We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Nano/Microparticles Encapsulation Via Covalent Drug Conjugation

Victoria Fasiku, Edith K. Amuhaya, Kingo M. Rajab and Calvin A. Omolo

Abstract

Advancement in chemistry holds a great promise in improving drug encapsulation that leads to superior drug delivery efficiency and the therapeutic efficacy of nano/micro-delivery systems. Drugs are being designed to specifically access the infection sites via covalent conjugation to nano/micro-delivery systems. This chapter focuses on techniques for achieving covalent encapsulation of drugs in nano/micro-delivery systems, how conjugation is applied to selectively influence pharmacokinetic profile, intracellular, and extracellular uptake, specific targeting to disease sites, binding to specific receptors, and controlled/sustained release. In addition, the effect of conjugation on drug efficacy and biosafety of the micro/nanoparticulate drug delivery systems are discussed.

Keywords: covalent conjugation, sustained release, smart responsive, targeted delivery

1. Introduction

For drugs to execute their effective mechanism of action, they require to reach its targeted site of action so that they can exert their intended action [1]. While conventional dosage formulations of drugs can achieve desired therapeutic concentrations, they are unable to effectively maintain the desired therapeutic concentrations, conferring a limited half-life thereby leading to ineffective treatment by the drugs [2]. Therefore, the development of novel drug delivery systems with the ability to improve on this limitation of the conventional drug delivery systems is needed.

Micro- and nano-carrier systems are among the approaches that have been successfully utilized for encapsulation of various types of drugs such as peptides, proteins, and low-molecular weight drugs [3–5]. These systems have been found to overcome limitations of conventional dosages forms such as improving solubility [6], bioavailability, and biodistribution of drugs [7], and targeting disease sites [8], hence contributing to a high proportion of the active drug reaching the targeted site. In addition, drug carrier systems protect the loaded drugs from premature degradation in the biological environment, thus enhancing bioavailability and cellular uptake. For effective delivery of drugs to occur, they have to be successfully loaded onto drug delivery systems as payloads. Two techniques are employed in

encapsulating drugs onto drug delivery systems. They include noncovalent physical encapsulation and covalent linking of the drugs to drug delivery systems.

Physical encapsulation of drugs into a carrier system involves hydrophobic interactions, electrostatic ionic interactions, and physical entrapping of drugs in the carrier matrix [9]. While the physical encapsulation of drugs into a carrier system is a popular technique, certain disadvantages are associated with it. For example, ionic complexation precipitation and dose dumping may take place if the effective attractions between the drug and the delivery system are reduced due to charge fluctuations and short-range attractions between monomers [10]. For hydrophobic drugs entrapped in the core of micelles, dose dumping may occur if micelles undergo hemodilution below the critical micelle concentration [11]. Moreover, challenges have been encountered in entrapping hydrophilic drugs in carrier systems using physical encapsulation [12]. These challenges have resulted in physical encapsulation with low loading efficiencies. Due to this, drug delivery scientists are resorting to covalently linking drugs to nano/micro-drug delivery system as an alternative method to physical encapsulation. This chapter discusses the techniques of covalent drug encapsulation, the mechanism of drug release from the covalent linkages, efficacy, and biosafety of drugs due to covalent conjugation and how disease site targeting via covalent conjugation is achieved.

2. Techniques of covalent drug encapsulation

Covalent drug conjugation involves attaching the drug into drug delivery systems via a physiologically labile bond [13]. A greater control over drug release is achieved by the covalent attachment of drugs to the drug delivery systems. Targeting and release of the drug from such systems is achieved through hydrolysable or biodegradable linkages between the payload and the micro/nanosystems [14]. Most commonly employed bio-hydrolysable bonds include amide, disulfide, ester, thiol, and carbamate bonds [15]. Conjugation of the drug to a delivery system may also include covalent linkers. The choice of a covalent linkers used is determined by its selectivity for drug release and the environment in which the drug should be released. Covalently conjugated drugs have exhibited the ability to release drugs by cleaving conjugated bonds under internal or external stimuli such as pH, redox potential, enzyme, light, and thermal energy [16].

The main advantages of covalent linking over physical encapsulation include the enhanced residence time of the drug in the body, slow release, improved biodistribution, and therapeutic efficacy, as well as reduced systemic toxicity [16, 17]. Covalent drug conjugation to micro and nanosystems is achieved via special bonds that are biodegradable or cleaved inside the body or a special environment at disease sites. Special linker moieties and functional groups of the drug dictate the success of conjugation on a nano/microsystem [18]. Based on the functional groups available on the delivery system and the drug being conjugated, several conjugation methods have been devised (**Figure 1**). The section below discusses the techniques of drug conjugations and their application in drug delivery.

2.1 Ester-linked drug conjugates

Ester bonds are widely used in conjugating drugs to drug delivery systems [19]. The ester bond is formed when a hydroxyl group and a carboxylic acid group react. Drugs with carboxylic groups can therefore be conjugated to hydroxyl groups of the drug delivery system and vice versa (**Figure 2**). Linkers or spacers such as a succinic anhydride may be employed to facilitate the conjugation [20]. Esterification of
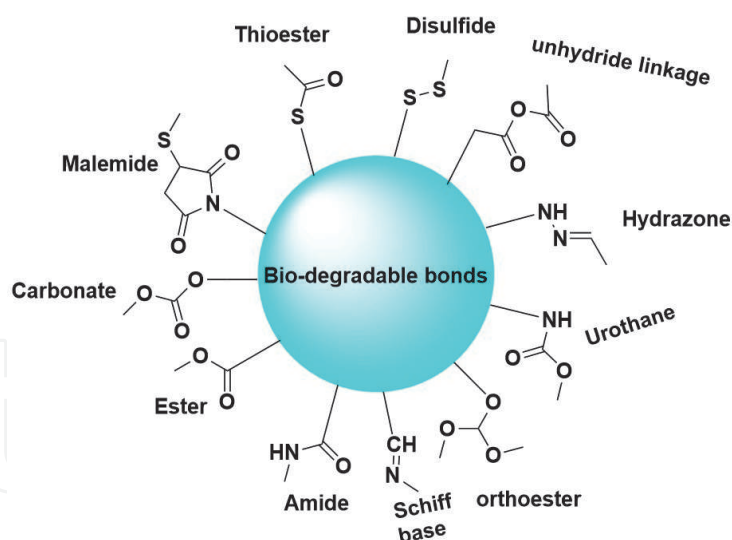
Figure 1.
Biodegradable bonds employed in covalent drug conjugation.
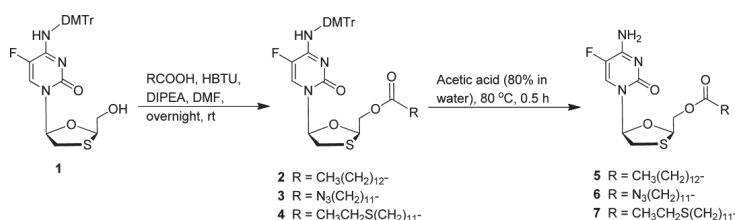
Figure 2.
Synthesis of the fatty acid ester-linked Emtricitabine. Adapted from [21]. DTMTr, 4,4'-dimethoxytrityl chloride; HBTU, hexafluorophosphate benzotriazole tetramethyl uronium; DPEA, N,N-diisopropylethylamine; DMF, dimethylformamide.

hydrophilic drugs with fatty acid is a popular technique to formulate self-assembling particulate prodrugs. This conjugation has shown to improve cellular uptake of hydrophilic drugs [22]. While viruses are intracellular obligate microorganisms, most of the drugs employed for their treatment are DNA nucleosides analogs which are highly hydrophilic with poor cellular uptake. Improving cellular uptake of these drugs usually improves their activity [23]. Agarwal et al. reported that conjugation of Emtricitabine (FTC) with myristic acid resulted to an analog that had 35.2 times higher activity than the nonconjugated drug against multidrug-resistant HIV viruses strain B-NNRTI and B-K65R [21]. These results indicated that antiretroviral ester conjugation with fatty acids could generate more potent analogs with a better resistance profile than its parent compound [24]. Similar results have been reported via esterification of fatty acids with lamivudine (3TC) [21] and acyclovir [25].

2.2 Amide and linked drug conjugates

Amide linkages can be used to covalently attach drugs to Nano/microcarriers using an anchor functionalized with carboxylic acid end groups [26]. Among the covalent linkages, amide bonds are the most widely used linkages to conjugate drugs to drug delivery systems. The conjugation is usually catalyzed by 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) or N, N′-dicyclohexylcarbodiimide (DCC) chemistry [27, 28]. The process involves reacting a carboxylic group with EDC and N-hydroxysuccinimidyl (NHS) to form an acyl amino ester that is subsequently reacted with an amine to create the amide bond.

EDC has good water solubility enabling its direct application in aqueous solutions without the addition of any organic compounds, thus making it suitable for the attachment of bioactive molecules to the carrier surface [29].

Several nano/micro-delivery systems with amide-linked drug conjugates have been widely reported with a great success. Such a system was reported by Yousefpour et al. (**Figure 3**) who conjugated doxorubicin and monoclonal antibody, trastuzumab to chitosan to form nanoparticles with high conjugation capacity, enhanced and selective uptake by human epidermal growth factor receptor 2 (Her2+) on cancer cells compared with the nonconjugated drug. Similar conjugation was reported by Kurtoglu et al. who conjugated Ibuprofen to PAMAM dendrimer and mPEG via amide linkages [30]. The drug conjugates showed better results when compared to bare drugs.

Monoclonal antibodies and derived therapeutics have also been linked with adverse effects and toxicity. The associated toxic effects of monoclonal antibodies
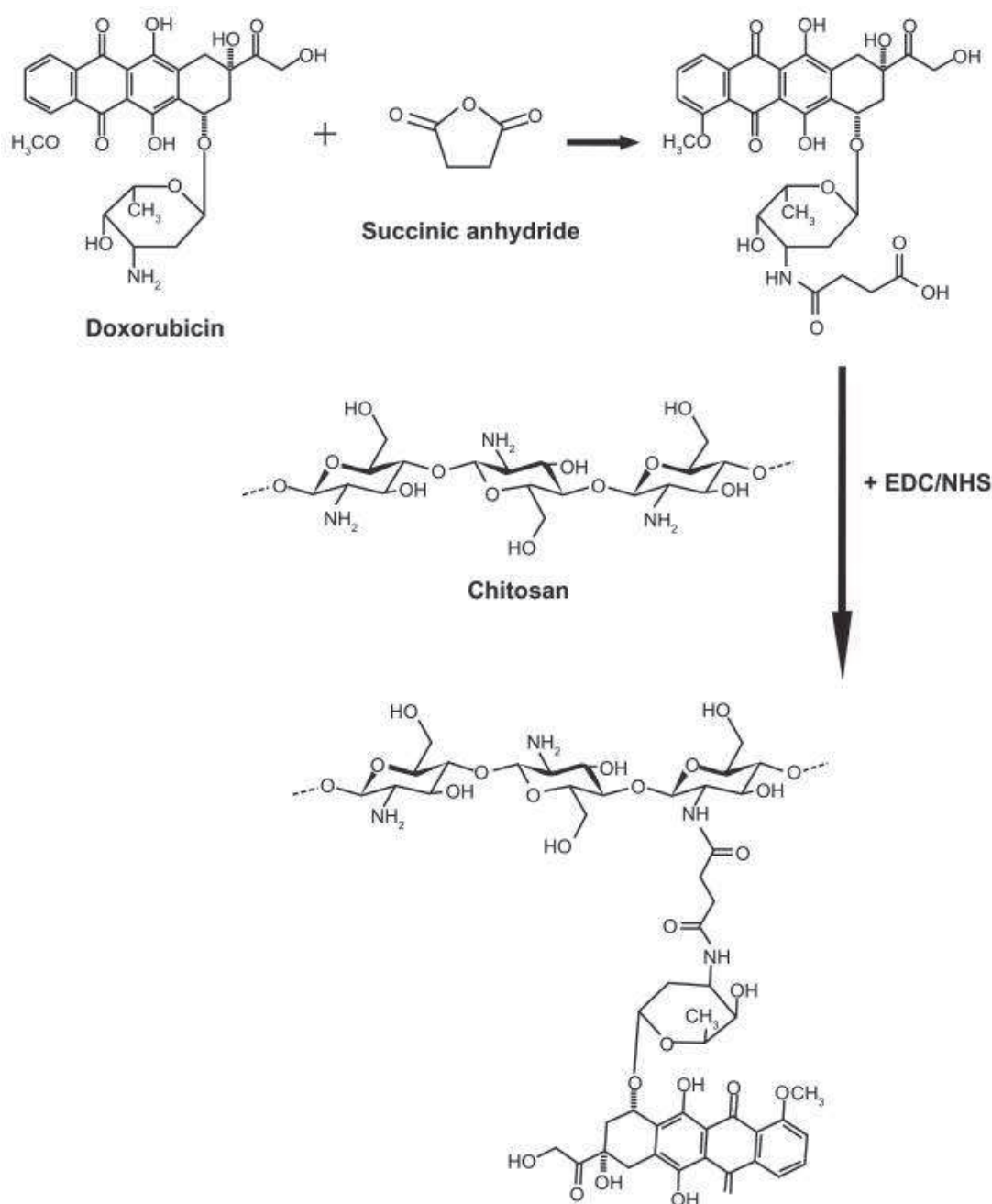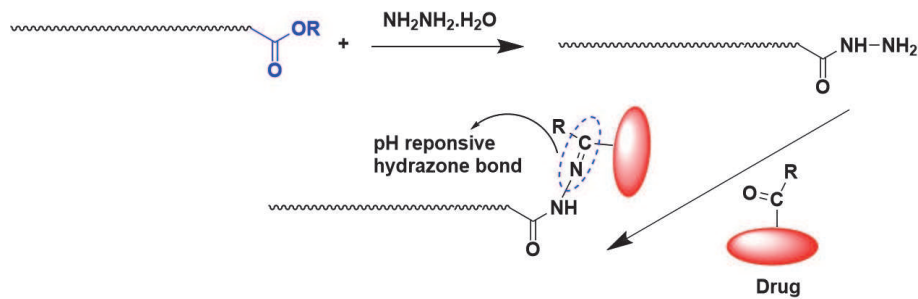

