

Review Article

ADVANCES OF HYDRAZONE LINKER IN POLYMERIC DRUG DELIVERY

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ABSTRACT

Polymeric drug delivery vehicles are known for the controlled release of the drug. More importantly, stimuli-responsive systems are emerged as a suitable platform for targeted drug delivery system, due to its properties such as low toxicity, improved patient compliance, and convenience. In the present article, an overview of the hydrazone linker used in drug delivery is discussed. Particularly, the role of hydrazone linker in anti-cancer drug delivery system is elaborated, as the hydrazone linker gets cleaved under cancerous cell environment at pH 5.

Keywords: Linkers, Stimuli-Responsive Materials, Drug Delivery

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INTRODUCTION

The polymers play the most important roles in today's chemistry world. Polymers are used for a large number of medical applications as drug carriers, as support or replacement of malfunctioning body parts or as a drug reservoir providing a local therapeutic effect. The most important applications of polymers in modern pharmaceuticals are the development of advanced drug delivery systems, commonly known as controlled release drug delivery systems. Controlled drug delivery is the most rapidly emerging area in chemical science and chemical engineering towards human health care. The use of polymeric vehicles in the field of drug delivery is to reduce toxicity, improved patient compliance, and convenience. In literature, there are reports in which stimuli-responsive drug delivery system was demonstrated. Stimuli-responsive polymers provide a potential application in the biomedical field [1].

Over the last decade, the development of polymeric materials in the field of drug delivery has emerged as a potential tool for controlled drug delivery. Controlled drug delivery is the most rapidly emerging area in chemical science and chemical engineering towards human health care. The use of polymeric vehicles in the field of drug delivery is to reduce toxicity, improved patient compliance, and convenience. The nanotechnology has made a significant impact on the development of drug delivery systems [1, 2]. A variety of biomaterials have been used as delivery vehicles to develop an effective drug delivery system in the therapeutic area. These systems can enhance the therapeutic activity by prolonging drug half-life, improving solubility of hydrophobic drugs, reducing potential immunogenicity, and releasing drugs in a sustained or stimuli-triggered fashion. This system may reduce the toxic side effects of drugs and the frequency of the drug administration. In addition, nanocarriers can passively accumulate in specific tissues (for example tumors) through the Enhanced Permeability and Retention (EPR) effect. Here, summarised some delivery systems that serve as important milestones throughout the history of drug delivery [3]. Further for tumor-targeted drug delivery to be most effective the drug should only be released once the drug carrier has localized within the diseased tissue, and therefore the premature release of the drug while circulating in the serum must be minimized. Linkages based on enzymatically labile peptides or acid-labile amides and hydrazones are the most commonly utilized means of covalent attachment between doxorubicin (DOX) and a carrier [4, 5].

In the conjugated drug delivery system, the drug is attached to the polymeric backbone through different stimuli-responsive linkers. In 1975, Helmut Ringsdorf has proposed a rational model for pharmacologically active polymers [6]. His concept of covalently bound polymer-drug conjugates still forms the basis for most of the work in this area. The Ringsdorf model primarily consists of three important concepts along with the biocompatible polymeric backbone, (1) a solubilizer, which serves the purpose of imparting

hydrophilicity and ensuring water solubility, (2) a drug, usually bound to the polymeric backbone via stimuli-responsive linkers, and (3) a targeting moiety whose function is to provide transport to a desired physiological destination or bind to a particular biological target. In the late 1970s, Duncan, Kopeck and Ringsdorf, propose the polymer-drug conjugate system in drug delivery for the first time [7]. With the polymer-drug conjugation, the drugs can also be protected from degradation, which results in the improvement in efficacy due to increased drug circulation times.

Over the last decade, the researcher has been developed the biomimetic materials building blocks by using bioorthogonal reactions. The recent advances in the field of polymer and biomaterials chemistry which is originally inspired by biological module design principles. Bioorthogonal defines the chemical transformations that occur between a pair of molecules with mutually reactive functional groups without significant interference from co-existing functionalities in physiological conditions. The most important features are biocompatibility, specificity, high yield, and fast reaction kinetics. Some examples of bioorthogonal reactions include Diels-Alder reaction, Huisgen's 1, 3 dipolar cycloaddition reaction, Copper-catalyzed azide-alkyne click reaction, Michael reaction, radical initiated thiol-ene reaction, tetrazine ligation, and the hydrazone/oxime chemistry [8, 9].

