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Revisão

INTELLIGENT DELIVERY OF ANTITUMOR DRUGS MEDIATED BY POLYMERIC NANOMICELLES: A REVIEW

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Cancer therapy represents a challenge, even with the current scientific developments. Over the years, several studies have shown that the application of antitumor drug nanocarriers is an efficient strategy; however, it requires further improvement. Several nanosystems have been developed, some of which are already in use, including liposomes. Polymer-based nanocarriers are still being adjusted to the peculiarities of the human body due to the biological barriers that were encountered by the first systems that were developed. Among them, the formation of corona proteins, clearance by the endothelial reticulum system and kidneys, activation of the immune system, lack of selectivity, and difficult release have been extensively studied and improved with the development of new devices. In this review, we explore the evolution of primary nanocarriers based on polymeric micelles and highlight the gaps that remain in this field to assist in the research of new systems with superior therapeutic indices.

Keywords: nanocarrier; polymeric micelle; nanomicelle; anticancer therapy.

INTRODUCTION

The use of nanotechnology began in the 20th century.[1] It is defined as the synthesis, handling, or application of materials with dimensions ranging between 1 and 100 nm and may present varied geometries (nanotubes, nanospheres, ellipsoids, etc.).[2]

Nanoparticles (NPs) have unique properties that allow them to overcome physical, chemical, and biological barriers, assuming a prominent role in several fields, including nanomedicine.[3] To date, more than 60,000 studies have been published related to this discipline, and approximately 93% of these are related to medical applications in humans.[4]

Nanomedicine involves the use of materials, drugs, and nanosystems to predict, prevent, diagnose, and treat diseases. One of its many applications is cancer treatment.[3] Cancer is characterized by the uncontrolled multiplication of cells with genetic changes, causing malfunction and death of healthy cells. According to the World Health Organization, over the past 20 years, the number of cancer diagnoses has doubled, reaching 19.3 million in 2020. Currently, approximately one in five people are diagnosed with a type of malignant neoplasm, and the disease causes approximately 17% of deaths worldwide.[5]

Cancer treatments include targeted therapy, hormone therapy, and immunotherapy, depending on the type and stage of development of the disease.[6] Targeted therapy employs certain characteristics of the cancer cells, such as overexpressed substances and genetic changes, to achieve selective treatment.[6] Targeted medications can act by blocking cell division and the blood vessels that feed the tumor, altering the protein composition, stimulating the immune system, or transporting anticancer agents to the tumor.[6]

The drugs used in chemotherapy are aggressive and have low specificity, causing several side effects and systemic toxicity.[7] Often, the administered active dosage becomes insufficient owing to the simultaneous interaction with healthy cells, and a larger amount of the active compound must be administered to present an efficient cytotoxic effect against tumor cells.[4]

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A strategy to minimize this aggravating factor is to alter the pharmacokinetics of antitumor drugs with nanostructured delivery vehicles that can regulate their biodistribution in the body and adapt to the peculiarities of cancer cells, allowing selectivity in drug delivery.[8] These systems act as carriers that trap drugs and lead them to the damaged cells through the biological environment, protecting them from degradation and allowing their facilitated passage to the intracellular environment, with minimal or no interaction with healthy cells.[3] Nanocarrier selectivity can be passively achieved through the enhanced permeability and retention (EPR) effect or via active targeting with the aid of ligands (marked as (2) in Figure 1) with affinity for cancer cells. EPR-assisted passive targeting (denoted as (2) in Figure 1) is an event caused by misalignments and leaks in the tumor vasculature that allow the escape of nanocarriers and their introduction into the tissue.[9] Once inside the tumor, the low lymphatic drainage and impaired circulation of the tissue help in the accumulation of the nanosystem, favoring its internalization by endocytosis (denoted as (3) in Figure 1). Endocytosis is a biological phenomenon characterized by the encapsulation of an extracellular body, which allows the entry of the nanocarrier through the formation of the endosome (denoted as (4) in Figure 1).[10]

As shown in Figure 1, inside the cells, the endosomal vesicle is ruptured (5) by a specific characteristic of the tumor environment, allowing the release of the drug (6) and resulting in cell death (7). Endosomal escape is not guaranteed, and only a small percentage of the drug can act against the tumor.[8,11] Research has shown that this leak can occur by the fusion of the nanocarrier with the membrane, osmotic rupture of the endocytic vesicle, swelling of the nanocarriers, and endocytic membrane destabilization.[10]

The nanomicelle size is an important factor in this regard, because current chemotherapy drugs possess a low molecular weight (< 500 g mol^{-1}) and degrade quickly in the bloodstream, losing a part of the dose administered prior to reaching the tumor.[8] Therefore, nanomicelles must have dimensions in the range of 20-100 nm to avoid entering healthy tissues and ensure accumulation only in the tumors.[12,13] Moreover, polymers must be biocompatible so that corona protein layers are not formed on their surfaces,