Figure 3.
Schematic description of the procedures for the amide conjugation between chitosan and doxorubicin to form a self-assembling nano-drug delivery system [29].

Drug	Drug delivery system	Main findings	Reference
Doxorubicin (DOX)	Self-assembled prodrugs	• Greater antitumor efficacy than free DOX. • High drug loading • Sustained drug release	[31]
Adriamycin	Micelles	• *In vivo* high anticancer activity • Low side effects	[32]
Camptothecin and Capecitabine	Nanofibers and spherical nanoaggregates	• Synergism of the co-conjugated drugs	
Penicillin V and Cephradine	Aggregates	• Similar activity to bare drugs • Low on side effects • Sustained release of the drug	[33]
DOX and trastuzumab	Nanoparticles	• Enhanced and selective uptake • High loading capacity • Reduction of drug side effects in Her2+ breast and ovarian cancers.	[29]
Gemcitabine	Self-assembled prodrug	• High loading capacity • Increased biological half-life of the loaded drug • Better activity than the bare drug	[34]

Table 1.
Drug loading via amide conjugation functionalized for varied clinical and research applications.

Figure 4.
Illustration of linking drugs to the delivery system via hydrazine bond.

(mAb) have limited their therapeutic application. However, antibody/drug covalent (ADC) conjugation-based platform has enabled selective delivery of a potent cytotoxic payload to target diseased cells, resulting in improved efficacy, reduced systemic toxicity, and preferable pharmacokinetics (PK), pharmacodynamics (PD), and biodistribution compared to traditional chemotherapy [35]. The success of such conjugations includes FDA approved Adcetris® which is a drug conjugate of Dolastatin 10 and monomethyl auristatin E (MMAE). The link between Dolastatin 10 and MMAE is N-terminal amine via the amide bond linked to a self-immolating spacer, p-aminobenzyloxycarbonyl (PABC).

Apart from ADCs, amide bonds have been employed to link mAb to other drug delivery surfaces such as liposomes. Liposomes with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol) (DSPE-PEG-COOH) linked with mAb have been reported to prepare immunoliposomes. The attachment of mAb to the liposomes was achieved via surface —COOH on the surface of liposomes and —NH_2 of the mAB. This conjugation enhanced significantly the blood residence time of the mAb [36]. Another amide conjugation between targeted ligand and liposome was reported where the peptide was covalently attached to the

carboxylic groups on the PEGylated liposomes to form a nanoparticulate system able to target the infarcted heart. The system was effective in *in vitro* testing against cardiac cells [37]. Due to their stability, the rate of hydrolysis of amide bonds is lower when compared to ester bonds. This slower rate of hydrolysis affects the release of drugs, thus affecting the activity of the conjugated drugs [31]. The amide conjugation of drugs to drug delivery systems is summarized in **Table 1**.

2.3 Hydrazones conjugates

Hydrazones are formed by the action of hydrazine on ketones or aldehydes functional groups [38]. Their basic structure is $R_1R_2C=NNH_2$ which is formed when oxygen in ketones and aldehydes is replaced with the $-NNH_2$ functional group. pH-sensitivity attributes of hydrazones bond have been used in the formation of stimuli responsive nano/micro-drug delivery system. At a lower pH, the bond decomposes efficiently while at basic pH, hydrazones are usually stable [39]. The instability of hydrazone bonds in acidic mean molecules can be cleaved in acidic intracellular environment of endosomes or lysosomes, tumor tissues, and bacterial infection sites. Hydrazone bonds have been successfully used to covalently load drugs into delivery systems resulting to pH-responsive nano/micro-dosage forms that can effectively target a disease that alters physiological pH to acidic (**Figure 4**) [38, 40, 41].

2.4 Thioether linkage

Thioether bond is formed from the reaction between the thiol group containing SH group and first carbon of maleimide that is attached to the drug carrier [42]. Conjugation via thioether bonds is favored technique as the bond is formed under mild conditions, at room temperature, and in aqueous solution [26]. Thioether linkage makes it possible to link peptides to a delivery system or drugs to peptide (**Figure 5**). Several drug delivery systems have been reported to employ thioether linkage as a means of covalently loading drug on to them. mAb trastuzumab and nanoparticle doped with doxorubicin were successfully loaded in a drug delivery system via thioether linkage [43]. DOX was conjugated to a drug delivery system via thioether bond through poly(ethylene glycol) polymer having two linkers of maleimide and n-hydroxysuccinimide (nhs). The conjugates showed better cancer uptake when compared to free DOX. The better uptake was attributed to better affinity of the system to the HER2 receptor of breast cancer cells. When compared to the free drug, the conjugated delivery system had a longer blood circulation with less toxic effects when compared to the free drug [28]. Similar results were reported by Park et al. who formulated immunoliposomes conjugated with monoclonal anti-bodies (mAB) [44]. Additionally, another liposomal covalent system has also been reported by Kirpotin et al.; in the system, a free thiol group was used to conjugate antibodies to the nanocarriers. The carrier showed increased cellular uptake resulting in better tumor reduction [45]. Thioether linkage has also been applied successfully on carbon nanotubes functionalized by folic acid. The system employed in targeted delivery of DOX against cancer [46]. From literature reports, thioether-
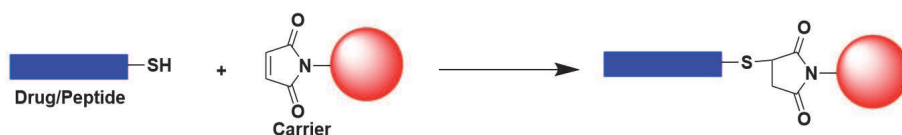
Figure 5.
Illustration showing maleimide thiol covalent linkage to drug delivery systems.

linked drug delivery systems can be effectively employed in targeted delivery of the conjugated drugs.

2.5 Disulfide linkage

Two thiol groups' conjugation results in the formation of a disulfide bond. One group originates from a nanocarrier and the other from a ligand [47]. Disulfide drug conjugates have shown to be stable in the extracellular environments but easily broken down in intracellular reductive intracellular environment. There is an increasing number of drug formulations that incorporate disulfide bonds being reported, for nano/micro-drug delivery system. Disulfide bonds are being designed to exploit differences in the reduction potential at disease location and the whole body at large [18]. Formulating environmentally responsive drug delivery systems has been made possible due to the desirable attribute of disulfide bonds. Lu et al. using a disulfide-bridged created mesoporous silica nanoparticle covalently loaded with folic acid (FA) and decorated bull serum albumin (BSA) for improved tissue biocompatibility and effective dual pH/glutathione (GSH) response drug releasing drug delivery system. Disulfides have also been employed as cleavable linkers in drug conjugates or to formulate stimuli-responsive carriers, and this has resulted in disulfides linker-based mAb drug covalent linkages (ADCs) in clinical trials [48].

2.6 Other covalent linkages

Other covalent linkages that include carbamate linkage, Schiff bases, and polycyclic linkages have also been employed to form covalent linkages between the drug and delivery systems. Reaction between a diene and dienophile results to cycloaddition via the Diels-Alder chemistry which forms bicyclic compounds. This chemistry can be utilized to form polycyclic linkages between the drug and the delivery system [49]. Whereas other hydrolyzable linkages include carbamate [50, 51], oximes [52], and Schiff bases [53, 54]. These types of linkages are specifically designed to make drug delivery systems have targeting ability due to the physiological changes brought about by the diseases.

3. Self-assembly of covalent conjugated drugs

Roughly 70% of new drug discoveries have shown poor aqueous solubility, while approximately 40% of the marketed immediate-release drugs are practically insoluble [55]. Additionally, the drugs that are highly soluble have been found to have membrane penetration difficulties [56]. Covalent modification of therapeutic compounds is therefore a strategy that enhances efficacies of the conjugated drugs by solving physicochemical problems associated with the drugs [57]. When hydrophobic drug molecules are attached to hydrophilic material or when hydrophilic drugs are attached to hydrophobic biomaterial or delivery systems, an amphiphilic system is formed. The resulting amphiphilic system can self-assemble into stable core-shell aggregates such as vesicles, classical micelles, unimolecular micelles, and nanorods [2, 58].

When amphiphiles are dispersed in water, the hydrophilic component of the amphiphile preferentially interacts with the aqueous phase (shell) while the hydrophobic portion tends to reside in the air or in the nonpolar solvent (core) in order to form stable assemblies [59]. Self-assemblies of drug conjugates are usually governed by forces such as hydrogen bonds, Van der Waals interactions, hydrophobic interactions, and electrostatic interactions [2]. Self-assembled drug conjugates often
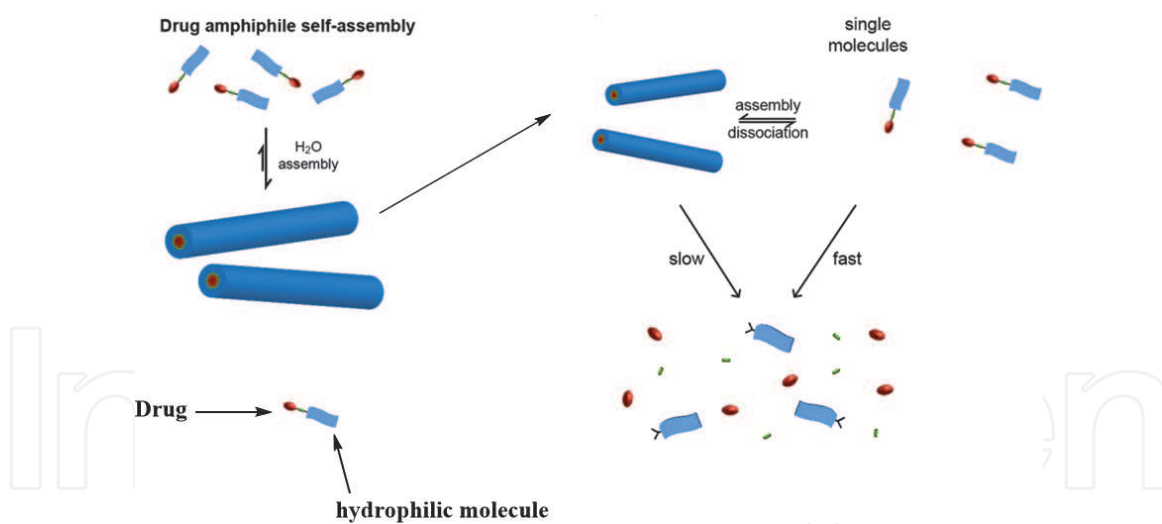
Figure 6.
Schematic representation of self-assembly of drug conjugates and subsequent release mechanism of the drug from the self-assembly. Adapted with permission from [60].

result to effective therapies as they possess better physicochemical properties that lead to enhanced drug penetration for highly hydrophilic and charged drug molecules [61], enhanced solubility for highly insoluble drugs, and increased residence time for drugs that are easily eliminated via the kidney [62]. For instance, most of anticancer drugs are hydrophobic in nature, and therefore, to produce self-assembled nanostructures with better therapeutic and formulation aspects, hydrophilic molecules or polymers are usually attached to them via a degradable linker to induce amphiphilicity and self-assembly (**Figure 6**) [61]. Self-assembly widely occurs in nature and has been borrowed by science to formulate self-assembled nano/micro-drug delivery systems with better therapeutic outcomes than original drug molecules [63].