Search criteria

In this review, articles were included from PubMed databases, Mendeley, Google Scholar, Research Gate, and Science Direct, and using several keywords for search: Linkers, Stimuli-Responsive Materials, Drug Delivery. The articles between the years of 1999-2018 were selected for review.

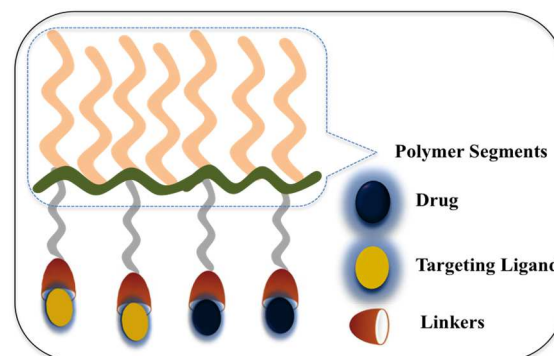


Fig. 1: Schematic representation for polymer-linker-drug conjugates [36]

pH-responsive linkers

Role of the linker in drug delivery is very important since drugs need to be come out from the polymer conjugates. These therapeutic drugs are often expected to release from the polymer backbone for it to then reach the cytosol and possibly other intracellular targets. Subsequently, an important approach for such design is to use the considerable drop in pH that molecules encounter as they are trafficked through endocytic pathways. These pathways are used by the cells to internalize molecules that will then encounter a drop from extracellular pH 7.3–7.5 to 4.5–5.0 in lysosomes.

In the literature, several methods for drug release have been employed, which are namely temperature sensitive, membrane-disruptive, acid-labile and enzyme-degradable materials. *N*-Isopropylacrylamide (NIPAAm) based polymers are often used in micelles, liposomes, and hydrogels for their temperature-sensitive behavior [10, 11]. Similarly, Skirtach *et al.* have synthesized thermo-responsive polyelectrolyte multilayered shells doped with metal nanoparticles, which disrupted the membrane and led to release when treated with a near-infrared laser [12]. The doxorubicin module cancer drug has also been conjugated to *N*-(2-Hydroxypropyl) methacrylamide (HPMA) with enzymatically degradable peptide linkers, as well as acid-labile linkers [13-15].

In pH-responsive drug delivery, research has been conducted on polymer conjugates, hydrogels, particles, micelles, dendrimers, and liposomes. Polymers that have been used to form pH-sensitive conjugates included dextran, alginic acid, carboxymethyl cellulose, poly (amino acids), HPMA and Poly (Ethylene Glycol) (PEG) [15]. Several pH-sensitive linkers have been studied in this context, including hydrazones [16, 17], *cis*-aconityl [18], orthoesters [19], and acetals [20] (fig. 1).

Hydrazone ligation

Schiff-base reactions are extensively used in the field of chemistry. These reactions have many important advantages, such as mild reaction conditions and high reaction rates. Carboxylic hydrazone, *cis*-aconityl, and acetal are common acid-labile bonds that have been investigated extensively for use in drug delivery. Imine and ketal bonds have also been studied to a lesser extent. Also, it is very helpful to protect various functional groups and employed for synthesizing a series of organic ligands. In the last two decades, in the field of polymer chemistry, Schiff's-bases can serve as potential pH-responsive linkers in polymer chains because of their sensitive responses to changes in the pH value. The formation of Schiff-base structure is reversible covalent bonds which provide various functions and applications. The most important property of the Schiff's-base reaction is the sensitivity towards acidic pH, So this pH-responsive nature is widely used in the field of drug delivery in polymer chemistry [21]. This approach offers the advantage of flexibility in design and the ratio of macromonomers used for copolymerization can be adjusted to have particles with the required properties.