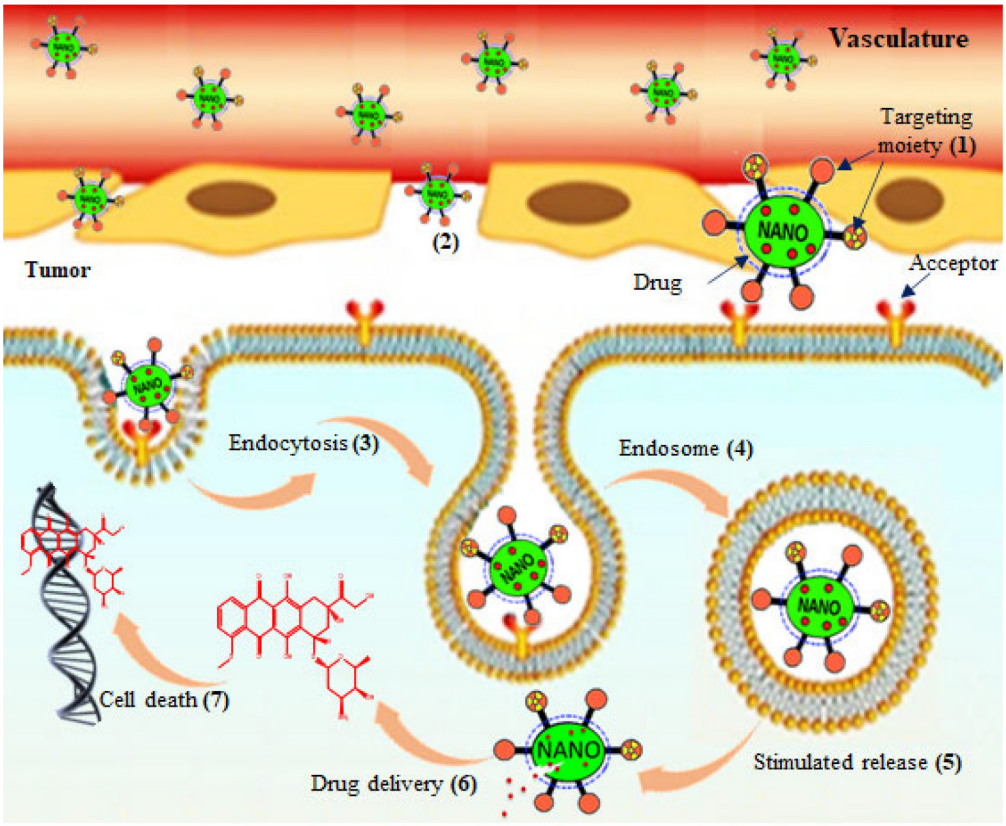

Figure 1. *Illustration of the capture, internalization, and release of the drug in a tumor environment[4] (adapted)*

which can induce macrophage recognition during circulation and promote early clearance of polymers or the activation of the immune system.[11]

Among the 140 antitumor drugs, more than 60% are natural products or are derived from natural products, belonging to several structural classes, including anthracyclines, enediynes, indolocarbazoles, isoprenoids, and polyketides. For example, paclitaxel, which was initially isolated from *Taxus brevifolia* extract, can also be obtained from certain fungi such as *Taxomyces adreanae*; vincristine can be obtained from *Catharanthus roseus*; irinotecan, a derivative of camptothecin, is an active compound that can be extracted from the *Camptotheca accuminata* tree; moreover, doxorubicin can be isolated from strains of *Streptomyces peuceticus* (Figure 2).[14]

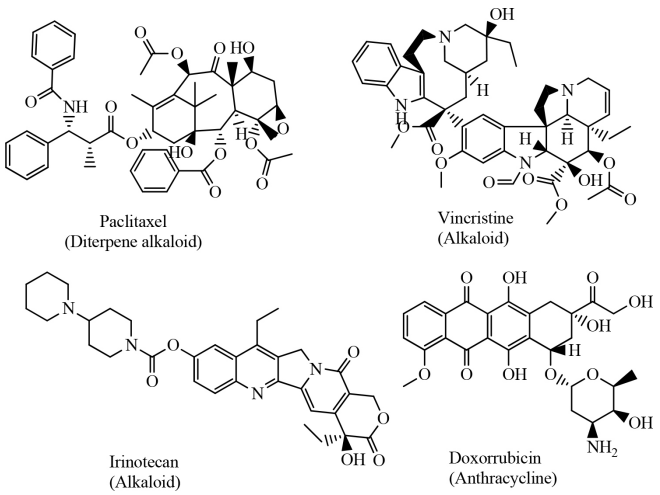

Figure 2. *Antitumor drugs isolated from natural products[14] (adapted)*

Nanocarriers can be prepared from lipids, proteins, and polymers, generating liposomes, inorganic NPs, and polymeric NPs (Figure 3), whose applications depend on their properties and synthesis methods.[9,15]