4. Mechanism of drug release from drug conjugates

Covalent linkages alter the absorption, distribution, metabolism, and elimination (ADME) properties of an active drug [64]. Before conjugation, it is paramount to have a complete understanding of the physicochemical, structural relationship activity of the drug candidates. It is also important to understand the ability of the attached groups to cleave, leaving and exposing the functional groups responsible for the activity of the drug [65]. Moreover, once the drug is cleaved from the delivery system, the delivery system should be inactive and nontoxic [66]. Most of the drawbacks from covalent linkages of drugs to the delivery systems are the inability of the drug to cleave from the delivery system. The inability of the drug to detach from the drug delivery system may lower the activity of the drug due to poor bioavailability [67]. Therefore, the chosen covalent linking technique should have the ability to easily cleave to enable the release of the drug.

Esterification is a common technique for conjugation because esterases are widely distributed in body tissues that easily cleave the ester bonds leaving the free drug to act. Esterase is a hydrolase enzyme that splits esters into an acid and alcohol in a chemical reaction with water called hydrolysis [68]. The easy cleaving of esters makes the use of ester linkages as an attractive technique. Breakage of amide bonds is via hydrolysis of the carbon-nitrogen bond, and this results in a carboxylic acid and either ammonia or an amine [69]. This cleavage of amides is

responsible for the drug release from the drug delivery systems. It is important to note that amides are stable bonds, which do not hydrolyze at physiological pH and body temperature.

Disulfide bonds usually exploit differences in the reduction potential at different locations within and upon cells to release the conjugated drugs. Due to this, different delivery platforms have been designed to achieve different targeted delivery strategies. Redox enzymes reduce disulfide bonds on and inside the cells resulting in drug release [18]. Other bonds like hydrazones, Schiff bases hydrolysis is catalyzed by acid environment. In an acid environment, the bond between the drug delivery system and the drug is broken to release the drug. Apart from direct drug linkage to the delivery systems [70], suitable linkers that are self-immolating such as p-aminobenzyloxycarbonyl (PABC) can also be involved in the reaction. The purpose of the linkers is to situate the cleavable delivery system away from the drug to allow facile release. Upon cleavage, the linkers rapidly fragment, leading to the release of the drug in a chemically unmodified form [71, 72] (**Table 2**).

5. Efficacy and biosafety of drugs due to covalent conjugation

Particulate drug delivery systems have recorded significant progress in the delivery of small molecular drugs; however, some challenges such as poor drug loading, formulation instability, premature drug leakage, and poor blood circulation are still encountered. This has led to the discovery of newer strategies that can be used to overcome these challenges. Over the years, various research groups have explored the efficacy and biosafety of drugs that were covalently conjugated to nano/micro-delivery systems [73]. These include systems developed from polymers, dendrimers, and peptides among others; usually, the drug is bond to the biomaterial via a linker. The efficacy of drugs covalently conjugated has been significantly improved in terms of drug loading capacity and stability amongst other benefits. Biomaterials such as polymers that are covalently bonded to drugs have shown to be useful drug carriers which help to hold drugs and are tunable to increase the efficacy of the drug [74, 75].

The other advantages drug conjugation provides include increased solubility of the drugs that are insoluble in water, thus enhancing a controlled release of the drug as there will be increased permeability through lipophilic tissues. This will, in turn, lead to an increased effective concentration of the drug at the targeted site [75]. Additionally, drug conjugated covalently to biomaterials are shielded from degradation or deactivation as well as increases the circulation time of the drug [76]. One or more of these advantages offered by drug conjugation has been reported by various groups of researchers. For instance, the efficacy of different poly(ethylene glycol) (PEG)-based anticancer drug conjugates has been extensively explored [73, 77, 78]. The details of the importance of nano/micro-delivery systems such as lysosomes, polymeric micelles, and polymeric nanoparticles in drug delivery applications have also been discussed [79].

In a study [80], hydrazine-based doxorubicin-polymer conjugates were synthesized into doxorubicin-loaded nanoparticles. It was reported that the bioactivity of the drug was largely retained *in vitro*, and there was a tremendous reduction in systemic toxicity of doxorubicin upon nanoparticle conjugation *in vivo* when compared to the physical formulation of the drug. In addition, the nanoparticles prevented the drug from disassembling upon interaction with serum proteins in the blood [81, 82]. The *in vitro* study showed that the doxorubicin-nanoparticle conjugate accelerated the release of the drug in acidic conditions and killed the cancer cell. In the same vein, Modarassi and colleagues investigated the drug release

Bond	Mechanism of cleavage	Method of hydrolysis	References
Esters		Cleavage in the body is catalyzed by esterases	[83]
Amides		The bond is cleaved via hydrolysis. Sometimes catalyzed by enzymes like esterase, hydrolase, serine and cysteine proteases, peptidases, antibody Fab-BL 125 and RNA	[84–90]
Hydrazones		Cleavage is via acid-catalyzed hydrolysis	[91]
Disulfide		Bond cleavage is achieved via acid and base catalysis and redox potential hydrolysis catalyzed by various classes of reductases enzyme in the presence of excess reduced glutathione (GSH) and thioredoxin Reductase	[18]
Thiol-maleimide		Drug release is via ring-opening hydrolysis and thiol exchange	[92, 93]

Table 2.
Cleavage mechanism resulting in drug release of common bonds employed in covalent conjugation.

behavior of doxorubicin that was conjugated onto the structure of nanoparticles. The conjugation was achieved via an acid-labile hydrazone linkage, and the effect of conjugation was compared with the nonconjugated drug [94] (**Figure 7**). The results of the *in vitro* investigation revealed that the release of doxorubicin was dependent on the amount of crosslinker. The higher the amount of crosslinkers, the lower the cumulative drug release in the physically loaded drug. On the other hand, drug conjugation showed that an increase in the amount crosslinker within the structure led to an increased rate and amount of drug released. This implies that the efficacy of drug conjugation with respect to drug release is superior to nonconjugated drugs.

Another drug PEG conjugate that has shown enhanced drug efficacy is the paclitaxel (PTX) conjugated with polyethylene glycol (PEG-B-PTX), synthesized by Dong et al. [95]. The antitumor efficacy of the stable micelle of about 50 nm, and 13.3 wt% drug load content was investigated against human glioma and breast cancer cells *in vitro*. The conjugate micelle exhibited improved antitumor effects when compared to the clinically used taxol. This result suggests that the drug conjugate can be a superior alternative for current clinically used PTX nanoformulations which has limitations such as poor *in vivo* stability, premature release, and little improvements in its antitumor efficacy [96, 97]. A detailed *in vivo*
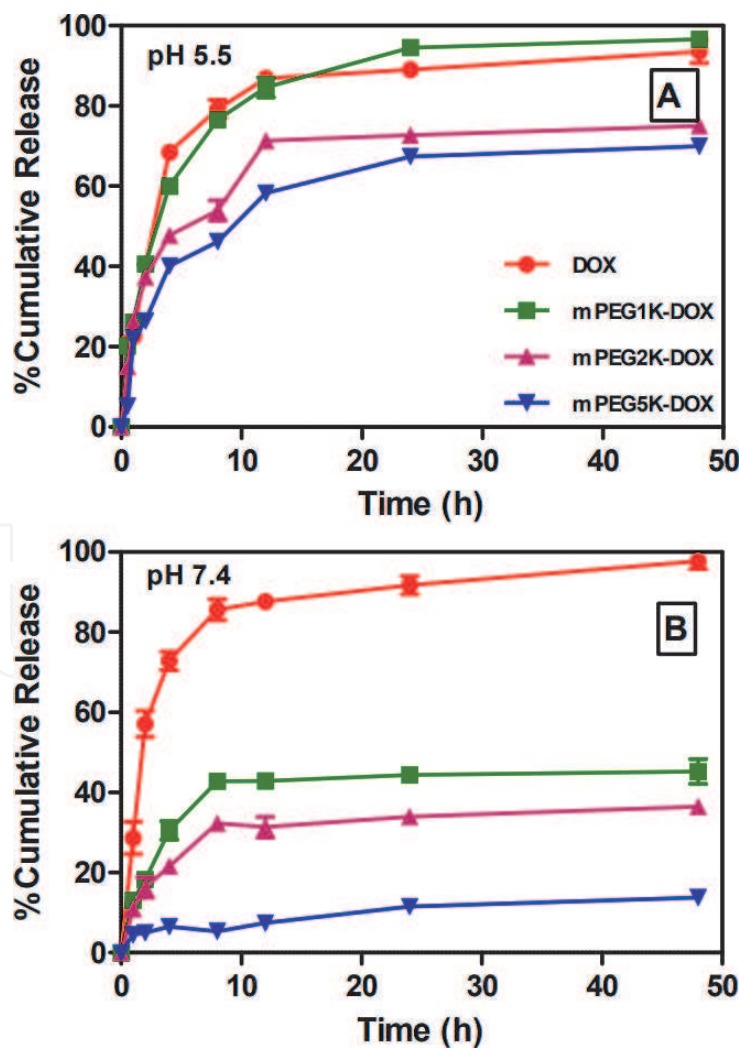
Figure 7.
A) The hydrazone acid-labile DOX release behavior from nano/microparticles at pH 7.4. (B) The hydrazone acid-labile DOX release behavior from nano/microparticles at pH 5.5. Sustained release slow drug release at pH 7.4 when compared to acidic pH. Overall slower release when compared to nonconjugated DOX. Adapted with permission from [98].

experiment by the same research group further revealed that PEG-B-PTX showed prolonged circulation time as well as enhanced *in vivo* antitumor efficacy (tumor inhibition rate of 89.4%) with low side effects. This observation can be attributed to the favorable pharmacokinetic profile and tumor-specific release of the drug from the drug conjugate. This promising study has prompted other groups of researchers to investigate the efficacy of other stimuli responsive PTX conjugates [99–103]. This same set of researchers [95] went further to demonstrate that hydrophobic drugs can be conjugated with a short water-soluble polymer or peptide chain to make the drug amphiphilic. It was proven that the self-assembled nanovehicles are suitable for the delivery of the drug and even co-delivery of other drugs as reported by other research groups [104, 105]. The effects of this approach are well-characterized chemical structures, accurate and reproducible drug loading efficiency (i.e., 100%), fixed, and high drug loading contents. Also, burst release of drugs associated with physically drug-loaded micelles can be prevented [106]. These attributes are very important and favorable for clinical translation; therefore, these conjugates have great potential for clinical application.

Furthermore, other studies have buttressed the potential excellent effect of drug conjugates in combating diseases as seen in the experiment done by Li et al. [107]. Camptothecin and doxorubicin were loaded onto a polymer, and the efficiency of the drug conjugate was explored. A synergistic drug delivery which improved the anti-cancer efficiency of the drugs was reported. The *in vitro* stability study showed that the drugs were stable with 80% drug loading after 4 weeks at a storage temperature of 4°C. In addition, the *in vitro* studies showed an approximately 30% increase in the cellular uptake of the conjugated drugs into the cancer cells when compared to the free drugs. It is noteworthy that a superior anticancer efficacy was observed in the combined drug conjugate, and the enhanced synergistic effects of about 23.9% was attributed to the good better stability profile, internalization by cells and pH response. Other benefits of the drug conjugate observed from this study include suitable sizes and good water solubility. This led to the greater penetration of the drugs into the solid tumors, thus improving the overall efficacy of the drug conjugate when compared to the free drugs. From these results, it is suggested that DOX-CPT conjugated to nano-delivery systems has the potential to provide synergistic anticancer treatment.

