful application of stimuli-responsive drug release, with multiple approved products including brentuximab vedotin (Adcetris, using cathepsin B-cleavable Val-Cit linker), ado-trastuzumab emtansine (Kadcyla, using lysosome-cleavable thioether linker), and trastuzumab deruxtecan (Enhertu, using cathepsin B-cleavable tetrapeptide linker), demonstrating that enzyme-responsive drug release can achieve regulatory approval and clinical benefit when coupled with precise antibody-mediated cellular targeting [62].

Table 3: Clinical status of stimuli-responsive drug delivery systems and enzyme-responsive antibody-drug conjugates (as of 2022)

Product	Stimulus	Drug	Indication	Clinical Status / Outcome
ThermoDox (Celsion)	Temperature (42°C)	Doxorubicin	Hepatocellular carcinoma	Phase 3 (HEAT study) — primary endpoint not met; subgroup benefit
Adcetris (Seattle Genetics)	Cathepsin B (enzyme)	MMAE (Val-Cit linker)	Hodgkin lymphoma, ALCL	FDA approved (2011) — clinical use
Kadcyla (Genentech/Roche)	Lysosomal (enzyme)	DM1 (thioether linker)	HER2+ breast cancer	FDA approved (2013) — clinical use
Enhertu (AstraZeneca/Daiichi)	Cathepsin B (enzyme)	DXd (tetrapeptide linker)	HER2+ breast, gastric cancer	FDA approved (2019) — clinical use
pH-responsive NP (investigational)	pH (tumor/endosomal)	Doxorubicin / siRNA	Various solid tumors	Phase 1/2 — multiple trials ongoing (2022)
GSH-responsive NP (investigational)	Redox (GSH)	Paclitaxel, platinum drugs	Ovarian, breast cancer	Phase 1 — early clinical data (2022)

MMAE: monomethyl auristatin E; DM1: emtansine; DXd: deruxtecan; ALCL: anaplastic large cell lymphoma; HER2: human epidermal growth factor receptor 2; GSH: glutathione.

13. Critical Analysis

The stimuli-responsive drug delivery field suffers from a well-documented and persistent disconnect between the volume and enthusiasm of preclinical publications and the extremely limited clinical translation achieved to date. A 2016 meta-analysis by Wilhelm and colleagues examined 117 published studies of nanoparticle tumor delivery and found that the median tumor delivery efficiency across all studies was only 0.7% of the injected dose, with no significant improvement observed over 10 years of research despite substantial investments in active targeting and stimuli-responsive design. This finding challenges the assumption, implicit in much of the stimuli-responsive literature, that nanoparticles accumulate at tumor sites in quantities sufficient to deliver therapeutically meaningful drug concentrations [63].

The in vitro conditions used to evaluate stimuli-responsive drug release in the published literature are frequently poorly matched to the actual in vivo tumor microenvironment. GSH-triggered release studies typically use 10 mM glutathione — a concentration at the upper end of estimates for intracellular cytoplasmic GSH — but ignore the fact that GSH is compartmentalized within cells and that nanoparticle-encapsulated drug must reach the cytoplasm before encountering this concentration. pH-triggered release studies use sharply buffered solutions at fixed pH values that do not recapitulate the pH gradients, spatial heterogeneity, and temporal variation of tumor pH in vivo. The consequence is systematic overestimation of stimulus-response selectivity in vitro that is not maintained in the complex in vivo environment [64].

The failure of ThermoDox in the HEAT phase 3 trial illustrates a broader challenge in stimuli-responsive cancer nanomedicine: the therapeutic benefit observed in carefully controlled preclinical tumor models in immunocompetent or immunodeficient mice does not reliably translate to clinical benefit in the heterogeneous, immunologically complex, and pharmacokinetically distinct human tumor environment. The HEAT trial failure was attributed in part to inadequate hyperthermia delivery in the clinical setting — many patients did not receive the full radiofrequency ablation treatment time required to heat the tumor to the liposome phase transition temperature — highlighting how exogenous stimuli-responsive systems are dependent on accurate and complete delivery of the triggering stimulus in clinical practice, a requirement that introduces additional failure modes not present in preclinical settings [65].

Enzyme-responsive antibody-drug conjugates represent the major exception to the clinical translation failure pattern of stimuli-responsive drug delivery, with multiple approved products demonstrating survival benefit in large randomized trials. Analysis of the factors distinguishing clinically successful enzyme-responsive ADCs from unsuccessful nanoparticle-based stimuli-responsive systems reveals that ADCs combine precision cellular targeting via antibody-antigen binding with intracellular enzyme-responsive drug release, providing two independent selectivity mechanisms. In contrast, most stimuli-responsive nanoparticles rely on EPR-based tumor accumulation — a mechanism that is now recognized as far less efficient and reliable in human tumors than the murine xenograft models in which most preclinical work is conducted [66].

14. Conclusion

Stimuli-responsive drug delivery systems represent a conceptually compelling and scientifically sophisticated approach to the fundamental challenge of achieving tumor-selective drug release while minimizing systemic toxicity in cancer therapy. By embedding the drug release trigger within the pathophysiological characteristics of the tumor microenvironment — its acidity, elevated glutathione, overexpressed proteases, and thermal properties — stimuli-responsive systems offer the theoretical possibility of pharmacologically active concentrations of drug at the tumor site with substantially reduced exposure of normal tissues.

The scientific achievements of the field are considerable: the chemical toolkit of pH-labile bonds, disulfide crosslinks, enzyme-cleavable peptide sequences, thermoresponsive polymers, and multi-stimuli AND gate systems provides diverse and increasingly sophisticated options for matching the release trigger to the disease context. The clinical validation of enzyme-responsive linker chemistry in approved antibody-drug conjugates including brentuximab vedotin, ado-trastuzumab emtansine, and trastuzumab deruxtecan demonstrates that stimuli-responsive drug release can deliver clinical benefit when combined with precise cellular targeting.

The critical appraisal presented in this review, however, reveals that the field must confront several uncomfortable realities: tumor nanoparticle delivery efficiency in humans is far lower than preclinical models suggest; in vitro stimuli conditions systematically overestimate in vivo trigger fidelity; and the EPR effect upon which most intravenous nanocarrier systems depend is insufficient in many human tumors to ensure meaningful drug accumulation at the release site. Addressing these challenges through improved tumor delivery strategies including active targeting, tumor stroma modulation, and locally administered stimuli-responsive depots, combined with more rigorous and clinically predictive preclinical pharmacokinetic models, will be essential to realize the therapeutic promise of stimuli-responsive drug delivery in the clinic.

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