Recent advances in hydrazone ligation

A hydrazone linkage can be utilized as an acid-labile bond for releasing the drug molecule from the conjugate upon a decrease in pH in tumor extracellular environments and in the lysosomes. This linker helps for efficient drug release in a mildly acidic environment i.e. at pH 5-6 because for example cancerous cell has pH 5 and using such linker the drug release at pH 5-6. Shunmugam *et al.* prepared doxorubicin with hydrazone linker by addition of doxorubicin hydrochloride, a ketone functional group at C-13 and norbornene hydrazide in methanol in presence of trifluoroacetic acid and the whole reaction set up was protected from light for 24 h. Hydrazone linkers are specifically interesting for their responsive acuteness in delivery behavior and also for its ability to the usually facile incorporation of hydrazides into delivery materials. Efficient drug releasing is obtained in this approach and they could release incorporated doxorubicin selectively in mildly acidic tumor sites due to the hydrazone linkers employed in the design [22].

The *cis*-aconityl bond has been utilized to form nano-aggregates between glycol and chitosan to deliver doxorubicin. About 20% of the drug was released from the delivery vehicle after 8 d. The

aggregates had an average size of 250 nm and were found to be retained in tumors *in vivo* studies due to the EPR effect. There was also significant accumulation in the kidneys since the aggregates were not targeted to the tumors. Some researchers have shown that the hydrazone bond was more effective than the *cis*-aconityl bond in cell culture experiments [18].

Prof. Frechet *et al.* proposed a dendritic system for drug delivery where intramolecular cyclization reaction between doxorubicin's C-14 hydroxyl and the carboxylate-substituted hydrazone rationalizes the seemingly anomalous hydrolysis kinetics seen for hydrazone carboxylate linked doxorubicin [23].

Next, Prof. Emrick has introduced an alternative approach for the PEG by using polyMPC. He has demonstrated the conjugation of the cancer drug doxorubicin to Poly (Methacryloyloxyethyl Phosphorylcholine) (polyMPC), linked by hydrazone linker, and polymerized it by using (1) a one-pot ATRP/click sequence, and (2) a post-polymerization conjugation strategy. DOX release from the polyMPC backbone was pH dependent (faster at pH 5.0 than at pH 7.4) owing to the hydrazone linkage [24]. An amphiphilic hyperbranched block copolymer micelle was developed. The inner hydrophobic layer was composed of a random copolymer of poly (ϵ -caprolactone) and poly (malic acid) (PMA-*co*-PCL) segments, while the outer hydrophilic shell was composed of PEG segments along with active tumor-targeting ligands Folate (FA). The drug was conjugated through pH-sensitive hydrazone linker which cleavable by the intracellular acidic environment [25].

Recently, Kim *et al.* reported the synthesis of a well-defined hyperbranched double hydrophilic block copolymer of Poly-(Ethylene Oxide)-hyperbranched-PolyGlycerol (PEO-*hb*-PG) to develop an efficient drug delivery system. Particularly, they have explored the pH-responsive hydrazone chemistry, where it has been demonstrated the hyperbranched PEO-*hb*-PG can form a self-assembled micellar structure on conjugation with the hydrophobic anticancer module drug doxorubicin. The DOX is linked to the polymer backbone by pH-sensitive-hydrazone bonds, which get cleaved under an acidic environment that resulted in the pH-responsive controlled release of doxorubicin. Specifically, the pH-responsive release of anti-cancer module drug doxorubicin and *in vitro* cytotoxicity studies revealed the controlled stimuli-responsive drug delivery system which helps to enhance efficiency. Also, the hyper-branched double hydrophilic block copolymers have important features such as enhanced biocompatibility, increased water solubility [26].

The caprolactone based stimuli-responsive drug delivery system was developed. A polymeric micelle system from degradable Poly (Ethylene Oxide)-block-Poly (ϵ -Caprolactone) (PEO-*b*-PCL) block copolymers with functional groups on both blocks were reported. The functional group on the PCL block was used to incorporate short polyamines for complexation with siRNA or to chemically conjugate DOX via a pH-sensitive hydrazone linkage [27]. Here, Caruso *et al.* demonstrated mesoporous silica templated particles for anticancer drug delivery using thiol-maleimide click chemistry of thiolated Poly(Methacrylic Acid) (PMA SH) and DOX is released from the particles through cleavage of the hydrazone bonds between Dox and PMA SH at endosomal/lysosomal pH [28].