The superior efficacy of drug-conjugated covalently compared to nonconjugated drugs has also been proven by Tang and coworkers via conjugating sorafenib with polyethylene glycol (PEG) nanoparticles. The efficacy of the drug conjugate (SFP) was evaluated on cancer cells in an *in vitro* antitumor experiment [108]. The result showed that SFP had an excellent antitumor activity due to the self-immolative release of the intact drug inside tumor cells caused by the GSH-responsive disulfide linker thus suggests that SFP may be a potential candidate for cancer treatment. Additionally, it was reported that the covalent drug conjugation prevented drug leakage and improved drug stability. SFP is therefore a promising nano/micro-carrier for safe drug delivery which may pave room for new opportunities to explore other drug conjugates. Among other studies, Daniel et al. have reported that drug conjugates especially polymer conjugates are potentially suitable for the delivery of antiviral drugs [109, 110]. A typical example is the synthesis of poly(lactide-co-glycolide) nanoparticles loaded with a combination of reverse transcriptase and protease inhibitors. Such drug conjugates have been reported to be effective in preventing the replication process of HIV replication [111, 112]. This implies that drug conjugates are a promising approach in improving the therapeutic efficacy of therapeutic agents, and this may encourage their clinical translation.

Irrespective of the excellent efficacy of any drug conjugates, before their application, it is very important that the biosafety is proven and confirmed to be harmless to the human system. Hence, it has become necessary that developed drug conjugates are evaluated for its biosafety. This has led to the investigation of the toxicity of different drug conjugates by various researchers. For instance, an *in vitro* cytotoxicity study was carried out by Tang and fellow workers, and the cytotoxicity of the drug-conjugate was assessed using the MTT assay method [108]. The study investigated the cytotoxicity effect of sorafenib-polyethylene glycol (PEG) nanoparticles conjugate (SFP) on Hela and HepG2, respectively, after incubation for 48 h at 37°C. The result showed a dose-dependent cytotoxic effect of free sorafenib (SF) and SFP on both cell lines, and no significant difference in cytotoxicity was observed for SF and SFP on both cell lines. Nonetheless, a higher cytotoxicity of SFP was displayed between the concentration ranges of 5–15 μM when compared to free SF. The higher *in vitro* cytotoxicity of SFP observed at those concentrations may be due to the increased intracellular localization of SFP nanoparticles. This suggests that the conjugation of sorafenib with a polyethylene glycol (PEG) nanoparticle does not have a negative influence on the toxicity as seen in the favorably cytotoxic effect on both cell lines. Also, the ability of SFP to serve as a biosafe anticancer therapeutic agent was further confirmed in the hemocompatibility and histological safety results. These nontoxic properties of the drug conjugate can be attributed to the outer PEG shell of SFP.

Furthermore, drawbacks such as dose-dependent toxicity have been reported with drug conjugates; therefore, in an attempt to minimize toxicity, Li et al. have evaluated the effect of combining drugs onto a nano-drug delivery system on toxicity [107]. The cell cytotoxicity of the DOX and CPT conjugate versus the free drugs showed enhanced uptake in the cancer cells but reduced in the normal cells exposed to the drug-conjugate. Additionally, it was reported that the side effects of the drugs (doxorubicin and camptothecin) employed in the study decreased by reducing the dosage of the drugs. The toxic side effects of the drugs were alleviated, and it was also observed that the multidrug resistance (MDR) was reversed. This may be attributed to the synergistic effects of multiple therapeutic agents [113, 114]. Remarkably, to assess the general biosafety of drug conjugates, Ibrahim et al. have gone further into *in vivo* investigation of the drug conjugates developed by their group [115]. The organs of mice exposed to these drug conjugates for 21 days were sliced and analyzed histologically. It was noted that exposure to the tissues (heart, liver, spleen, lung, and kidney) did not result in any tissue damage; hence, the biocompatibility of the formulations was confirmed. Other studies that reported little or no toxic side effects of drug conjugates are Dong and Lu with their co-workers, respectively [96, 116].

In the cytotoxicity study carried by Lu et al., pullulan which is a natural biocompatible polysaccharide was used to synthesize a novel pH-sensitive nanoparticle drug delivery system for the delivery of doxorubicin (DOX) [116]. The chemical structure of the pullulan/DOX conjugate nanoparticles was assessed using FTIR and 1H NMR and further investigated for *in vitro* drug release and cytotoxicity activities, respectively. The result of the release behavior *in vitro* showed that a faster release of DOX was released from the drug delivery at pH 5.0 than at pH 7.4. It was observed that lower concentrations of the drug (DOX) were more cytotoxic to 4 T1 cells than pullulan/DOX conjugate nanoparticles at a concentration range of 0.01–5 mg/l. This may be due to the ability of free DOX to readily transport into the cells via passive diffusion [117]. On the other hand, low cytotoxicity was reported for DOX released from the nanoparticle. This may be due to a time-consuming DOX release from nanoparticles and delayed nuclear uptake in 4 T1 cells [118]. Lin et al.
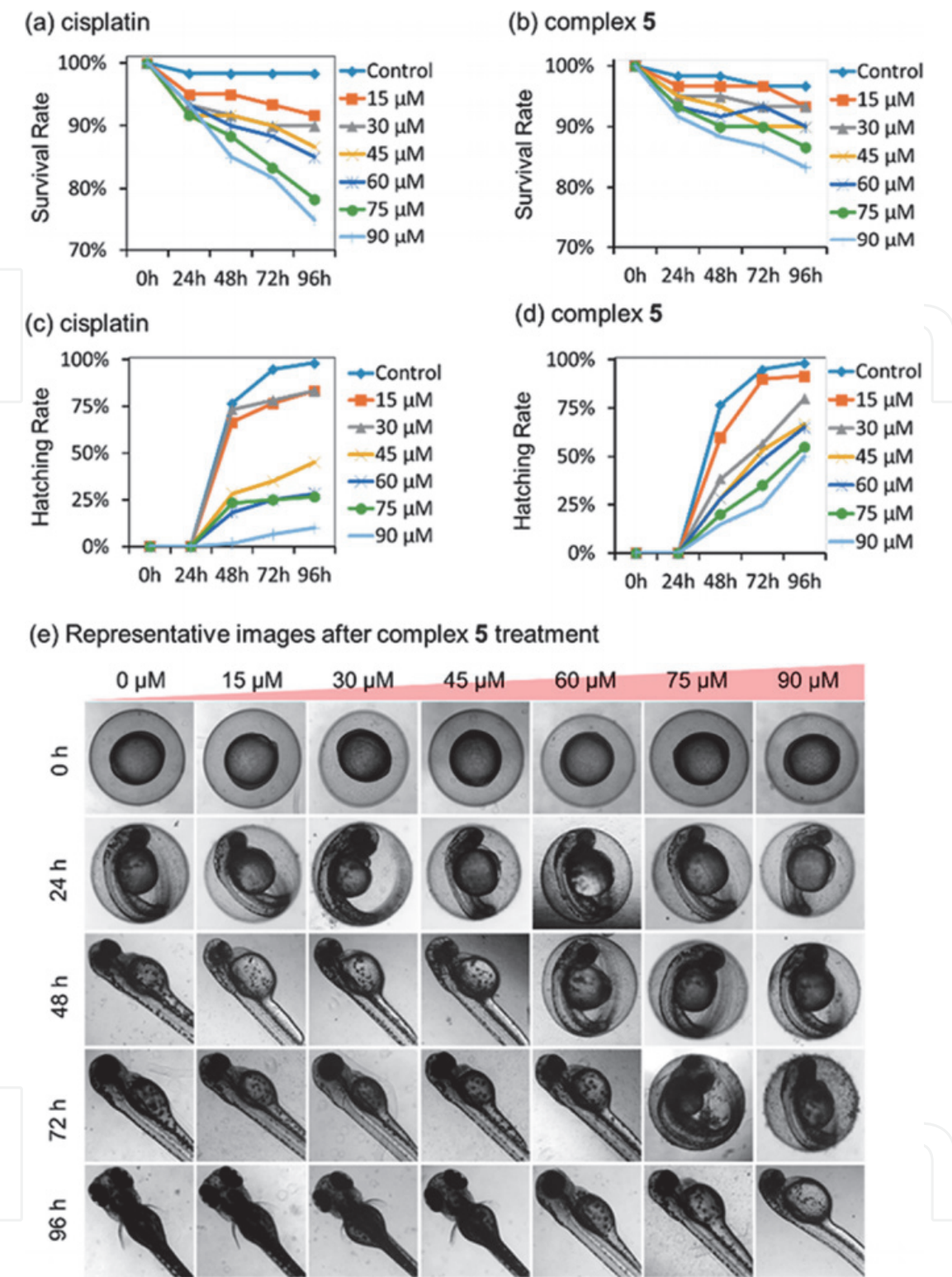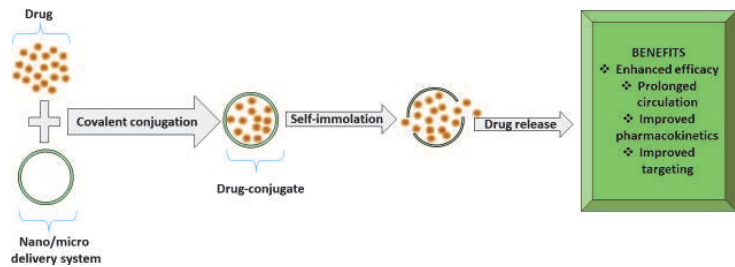
Figure 8.
Biosafety evaluation of free cisplatin and prodrug cisplatin using zebrafish embryos. Survival rates of embryos in the presence of (a) the bare drug and (b) prodrug. Hatching rates of zebrafish embryos after the exposure to (c) the bare drug (d) prodrug. (e) Pictogram representation the embryos with treatment of prodrug at different concentrations over a period of 96 hours.

improved biosafety of profile of cisplatin by converting it a prodrug. The prodrug has higher survival rate of the zebrafish embryos [119] (**Figure 8).**

From the observations made in these studies, it is clear that drug conjugation impacts positively on the efficacy of the drug as well as its biosafety/toxicity. The biosafety concerns are greatly eliminated or minimized by conjugating drugs to biomaterials. Some unwanted side effects, toxicity, and organ damage associated with the fluctuations that arise from periodic drug administration can be avoided by

drug conjugation [120]. Surprisingly, it appears that a substantial number of anti-cancer drugs and polymers are the most explored in covalent drug conjugation compared to other therapeutic drugs and biomaterials. This may be because polymer-drug conjugates provide more advantages in enhancing stability, increasing water solubility, and prolonging blood circulation [121–123]. Despite these advantages, certain drawbacks such as difficulty to accurately control the reaction site and the degree of conjugation have been associated with polymer-drug conjugates. Thus, the ability to reduce the heterogeneity and batch-to-batch difference of the product remains a challenge [75, 124]. It is therefore suggested that more studies to be done to overcome these challenges; this will provide more information on the efficacy and biosafety of drug conjugates (**Figure 9**). Furthermore, reports from these studies revealed that the efficacy and biosafety of the drugs conjugated onto various nano/micro-delivery systems were significantly enhanced when compared to the free drugs. Other nano/micro-delivery systems and drugs that have been explored are summarized in **Table 3**. The table highlights the key findings of various nano/micro-delivery systems that have been reported by different groups of scientists.

Figure 9.
Key benefits derived from drug conjugation.

Nano/micro-delivery system	Drug	Target	Outcome	References
Polyglutamic acid	Paclitaxel	Nonsmall-cell lung cancer (NSCLC)	Paclitaxel poliglumex reduced the systemic exposure to peak concentrations of free paclitaxel. In addition, the drug-conjugate produced similar survival to docetaxel as second-line treatment in NSCLC with less febrile neutropenia and alopecia and greater ease of administration.	[125]
PEG-b-PCC poly(2-methyl-2-carboxyl-propylene carbonate) polymer	Gemcitabine and dodecanol	Pancreatic cancer	*In vivo* studies showed a significant increase in the effectiveness of drug-conjugate when compared to the free drug.	[126]
Hyaluronic acid (HA)	Cisplatin	H1299, H358 cell lines and mice	HA-cisplatin conjugate bounded to CD44 expressing cancer cell lines (H1299 and H358). The drug-conjugate was more effective in killing lung tumors in mice when compared to the free drug	[127]

Nano/micro-delivery system	Drug	Target	Outcome	References
Poly(styrene-comaleic acid)	Neocarzinostatin	Liver and renal cancer	Arterial infusion therapy with poly(styrene-co-maleic acid)-conjugated neocarzinostatin SMANCS/Lpd showed to be effective for large renal cell carcinoma	[128]
Poly(methyl methacrylate) (PMMA) polymer	Gemcitabine	A549 cell-derived xenograft murine model	*In vivo* experiment showed that the Gem-conjugates reduced tumor growth by 68% with little toxicity while free Gem had no effect but significant toxicity.	[129]
PEG	Interferon α2a/b	Hepatitis B	The HBsAg clearance rate was significantly greater in the group treated with drug-conjugate compared to the standard therapy group at 24 and 48 weeks post-treatment (33.3% vs. 10.5% and 35.7% vs. 10.5%, respectively; $P < 0.05$ for both).	[130]
Glycol chitosan (GC)	Heparin	Lungs, mice	GC-heparin conjugates were safe in the lungs and revealed comparable blood coagulation times compared to free heparin	[131]
Chimeric peptides (CPs)	Doxorubicin	4 T1 and Lewis lung cancers	Increased intratumoral accumulation of the conjugate with a curative effect in 60% of the treated mice was observed	[132]
Poly(amidoamine) (PAMAM)	Doxorubicin	Mice bearing melanoma (B16-F10) lung metastases	Prolonged lung retention of drug-dendrimer conjugate compared to free drug. Improved chemotherapeutic activity on the lung of mice compared to the free drug	[133]
Poly(2-ethyl-2-oxazoline)	Rotigotine	Parkinson disease	POZ-conjugated rotigotine showed the potential to be viable for subcutaneous treatment for PD patients.	[134]
Glycol chitosan	Doxorubicin		Drug-conjugate accumulated within tumors via the EPR effect, and a significant antitumor activity was observed compared to free doxorubicin	[135]
Carbopol®(CP)	Calcitonin	A549 cells; rats	Drug-conjugate showed no signs of toxicity and maximally lowered blood calcium levels when compared to calcitonin alone	[136]
Carboxymethylcellulose	Docetaxel	EMT-6 breast cancer	Biodistribution studies showed a 5.5-fold greater tumor accumulation of drug-	[137]

Nano/micro-delivery system	Drug	Target	Outcome	References
			conjugate compared to the clinically administered DTX formulation (Taxotere). Drug-conjugate also showed a two-fold improvement in anticancer activity in a murine EMT-6 breast cancer model compared to Taxotere.	
Hyaluronic acid,	Paclitaxel (PTX)	Breast cancer	PTX conjugate showed more enhanced *in vivo* tumor inhibition effects compared to free PTX.	[138, 139]
PEG-b-poly(glutamic acid) micelle	Oxaliplatin	Mouse model of human carcinoma cell line KB.	The antitumor efficacy of drug-conjugate was superior to that of oxaliplatin. Also, the animals did not develop acute cold hypersensitivity, which is frequently experienced by patients after oxaliplatin administration.	[140]
PEG	Alendronate	Lung mucosal	The drug-conjugate suppressed lung mucosal toxicity after pulmonary delivery, whereas the administration of the free drug-induced significant toxicity.	[141]

Table 3.
*Key observations made from other drug conjugates.*

6. Effect of covalent drugs conjugation on the pharmacokinetic profile of the drug

The pharmacokinetic profile of drugs is a very crucial aspect that is considered for clinical application. It is interesting to observe that the pharmacokinetic profile of drugs is becoming better due to covalent drug conjugation as demonstrated by drugs encapsulated to nano/micro-delivery systems. The conjugation of drugs to biomaterials has opened opportunities to alter the pharmacokinetics and biodistribution of the drugs within the human body [120]. The alteration of the pharmacokinetics of the drug offers advantages such as prevention of the rapid clearance or metabolism of the drug. In addition, the drugs are carried to the targeted site of pharmacological action. Drug conjugation to nano/micro-delivery system has shown to be a powerful technique that can alter the pharmacokinetic profile of the drug, thus minimize the side effect of various anticancer drugs such as doxorubicin.

One of the studies that reported the enhanced pharmacokinetic properties of anticancer drug encapsulated to a nanoparticle is that conducted by Vandriess et al. [80]. It was observed that the nanoparticles containing the drug enhanced the drug accumulation and a subsequent reduction of tumor growth in an *in vivo* zebrafish model. Also, another study has revealed that self-assembling drug polymer conjugates which allows a covalent attachment of the drug to the hydrophilic part of the polymer can improve the pharmacokinetic profile of drugs. Thus, covalently

conjugating drugs to delivery systems improves pharmacokinetic profiles and physicochemical problems associated with certain free drugs [142]. Benefits from covalent conjugations include prevention of rapid renal clearance, and improved drug solubility is derived. It is however important to note that the attachment of a large number of hydrophobic drugs to nanocarriers such as polymers may result in unwanted aggregation and precipitation in some cases [143].

In one of the first studies, research groups such as Kataoka et al. took advantage of the self-aggregation behavior of drug conjugates to develop a micelle forming drug conjugate [144]. In their study, doxorubicin (DOX) was conjugated to a poly (ethylene glycol)-poly(aspartic acid) block copolymer (PEG-b-P(Asp(DOX)), and the pharmacokinetic profile was investigated. It was observed that there was no interaction between the drug and serum albumin, which is known to bind to the DOX. The inability for the drug and the serum albumin to interact indicates the shielding ability of the nano/micro-delivery system which led to a good biodistribution and therapeutic effect of the drug. Other subsequent studies by the same research group aimed to improve the synthesis [145] and pharmacokinetics profile [146, 147] of the same drug-polymer conjugate. The drug conjugate was designed via an amide bond which can only cleave to release the drug by enzymatic action. The results showed that the drug conjugate had a better pharmacokinetic profile when compared to the free DOX. Interestingly, the conjugate was better tolerated despite needing a higher dose to achieve the same effect as free DOX.
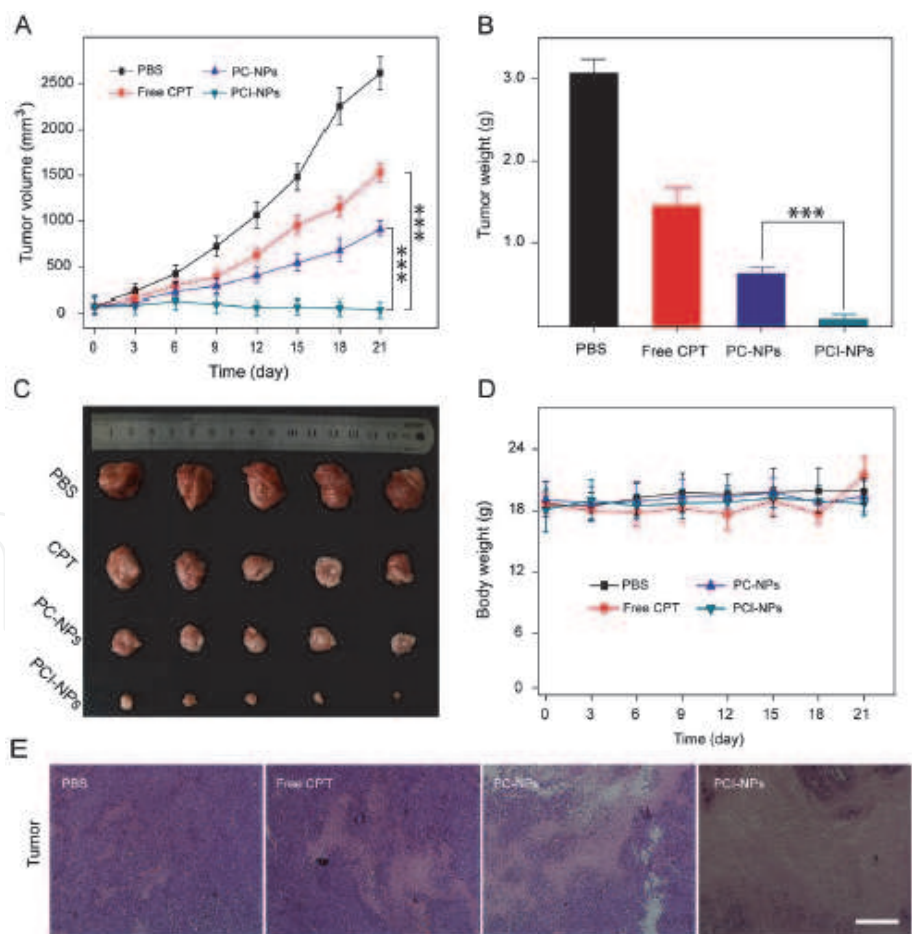
Figure 10.
Control (phosphate buffer system), camptothecin (CPT), and micellar nanoparticles drug conjugates (PC-NPs and PCI-NPs) effectiveness on 22 tumor-bearing BALB/c mice after (A) graphs displaying tumor growth inhibition (B) effect on tumor shrinkage after treatment with PBS, CPT, PC-NPs, and PCI-NPs at 21st day post-treatment of the mice (C) micro-photographs of the harvested tumors. (D) Changes in the bodyweight of the mice during treatment. (E) H&E histological images of different treatment groups. Adapted with permission from [115].

18

Similarly, more recent studies have further shown that covalent linked prodrugs improve the pharmacokinetics and biodistribution of the conjugated drug as reported by Ibrahim and co-workers. H22 tumor model was induced in BALB/c [115]; the mice were exposed to conjugated drug delivery system and free-drug camptothecin (CPT), respectively. From the results, conjugates had a significantly had better pharmacokinetic profile than the free drug, this also led to increased accumulation of the drug within the tumor tissues and consequently better activity. This observation can be associated with the enhanced permeability and retention (EPR) effect provided by the nano/micro-delivery system. An additional observation which is the biodistribution effect of the drug conjugate is seen in the outstanding inhibition of tumor growth that resulted in better tumor shrinkage when compared to other groups (**Figure 10**). After 21 days of treatment, the drug-conjugated delivery system had a 33-fold less of tumor when compared to the untreated group. It is presumed that the acidity of the tumor contributed to the enhanced anticancer effect by the drug conjugated delivery system after cellular uptake of the nanoparticles, and the drug was released in the cytosol. This might have been the reason for the overall better activity of the conjugated drugs compared to the free drug. This was attributed to the extended blood circulation and better accumulation of conjugates in tumor compared to free CPT. Overall, these studies suggest that by covalently conjugating drugs into nano/micro-delivery systems, and a more enhanced pharmacokinetic profile of drugs can be obtained.

7. Disease site targeting via covalent conjugation

The delivery of drugs to sites of target, where the action of the drug is required, is challenging due to various physicochemical, biopharmaceutical, and pharmacokinetic barriers the drug may face [143]. In order to address these issues, new approaches such as drug covalently conjugated to nano/micro-delivery systems are being explored which alters the pharmacokinetic properties of the drug. This is achieved by using drug various carriers such as polymers [148, 149] liposomes [150], and dendrimers [151] that are capable of protecting the payload drug and delivering it to the disease site. The nano/micro-delivery systems help in accumulating the drug in the tumor site and prolong the circulation time [80]. This consequently leads to a successful drug target to the specific disease site. Drug conjugates have been widely utilized in the field of cancer therapy because they can passively target cancer disease sites by permeating and retain the drug via tumor's leaky vasculature [152]. Apart from the ability of the nano/micro-delivery system to protect the drug from degradative processes such as hydrolysis and metabolism before arriving at the target site, the drug is able to accumulate in the targeted site. This ability is a major advantage that drug conjugates proffers, which enhances their antitumor activity. Additionally, the ability of the drug conjugate to disassemble provides the opportunity to tune the drug release rate at the target site. More so, the tuning or decorating the surface of the nanostructure (drug conjugate) can lead to enhanced tumor targeting compared to drugs in their free form [143]. The enhanced drug efficacy reported in the studies discussed earlier is closely associated with the ability of the drug conjugate to target the specific disease site.

The advantages of covalently conjugating drugs to nano/micro-delivery systems to target disease sites or site of infections has shown effective by results obtained by various researchers who employed different therapeutic agents and nano/micro-delivery systems in targeting specific disease sites as highlighted in **Table 3**. In all the reported study on drug targeting using drug conjugates, a superior targeting of drugs to disease site was displayed by the drug encapsulated to nano/micro-delivery

systems via covalent conjugation compared to the free drug. Thus, it implies that covalently conjugated drug-nano/micro-delivery systems have the potential to specifically target disease site; hence, it can be used to treat and manage diseases that appear to be challenging to combat.