Next, Kataoka *et al.* have been systematically developed a novel intracellular pH-sensitive polymeric micelle drug carrier that controls the systemic, local, and subcellular distributions of pharmacologically active drugs. The amphiphilic block copolymers micelles were prepared from poly(ethylene glycol)-poly-(aspartate hydrazone adriamycin), in which the anticancer drug, adriamycin, was conjugated to the hydrophobic segments through acid-sensitive hydrazone linkers. By this polymeric design, the micelles can stably preserve drugs under physiological conditions (pH 7.4) and selectively release them by sensing the intracellular pH decrease in endosomes and lysosomes (pH 5-6) [29]. Furthermore, the same group has been explored the conjugation of a drug cocktail with folate. They have demonstrated the effects of folate on cytotoxicity, biodistribution, anticancer activity, and pharmacological properties. The folate concentration on the surface of the micelles was controlled by precise synthesis of two different amphiphilic block

copolymers that self-assemble into spherical micelles, folate poly(ethylene glycol)-poly(aspartate-hydrazone-adriamycin) with γ -carboxylic acid activated folate and methoxy poly(ethylene glycol)-poly(aspartate-hydrazone-adriamycin) without folate [30, 31].

Next, Prof. Stenzel *et al.* reported a new acid-degradable polymer-platinum conjugate, which was prepared by post modification of a PEOGEMEMA-*b*-PHEMA block copolymer obtained by RAFT polymerization. They reported a hydrazone linkage, susceptible to hydrolytic cleavage, was formed by reaction of the carbonyl group of a diamino ligand with the hydrazide-modified copolymer. The degradation of the hydrazone bond was observed through Nuclear Magnetic Resonance (NMR) kinetic studies. The degradation was observed more than 10 times faster at pH 5.5 than at pH 7.4. The platinum drug was introduced onto the copolymer by permanent conjugation onto the diamino conjugation sites which constitute a promising system for a triggered release of platinum drugs [32].

Yoo *et al.* have conjugated doxorubicin to a copolymer of poly (lactic acid) and methoxy-PEG with acetal and hydrazone bonds. The micelles formed from the hydrazone-linked doxorubicin conjugate had the higher overall release and greater cytotoxicity in cell culture studies. A pH-sensitive polymer-doxorubicin conjugate would be more cytotoxic since it would release the drug in a free form. Also, due to the EPR effect, drug conjugates would tend to accumulate in tumors, and a pH-sensitive conjugate would release the drug inside the tumor. Therefore, in addition to greater efficacy after cellular delivery, the pH-sensitive conjugates would also potentially be helpful specifically in treating tumors [33].

Recently, Prof. Shunmugam *et al.* reported a wide variety of polymeric systems of hydrazone linker in anti-cancer as well as anti-tuberculosis drug delivery systems. Ring-opening metathesis polymerization techniques were utilized to make the polymer. The drug was attached to the norbornene backbone through hydrazone linker and its acid responsive drug release was demonstrated. The role of hydrazone linker in anti-cancer drug delivery system is elaborated, as the hydrazone linker gets cleaved under cancerous cell environment at \sim pH 5 [33-40].

CONCLUSION

In summary, the current review article is dealt with the role and utilization of hydrazone linker in the drug delivery field. The pH of a cancerous cell is acid in nature, particularly the polymeric micelles formed from the hydrazone-linked doxorubicin conjugate has the higher overall release and greater cytotoxicity in cell culture studies. A pH-sensitive polymer-doxorubicin conjugate would be more cytotoxic since it would release the drug in a free form. The polymer attached with the drug through hydrazone linker has gained its own importance due to the nature of cleavage of hydrazone bond at acid pH that of similar to cancerous cell pH. The important application of such linkers in next level of targeted drug delivery is highly utilized, due to the EPR effect, drug conjugates would tend to accumulate in tumors, and a pH-sensitive conjugate would release the drug inside the tumor.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The author declares no conflict of interest

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