8. Conclusions and future perspectives

Drug encapsulation to nano/micro-delivery systems is a field in nanotechnology that has been growing substantially over the last two decades. Specifically, the covalent conjugation of drugs to different nano/micro-delivery systems is one of the drug encapsulation techniques that is gaining increasing attention. Various nanocarriers are currently developed and explored for the delivery of a wide range of therapeutic agents such as peptides, small molecules, and drugs. The several advantages offered by covalently conjugating this therapeutics into nanocarriers have made them gradually more attractive. These advantages include prolonged circulation, controlled release, improved solubility, reduced immunogenicity, specific site targeting, enhanced biosafety pharmacokinetics and biodistribution, and combination or concurrent integration of therapeutics in a single carrier. Due to the great strides that have been achieved in the development of effective drug delivery systems via covalent conjugation and their immense potential, some of these conjugates are gaining entrance into the market. Therefore, it is anticipated that covalent conjugation will continue to advance to facilitate the translation of current research findings into innovative treatments for a broad range of diseases. However, it is still very important to have a comprehensive knowledge of the ideal physiochemical properties, safety, drug release rates and efficacy, pharmacokinetic behavior, and clearance kinetics of these systems before preclinical development and clinical translation. Hence, more efforts and focused research are required to address the knowledge gaps and provide desired information that can accelerate their clinical translation and application in diverse fields of biomedicine. The computational and theoretical modeling approach can also be employed to correlate and answer certain outcomes by providing concrete design parameters.

Author details

Victoria Fasiku[1], Edith K. Amuhaya[2]*, Kingo M. Rajab[3] and Calvin A. Omolo[2]*

1 Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

2 Department of Pharmaceutics, School of Pharmacy and Health Sciences, United States International University-Africa, Nairobi, Kenya

3 Department of Biomedical Sciences, College of Health Sciences, University of Dodoma, Dodoma, Tanzania

*Address all correspondence to: eamuhaya@usiu.ac.ke; comolo@usiu.ac.ke

IntechOpen

References

[1] Petros RA, DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nature Reviews Drug Discovery. 2010;**9**: 615-627

[2] Omolo CA, Kalhapure RS, Agrawal N, Jadhav M, Rambharose S, Mocktar C, et al. A hybrid of mPEG-b-PCL and G1-PEA dendrimer for enhancing delivery of antibiotics. Journal of Controlled Release. 2018;**290**: 112-128

[3] Hassan D, Omolo CA, Gannimani R, Waddad AY, Mocktar C, Rambharose S, et al. Delivery of novel vancomycin nanoplexes for combating methicillin resistant Staphylococcus aureus (MRSA) infections. International Journal of Pharmaceutics. 2019;**558**: 143-156

[4] Omolo CA, Megrab NA, Kalhapure RS, Agrawal N, Jadhav M, Mocktar C, et al. Liposomes with pH responsive 'on and off' switches for targeted and intracellular delivery of antibiotics. Journal of Liposome Research. 2019:1-19

[5] Ulbrich K, Hola K, Subr V, Bakandritsos A, Tucek J, Zboril R. Targeted drug delivery with polymers and magnetic nanoparticles: Covalent and noncovalent approaches, release control, and clinical studies. Chemical Reviews. 2016;**116**:5338-5431

[6] Omolo CA, Kalhapure RS, Agrawal N, Rambharose S, Mocktar C, Govender T. Formulation and molecular dynamics simulations of a fusidic acid nanosuspension for simultaneously enhancing solubility and antibacterial activity. Molecular Pharmaceutics. 2018;**15**:3512-3526

[7] das Neves J, Araújo F, Andrade F, Amiji M, Bahia MF, Sarmento B. Biodistribution and pharmacokinetics of dapivirine-loaded nanoparticles after vaginal delivery in mice. Pharmaceutical Research. 2014;**31**:1834-1845

[8] Maji R, Omolo CA, Agrawal N, Maduray K, Hassan D, Mokhtar C, et al. pH-responsive lipid–dendrimer hybrid nanoparticles: An approach to target and eliminate intracellular pathogens. Molecular Pharmaceutics. 2019;**16**: 4594-4609

[9] Lee JH, Yeo Y. Controlled drug release from pharmaceutical nanocarriers. Chemical Engineering Science. 2015;**125**:75-84

[10] Weinbreck F, de Vries R, Schrooyen P, de Kruif CG. Complex coacervation of whey proteins and gum arabic. Biomacromolecules. 2003;**4**: 293-303

[11] Chiappetta DA, Sosnik A. Poly (ethylene oxide)–poly (propylene oxide) block copolymer micelles as drug delivery agents: Improved hydrosolubility, stability and bioavailability of drugs. European Journal of Pharmaceutics and Biopharmaceutics. 2007;**66**:303-317

[12] Vrignaud S, Benoit J-P, Saulnier P. Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles. Biomaterials. 2011;**32**: 8593-8604

[13] Elvira C, Gallardo A, San Roman J, Cifuentes A. Covalent polymer-drug conjugates. Molecules. 2005;**10**:114-125

[14] D'Emanuele A, Attwood D. Dendrimer-drug interactions. Advanced Drug Delivery Reviews. 2005;**57**: 2147-2162

[15] Kim S-W, Lee YK, Kim S-H, Park J-Y, Lee DU, Hong JH, et al. Covalent, non-covalent, encapsulated nanodrug regulate the fate of intra- and extracellular

trafficking : Impact on cancer and normal cells. Scientific Reports. 2017;**7**:1-17. Article id: 6454

[16] Kaminskas LM, McLeod VM, Porter CJH, Boyd BJ. Association of chemotherapeutic drugs with dendrimer nanocarriers: An assessment of the merits of covalent conjugation compared to noncovalent encapsulation. Molecular Pharmaceutics. 2012;**9**:355-373

[17] Kaminskas LM, Boyd BJ, Karellas P, Krippner GY, Lessene R, Kelly B, et al. The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of pegylated poly L-lysine dendrimers. Molecular Pharmaceutics. 2008;**5**:449-463

[18] Saito G, Swanson JA, Lee KD. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: Role and site of cellular reducing activities. Advanced Drug Delivery Reviews. 2003;**55**:199-215

[19] Lele B, Hoffman A. Mucoadhesive drug carriers based on complexes of poly (acrylic acid) and PEGylated drugs having hydrolysable PEG–anhydride–drug linkages. Journal of Controlled Release. 2000;**69**:237-248

[20] Khandare J, Minko T. Polymer–drug conjugates: Progress in polymeric prodrugs. Progress in Polymer Science. 2006;**31**:359-397

[21] Agarwal HK, Chhikara BS, Hanley MJ, Ye G, Doncel GF, Parang K. Synthesis and biological evaluation of fatty acyl ester derivatives of (−)-2′,3′-dideoxy-3′-thiacytidine. Journal of Medicinal Chemistry. 2012;**55**: 4861-4871

[22] Debart F, Abes S, Deglane G, Moulton HM, Clair P, Gait MJ, et al. Chemical modifications to improve the cellular uptake of oligonucleotides. Current Topics in Medicinal Chemistry. 2007;**7**:727-737

[23] Agarwal HK, Chhikara BS, Bhavaraju S, Mandal D, Doncel GF, Parang K. Emtricitabine prodrugs with improved anti-HIV activity and cellular uptake. Molecular Pharmaceutics. 2013;**10**:467-476

[24] Takalani F, Kumar P, Kondiah PP, Choonara YE, Pillay V. Lipid–drug conjugates and associated carrier strategies for enhanced antiretroviral drug delivery. Pharmaceutical Development and Technology. 2020;**25**: 267-280

[25] Adhikari P, Pal P, Das AK, Ray S, Bhattacharjee A, Mazumder B. Nano lipid-drug conjugate: An integrated review. International Journal of Pharmaceutics. 2017;**529**:629-641

[26] Nobs L, Buchegger F, Gurny R, Allémann E. Current methods for attaching targeting ligands to liposomes and nanoparticles. Journal of Pharmaceutical Sciences. 2004;**93**: 1980-1992

[27] Hoare DG, Koshland DE. A method for the quantitative modification and estimation of carboxylic acid groups in proteins. Journal of Biological Chemistry. 1967;**242**:2447-2453

[28] Werengowska-Ciećwierz K, Wiśniewski M, Terzyk AP, Furmaniak S. The chemistry of bioconjugation in nanoparticles-based drug delivery system. Advances in Condensed Matter Physics. 2015;**2015** (2015):198175-198202

[29] Yousefpour P, Atyabi F, Vasheghani-Farahani E, Movahedi A-AM, Dinarvand R. Targeted delivery of doxorubicin-utilizing chitosan nanoparticles surface-functionalized with anti-Her2 trastuzumab. International Journal of Nanomedicine. 2011;**6**:1977-1990

[30] Kurtoglu YE, Mishra MK, Kannan S, Kannan RM. Drug release characteristics

of PAMAM dendrimer–drug conjugates with different linkers. International Journal of Pharmaceutics. 2010;**384**: 189-194

[31] Duhem N, Danhier F, Pourcelle V, Schumers J-M, Bertrand O, LeDuff CS, et al. Self-assembling doxorubicin–tocopherol succinate prodrug as a new drug delivery system: Synthesis, characterization, and in vitro and in vivo anticancer activity. Bioconjugate Chemistry. 2014;**25**:72-81

[32] Yokoyama M, Miyauchi M, Yamada N, Okano T, Sakurai Y, Kataoka K, et al. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly (aspartic acid) block copolymer. Cancer Research. 1990;**50**:1693

[33] Nathan A, Zalipsky S, Ertel SI, Agathos SN, Yarmush ML, Kohn J. Copolymers of lysine and polyethylene glycol: A new family of functionalized drug carriers. Bioconjugate Chemistry. 1993;**4**:54-62

[34] Gupta A, Asthana S, Konwar R, Chourasia M. An insight into potential of nanoparticles-assisted chemotherapy of cancer using gemcitabine and its fatty acid prodrug: A comparative study. Journal of Biomedical Nanotechnology. 2013;**9**:915-925

[35] Kizhedath A, Wilkinson S, Glassey J. Applicability of predictive toxicology methods for monoclonal antibody therapeutics: Status quo and scope. Archives of Toxicology. 2017;**91**: 1595-1612

[36] Maruyama K, Takizawa T, Yuda T, Kennel SJ, Huang L, Iwatsuru M. Targetability of novel immunoliposomes modified with amphipathic poly(ethylene glycol)s conjugated at their distal terminals to monoclonal antibodies. Biochimica et Biophysica Acta. 1995;**1234**:74-80

[37] Dvir T, Bauer M, Schroeder A, Tsui JH, Anderson DG, Langer R, et al. Nanoparticles targeting the infarcted heart. Nano Letters. 2011;**11**:4411-4414

[38] Sonawane SJ, Kalhapure RS, Govender T. Hydrazone linkages in pH responsive drug delivery systems. European Journal of Pharmaceutical Sciences. 2017;**99**:45-65

[39] Matson JB, Stupp SI. Drug release from hydrazone-containing peptide amphiphiles. Chemical Communications. 2011;**47**:7962-7964

[40] Vijayakameswara Rao N, Mane S, Kishore A, Sarma JD, Shunmugam R, et al. Norbornene derived doxorubicin copolymers as drug carriers with pH responsive hydrazone linker. Biomacromolecules. 2012;**13**:221-230

[41] Gu Y-J, Cheng J, Jin J, Cheng SH, Wong W-T. Development and evaluation of pH-responsive single-walled carbon nanotube-doxorubicin complexes in cancer cells. International Journal of Nanomedicine. 2011;**6**:2889

[42] Christie RJ, Fleming R, Bezabeh B, Woods R, Mao S, Harper J, et al. Stabilization of cysteine-linked antibody drug conjugates with N-aryl maleimides. Journal of Controlled Release. 2015;**220**:660-670

[43] Anhorn MG, Wagner S, Kreuter J, Langer K, von Briesen H. Specific targeting of HER2 overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles. Bioconjugate Chemistry. 2008;**19**(12):2321-2331

[44] Park JW, Hong K, Carter P, Asgari H, Guo LY, Keller GA, et al. Development of anti-p185(HER2) immunoliposomes for cancer therapy. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**:1327-1331

[45] Kirpotin D, Park JW, Hong K, Zalipsky S, Li WL, Carter P, et al. Sterically stabilized anti-HER2 immunoliposomes: Design and targeting to human breast cancer cells in vitro. Biochemistry. 1997;**36**:66-75

[46] Lu YJ, Wei KC, Ma CCM, Yang SY, Chen JP. Dual targeted delivery of doxorubicin to cancer cells using folate-conjugated magnetic multi-walled carbon nanotubes. Colloids and Surfaces B: Biointerfaces. 2012;**89**:1-9

[47] West K, Otto S. Reversible covalent chemistry in drug delivery. Current Drug Discovery Technologies. 2005;**2**: 123-160

[48] Chari RV. Expanding the Reach of Antibody-Drug Conjugates. ACS Medicinal Chemistry Letters. 2016:**7** (11):974-976

[49] Rau H, Baron J, Hersel U, Krusch M. Prodrugs Containing an Aromatic Amine Connected by an Amido Bond to a Linker, in Google Patents. 2015

[50] Gawali SL, Barick KC, Shetake NG, Rajan V, Pandey BN, Kumar NN, et al. pH-labile magnetic nanocarriers for intracellular drug delivery to tumor cells. ACS Omega. 2019;**4**:11728-11736

[51] Ghosh AK, Brindisi M. Organic carbamates in drug design and medicinal chemistry. Journal of Medicinal Chemistry. 2015;**58**: 2895-2940

[52] Kölmel DK, Kool ET. Oximes and hydrazones in bioconjugation: Mechanism and catalysis. Chemical Reviews. 2017;**117**:10358-10376

[53] Fancui M, Quanxin W, Zhuangzhuang Q, Yan Y. Micelles prepared with cinnamaldehyde and vancomycin by Schiff base reaction. International Journal of New Technology and Research. 2018;**4**

[54] Xiao N, Liang H, Lu J. Degradable and biocompatible aldehyde-functionalized glycopolymer conjugated with doxorubicin via acid-labile Schiff base linkage for pH-triggered drug release. Soft Matter. 2011;**7**: 10834-10840

[55] Bhakay A, Rahman M, Dave RN, Bilgili EJP. Bioavailability enhancement of poorly water-soluble drugs via nanocomposites: Formulation–processing aspects and challenges. 2018; **10**:86

[56] Benedetto Tiz D, Kikelj D, Zidar N. Overcoming problems of poor drug penetration into bacteria: Challenges and strategies for medicinal chemists. Expert Opinion on Drug Discovery. 2018;**13**:497-507

[57] Lock LL, LaComb M, Schwarz K, Cheetham AG, Lin Y-A, Zhang P, et al. Self-assembly of natural and synthetic drug amphiphiles into discrete supramolecular nanostructures. Faraday Discussions. 2013;**166**:285-301

[58] Omolo CA, Kalhapure RS, Jadhav M, Rambharose S, Mocktar C, Ndesendo VM, et al. Pegylated oleic acid: A promising amphiphilic polymer for nano-antibiotic delivery. 2017;**112**: 96-108

[59] Su H, Zhang P, Cheetham AG, Koo JM, Lin R, Masood A, et al. Supramolecular crafting of self-assembling camptothecin prodrugs with enhanced efficacy against primary cancer cells. Theranostics. 2016;**6**:1065

[60] Cheetham AG, Zhang P, Lin YA, Lock LL, Cui H. Supramolecular nanostructures formed by anticancer drug assembly. Journal of the American Chemical Society. 2013;**135**:2907-2910

[61] Ma X, Bai S, Zhang X, Ma X, Jia D, Shi X, et al. Enhanced tumor penetration and chemotherapy efficiency by covalent self-assembled

nanomicelle responsive to tumor microenvironment. Biomacromolecules. 2019;**20**:2637-2648

[62] Wang Y, Cheetham AG, Angacian G, Su H, Xie L, Cui H. Peptide–drug conjugates as effective prodrug strategies for targeted delivery. Advanced Drug Delivery Reviews. 2017;**110**:112-126

[63] Lu Y, Lin J, Wang L, Zhang L, Cai C. Self-assembly of copolymer micelles: Higher-level assembly for constructing hierarchical structure. Chemical Reviews. 2020

[64] Amitay Y, Shmeeda H, Patil Y, Gorin J, Tzemach D, Mak L, et al. Pharmacologic studies of a prodrug of mitomycin C in pegylated liposomes (Promitil®): High stability in plasma and rapid thiolytic prodrug activation in tissues. Pharmaceutical Research. 2016; **33**:686-700

[65] Karaman R. Prodrugs design based on inter- and intramolecular chemical processes. Chemical Biology & Drug Design. 2013;**82**:643-668

[66] Scicluna MC, Vella-Zarb L. Evolution of nanocarrier drug-delivery systems and recent advancements in covalent organic framework–drug systems. ACS Applied Nano Materials. 2020;**3**:3097-3115

[67] Taresco V, Alexander C, Singh N, Pearce AK. Stimuli-responsive prodrug chemistries for drug delivery. Advanced Therapeutics. 2018;**1**:1800030

[68] Tian L, Yang Y, Wysocki LM, Arnold AC, Hu A, Ravichandran B, et al. Selective esterase–ester pair for targeting small molecules with cellular specificity. Proceedings of the National Academy of Sciences. 2012;**109**:4756-4761

[69] Ding C, Li Z. A review of drug release mechanisms from nanocarrier systems. Materials Science and Engineering: C. 2017;**76**:1440-1453

[70] Wang Z, Deng X, Ding J, Zhou W, Zheng X, Tang G. Mechanisms of drug release in pH-sensitive micelles for tumour targeted drug delivery system: A review. International Journal of Pharmaceutics. 2018;**535**:253-260

[71] Dillon KM, Powell CR, Matson JB. Self-immolative prodrugs: Effective tools for the controlled release of sulfur signaling species. Synlett. 2019;**30**: 525-531

[72] Meng X, Gao M, Deng J, Lu D, Fan A, Ding D, et al. Self-immolative micellar drug delivery: The linker matters. Nano Research. 2018;**11**: 6177-6189

[73] Senevirathne SA, Washington KE, Biewer MC, Stefan MC. PEG based anti-cancer drug conjugated prodrug micelles for the delivery of anti-cancer agents. Journal of Materials Chemistry B. 2016;**4**:360-370

[74] Lee E, Kim H, Lee I-H, Jon S. In vivo antitumor effects of chitosan-conjugated docetaxel after oral administration. Journal of Controlled Release. 2009;**140**:79-85

[75] Duncan R, Vicent MJ. Do HPMA copolymer conjugates have a future as clinically useful nanomedicines? A critical overview of current status and future opportunities. Advanced Drug Delivery Reviews. 2010;**62**:272-282

[76] Jung B, Jeong Y-C, Min J-H, Kim J-E, Song Y-J, Park J-K, et al. Tumor-binding prodrug micelles of polymer–drug conjugates for anticancer therapy in HeLa cells. Journal of Materials Chemistry. 2012;**22**:9385-9394

[77] Konkle BA, Stasyshyn O, Chowdary P, Bevan DH, Mant T, Shima M, et al. Pegylated, full-length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A. Blood, The Journal of the

American Society of Hematology. 2015;**126**:1078-1085

[78] Coyle T, Reding MT, Lin J, Michaels L, Shah A, Powell J. Phase I study of BAY 94-9027, a PEG ylated B-domain-deleted recombinant factor VIII with an extended half-life, in subjects with hemophilia A. Journal of Thrombosis and Haemostasis. 2014;**12**: 488-496

[79] Devarajan PV, Dawre SM, Dutta R. Infectious diseases: Need for targeted drug delivery. In: Targeted Drug Delivery: Concepts and Design. Springer; 2015. pp. 113-148

[80] Van Driessche A, Kocere A, Everaert H, Nuhn L, Van Herck S, Griffiths G, et al. pH-sensitive hydrazone-linked doxorubicin nanogels via polymeric-activated Ester scaffolds: Synthesis, assembly, and in vitro and in vivo evaluation in tumor-bearing zebrafish. Chemistry of Materials. 2018; **30**:8587-8596

[81] Cao X, Le C, Lim K, Thi H, Kim G, Gal Y. Redox-responsive core cross-linked prodrug micelles prepared by click chemistry for pH-triggered doxorubicin delivery. Express Polymer Letters. 2017;**11**:832-845

[82] Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: Design, development and clinical translation. Chemical Society Reviews. 2012;**41**:2971-3010

[83] Augustijns P, Annaert P, Heylen P, Van den Mooter G, Kinget R. Drug absorption studies of prodrug esters using the Caco-2 model: Evaluation of ester hydrolysis and transepithelial transport. International Journal of Pharmaceutics. 1998;**166**:45-53

[84] Amsberry KL, Gerstenberger AE, Borchardt RT. Amine prodrugs which utilize hydroxy amide lactonization. II.

A potential esterase-sensitive amide prodrug. Pharmaceutical Research. 1991;**8**:455-461

[85] Meinig JM, Ferrara SJ, Banerji T, Banerji T, Sanford-Crane HS, Bourdette D, et al. Targeting fatty-acid amide hydrolase with prodrugs for CNS-selective therapy. ACS Chemical Neuroscience. 2017;**8**:2468-2476

[86] Kraut J. Serine proteases: Structure and mechanism of catalysis. Annual Review of Biochemistry. 1977;**46**: 331-358

[87] Otto HH, Schirmeister T. Cysteine proteases and their inhibitors. Chemical Reviews. 1997;**97**:133-172

[88] Häse C, Finkelstein RA. Bacterial extracellular zinc-containing metalloproteases. Microbiology and Molecular Biology Reviews. 1993;**57**: 823-837

[89] Martin MT, Angeles TS, Sugasawara R, Aman NI, Napper AD, Darsley MJ, et al. Antibody-catalyzed hydrolysis of an unsubstituted amide. Journal of the American Chemical Society. 1994;**116**:6508-6512

[90] Dai X, De Mesmaeker A, Joyce GF. Cleavage of an amide bond by a ribozyme. Science. 1995;**267**:237-240

[91] Kalia J, Raines RT. Hydrolytic stability of hydrazones and oximes. Angewandte Chemie (International Ed. in English). 2008;**47**:7523-7526

[92] Ross PL, Wolfe JL. Physical and chemical stability of antibody drug conjugates: Current status. Journal of Pharmaceutical Sciences. 2016;**105**: 391-397

[93] Fontaine SD, Reid R, Robinson L, Ashley GW, Santi DV. Long-term stabilization of Maleimide–Thiol conjugates. Bioconjugate Chemistry. 2015;**26**:145-152

[94] Modarresi-Saryazdi SM, Haddadi-Asl V, Salami-Kalajahi M. N, N'-methylenebis (acrylamide)-crosslinked poly (acrylic acid) particles as doxorubicin carriers: A comparison between release behavior of physically loaded drug and conjugated drug via acid-labile hydrazone linkage. Journal of Biomedical Materials Research Part A. 2018;**106**:342-348

[95] Dong C, Zhou Q, Xiang J, Liu F, Zhou Z, Shen Y. Self-assembly of oxidation-responsive polyethylene glycol-paclitaxel prodrug for cancer chemotherapy. Journal of Controlled Release. 2020;**321**:529-539

[96] Stirland DL, Nichols JW, Miura S, Bae YH. Mind the gap: A survey of how cancer drug carriers are susceptible to the gap between research and practice. Journal of Controlled Release. 2013;**172**: 1045-1064

[97] Sun Q, Zhou Z, Qiu N, Shen Y. Rational design of cancer nanomedicine: Nanoproperty integration and synchronization. Advanced Materials. 2017;**29**:1606628

[98] Rao KSVK, Zhong Q, Bielski ER, da Rocha SRP. Nanoparticles of pH-responsive, PEG–doxorubicin conjugates: Interaction with an in vitro model of lung adenocarcinoma and their direct formulation in propellant-based portable inhalers. Molecular Pharmaceutics. 2017;**14**: 3866-3878

[99] Chakroun RW, Wang F, Lin R, Wang Y, Su H, Pompa D, et al. Fine-tuning the linear release rate of paclitaxel-bearing supramolecular filament hydrogels through molecular engineering. ACS Nano. 2019;**13**: 7780-7790

[100] Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nature Materials. 2013; **12**:991-1003

[101] Zhou Z, Ma X, Murphy CJ, Jin E, Sun Q, Shen Y, et al. Molecularly precise dendrimer–drug conjugates with tunable drug release for cancer therapy. Angewandte Chemie International Edition. 2014;**53**:10949-10955

[102] Zhou Q, Shao S, Wang J, Xu C, Xiang J, Piao Y, et al. Enzyme-activatable polymer–drug conjugate augments tumour penetration and treatment efficacy. Nature Nanotechnology. 2019;**14**:799-809

[103] Bhattacharyya J, Bellucci JJ, Weitzhandler I, McDaniel JR, Spasojevic I, Li X, et al. A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models. Nature Communications. 2015;**6**:1-12

[104] Wang J, Sun X, Mao W, Sun W, Tang J, Sui M, et al. Tumor redox heterogeneity-responsive prodrug nanocapsules for cancer chemotherapy. Advanced Materials. 2013;**25**:3670-3676

[105] Wang J, Hu S, Mao W, Xiang J, Zhou Z, Liu X, et al. Assemblies of peptide-Cytotoxin conjugates for tumor-homing chemotherapy. Advanced Functional Materials. 2019; **29**:1807446

[106] Shen Y, Jin E, Zhang B, Murphy CJ, Sui M, Zhao J, et al. Prodrugs forming high drug loading multifunctional nanocapsules for intracellular cancer drug delivery. Journal of the American Chemical Society. 2010;**132**:4259-4265

[107] Li J, Hu Z-E, Yang X-L, Wu W-X, Xing X, Gu B, et al. GSH/pH dual-responsive biodegradable camptothecin polymeric prodrugs combined with doxorubicin for synergistic anticancer efficiency. Biomaterials Science. 2019;**7**: 3277-3286

[108] Tang J, Zeng Z, Yan J, Chen C, Liu J, Feng X. Quantitative and high

drug loading of self-assembled prodrug with defined molecular structures for effective cancer therapy. Journal of Controlled Release. 2019;**307**:90-97

[109] Danial M, Telwatte S, Tyssen D, Cosson S, Tachedjian G, Moad G, et al. Combination anti-HIV therapy via tandem release of prodrugs from macromolecular carriers. Polymer Chemistry. 2016;**7**:7477-7487

[110] Danial M, Andersen AH, Zuwala K, Cosson S, Riber CF, Smith AA, et al. Triple activity of lamivudine releasing sulfonated polymers against HIV-1. Molecular Pharmaceutics. 2016;**13**: 2397-2410

[111] Shibata A, McMullen E, Pham A, Belshan M, Sanford B, Zhou Y, et al. Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment. AIDS Research and Human Retroviruses. 2013;**29**: 746-754

[112] Jiang Y, Cao S, Bright DK, Bever AM, Blakney AK, Suydam IT, et al. Nanoparticle-based ARV drug combinations for synergistic inhibition of cell-free and cell–cell HIV transmission. Molecular Pharmaceutics. 2015;**12**:4363-4374

[113] Xiao Y, Liu J, Guo M, Zhou H, Jin J, Liu J, et al. Synergistic combination chemotherapy using carrier-free celastrol and doxorubicin nanocrystals for overcoming drug resistance. Nanoscale. 2018;**10**:12639-12649

[114] Zhao C-Y, Cheng R, Yang Z, Tian Z-M. Nanotechnology for cancer therapy based on chemotherapy. Molecules. 2018;**23**:826

[115] Ibrahim A, Twizeyimana E, Lu N, Ke W, Mukerabigwi JF, Mohammed F, et al. Reduction-responsive polymer prodrug micelles with enhanced endosomal escape capability for efficient intracellular translocation and

drug release. ACS Applied Bio Materials. 2019;**2**:5099-5109

[116] Lu D, Wen X, Liang J, Gu Z, Zhang X, Fan Y. A pH-sensitive nano drug delivery system derived from pullulan/doxorubicin conjugate. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2009;**89**:177-183

[117] Yoo HS, Lee KH, Oh JE, Park TG. In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin–PLGA conjugates. Journal of Controlled Release. 2000;**68**:419-431

[118] Shuai X, Ai H, Nasongkla N, Kim S, Gao J. Micellar carriers based on block copolymers of poly (ε-caprolactone) and poly (ethylene glycol) for doxorubicin delivery. Journal of Controlled Release. 2004;**98**:415-426

[119] Ma L, Lin X, Li C, Xu Z, Chan C-Y, Tse M-K, et al. A cancer cell-selective and low-toxic bifunctional heterodinuclear Pt(IV)–Ru(II) anticancer prodrug. Inorganic Chemistry. 2018;**57**:2917-2924

[120] Larson N, Ghandehari H. Polymeric conjugates for drug delivery. Chemistry of Materials. 2012;**24**:840-853

[121] Du J-Z, Du X-J, Mao C-Q, Wang J. Tailor-made dual pH-sensitive polymer–doxorubicin nanoparticles for efficient anticancer drug delivery. Journal of the American Chemical Society. 2011;**133**:17560-17563

[122] Cai K, He X, Song Z, Yin Q, Zhang Y, Uckun FM, et al. Dimeric drug polymeric nanoparticles with exceptionally high drug loading and quantitative loading efficiency. Journal of the American Chemical Society. 2015; **137**:3458-3461

[123] Su L, Li R, Khan S, Clanton R, Zhang F, Lin Y-N, et al. Chemical design of both a glutathione-sensitive dimeric drug guest and a glucose-derived nanocarrier host to achieve enhanced osteosarcoma lung metastatic anticancer selectivity. Journal of the American Chemical Society. 2018;**140**:1438-1446

[124] Miller K, Erez R, Segal E, Shabat D, Satchi-Fainaro R. Targeting bone metastases with a bispecific anticancer and antiangiogenic polymer–alendronate–taxane conjugate. Angewandte Chemie International Edition. 2009;**48**:2949-2954

[125] Paz-Ares L, Ross H, O'brien M, Riviere A, Gatzemeier U, Von Pawel J, et al. Phase III trial comparing paclitaxel poliglumex vs docetaxel in the second-line treatment of non-small-cell lung cancer. British Journal of Cancer. 2008;**98**:1608-1613

[126] Chitkara D, Mittal A, Behrman SW, Kumar N, Mahato RI. Self-assembling, amphiphilic polymer–gemcitabine conjugate shows enhanced antitumor efficacy against human pancreatic adenocarcinoma. Bioconjugate Chemistry. 2013;**24**:1161-1173

[127] Ishiguro S, Cai S, Uppalapati D, Turner K, Zhang T, Forrest WC, et al. Intratracheal administration of hyaluronan-cisplatin conjugate nanoparticles significantly attenuates lung cancer growth in mice. Pharmaceutical Research. 2016;**33**:2517-2529

[128] Tsuchiya K, Uchida T, Kobayashi M, Maeda H, Konno T, Yamanaka H. Tumor-targeted chemotherapy with SMANCS in lipiodol for renal cell carcinoma: Longer survival with larger size tumors. Urology. 2000;**55**:495-500

[129] Wang W, Li C, Zhang J, Dong A, Kong D. Tailor-made gemcitabine prodrug nanoparticles from well-defined drug–polymer amphiphiles prepared by controlled living radical polymerization for cancer

chemotherapy. Journal of Materials Chemistry B. 2014;**2**:1891-1901

[130] Chen X, Chen X, Chen W, Ma X, Huang J, Chen R. Extended peginterferon alfa-2a (Pegasys) therapy in Chinese patients with HBeAg-negative chronic hepatitis B. Journal of Medical Virology. 2014;**86**:1705-1713

[131] Trapani A, Di Gioia S, Ditaranto N, Cioffi N, Goycoolea FM, Carbone A, et al. Systemic heparin delivery by the pulmonary route using chitosan and glycol chitosan nanoparticles. International Journal of Pharmaceutics. 2013;**447**:115-123

[132] Mastria EM, Chen M, McDaniel JR, Li X, Hyun J, Dewhirst MW, et al. Doxorubicin-conjugated polypeptide nanoparticles inhibit metastasis in two murine models of carcinoma. Journal of Controlled Release. 2015;**208**:52-58

[133] Zhong Q, Bielski ER, Rodrigues LS, Brown MR, Reineke JJ, da Rocha SR. Conjugation to poly (amidoamine) dendrimers and pulmonary delivery reduce cardiac accumulation and enhance antitumor activity of doxorubicin in lung metastasis. Molecular Pharmaceutics. 2016;**13**:2363-2375

[134] Eskow Jaunarajs KL, Standaert DG, Viegas TX, Bentley MD, Fang Z, Dizman B, et al. Rotigotine polyoxazoline conjugate SER-214 provides robust and sustained antiparkinsonian benefit. Movement Disorders. 2013;**28**:1675-1682

[135] Park JH, Kwon S, Lee M, Chung H, Kim J-H, Kim Y-S, et al. Self-assembled nanoparticles based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin: in vivo biodistribution and anti-tumor activity. Biomaterials. 2006;**27**:119-126

[136] Murata M, Yonamine T, Tanaka S, Tahara K, Tozuka Y, Takeuchi H. Surface modification of liposomes using polymer-wheat germ agglutinin conjugates to improve the absorption of peptide drugs

by pulmonary administration. Journal of Pharmaceutical Sciences. 2013;**102**: 1281-1289

[137] Ernsting MJ, Tang W-L, MacCallum NW, Li S-D. Preclinical pharmacokinetic, biodistribution, and anti-cancer efficacy studies of a docetaxel-carboxymethylcellulose nanoparticle in mouse models. Biomaterials. 2012;**33**:1445-1454

[138] Wang H, Wei J, Yang C, Zhao H, Li D, Yin Z, et al. The inhibition of tumor growth and metastasis by self-assembled nanofibers of taxol. Biomaterials. 2012;**33**:5848-5853

[139] Yang C, Bian M, Yang Z. A polymer additive boosts the anti-cancer efficacy of supramolecular nanofibers of taxol. Biomaterials Science. 2014;**2**:651-654

[140] Ueno T, Endo K, Hori K, Ozaki N, Tsuji A, Kondo S, et al. Assessment of antitumor activity and acute peripheral neuropathy of 1, 2-diaminocyclohexane platinum (II)-incorporating micelles (NC-4016). International Journal of Nanomedicine. 2014;**9**:3005

[141] Katsumi H, Takashima M, Sano J-I, Nishiyama K, Kitamura N, Sakane T, et al. Development of polyethylene glycol-conjugated alendronate, a novel nitrogen-containing bisphosphonate derivative: Evaluation of absorption, safety, and effects after intrapulmonary administration in rats. Journal of Pharmaceutical Sciences. 2011;**100**: 3783-3792

[142] Duncan R. Polymer conjugates as anticancer nanomedicines. Nature Reviews Cancer. 2006;**6**:688-701

[143] Cheetham AG, Chakroun RW, Ma W, Cui H. Self-assembling prodrugs. Chemical Society Reviews. 2017;**46**: 6638-6663

[144] Yokoyama M, Miyauchi M, Yamada N, Okano T, Sakurai Y, Kataoka K, et al. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly (ethylene glycol)-poly (aspartic acid) block copolymer. Cancer Research. 1990;**50**:1693-1700

[145] Yokoyama M, Okano T, Sakurai Y, Kataoka K. Improved synthesis of adriamycin-conjugated poly (ethylene oxide)-poly (aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin. Journal of Controlled Release. 1994;**32**:269-277

[146] Kwon GS, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Biodistribution of micelle-forming polymer–drug conjugates. Pharmaceutical Research. 1993;**10**:970-974

[147] Kwon G, Suwa S, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly (ethylene oxide-aspartate) block copolymer-adriamycin conjugates. Journal of Controlled Release. 1994;**29**:17-23

[148] Croy S, Kwon G. Polymeric micelles for drug delivery. Current Pharmaceutical Design. 2006;**12**: 4669-4684

[149] Ahmad Z, Shah A, Siddiq M, Kraatz H. Polymeric micelles as drug delivery vehicles. RSC Advances. 2014; **4**:17028-17038. Go to original source

[150] Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chemical Reviews. 2015;**115**:10938-10966

[151] Medina SH, El-Sayed ME. Dendrimers as carriers for delivery of chemotherapeutic agents. Chemical Reviews. 2009;**109**:3141-3157

[152] Torchilin V. Tumor delivery of macromolecular drugs based on the EPR effect. Advanced Drug Delivery Reviews. 2011;**63**:131-135