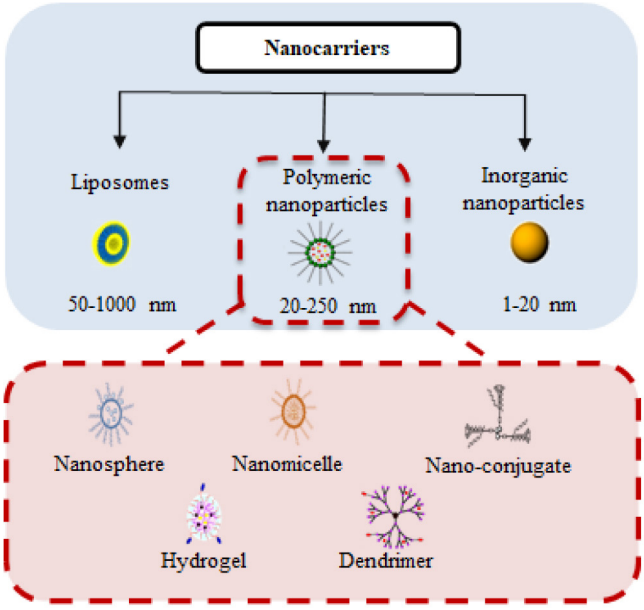

Figure 3. *Types of nanocarriers[7,15]*

Lipids can be used to produce two types of nanocarriers: liposomes, formed by a lipid bilayer with an aqueous nucleus, and lipid NPs, composed of a lipid monolayer with a solid lipid center.[15] Liposomal nanocarriers have been commercialized for the delivery of vincristine, irinotecan, and doxorubicin.[6,16]

The properties of metallic NPs have enabled their use as contrast agents and disease trackers, and are also used for photothermal applications and chemotherapy.[17] The functionalization of their surfaces and their combination with different metals has expanded their use in breast cancer therapy. However, their toxicity requires more extensive evaluation.[15] Recently, gold NPs were functionalized with polyethylene glycol (PEG) to transport and release cisplatin into cancerous tumors. This system allows the administration of lower dosages of the drug, which contributes to the reduction of side effects associated with this type of treatment.[18]

Polymeric NPs represent an important group of nanocarriers owing to their biodegradability and biocompatibility. They can trap drugs by immobilization, encapsulation, or conjugation, which enables the generation of different structures, mechanisms of direction, and drug delivery.[7,15] They can be synthesized from natural polymers, semisynthetic materials, or synthetic materials, and may present hydrophilic or hydrophobic characteristics and assume the structures of nanospheres, nanomicelles, nanoconjugates, hydrogels, and dendrimers. Biopolymers are more appropriate for drug delivery because they have a greater affinity for biological systems and are easily degraded by them. However, possible changes in their structure for better adaptation to the biological environment may not be successful, thus limiting their application. Because their synthesis can be directed exclusively based on the conditions necessary for the performance of the desired function, semisynthetic and synthetic polymers allow their structure and physicochemical properties to be better adjusted to the profile of the drug, organism, and target cells.[7]

Conjugates can also be easily identified using linear polymers, which are nanocarriers that are covalently coupled to the drug, whose release is triggered by the rupture of this bond. Some tests have been performed with several polymers; however, thus far, the polymers that are predominantly used for this type of system are PEG and n-(2-hydroxypropyl) metacrylamide copolymers. PEG is predominantly used in this research as it has good water solubility, absence of toxicity, antigenicity, immunogenicity, a flexible chain, and has already been approved by the Food and Drug Administration (FDA) as it is in phase II of clinical trials, in combination with irinotecan, for the treatment of gastric and metastatic breast tumors.[8,19]

Polymeric nanocarriers can form nanomicelles, which are widely used in this field because they allow the accommodation of hydrophobic drugs and their passage through circulation. They are defined as amphiphilic copolymers with hydrophilic and hydrophobic parts that reorganize in an aqueous medium when their critical micelle concentration is reached, forming structures with dimensions of 5-100 nm. The hydrophobic chain is centralized to accommodate drugs with low solubility in water, generating a capsule surrounded by a hydrophilic layer that may or may not be functionalized with molecules that aid in cancer cell selectivity.[8,20,21] The ability of polymer micelles to release, internalize, and biodistribute drugs is related to the properties of the polymers used, and more than one polymeric block can be used for better control of tumor transport and uptake.[7]

Currently, the FDA and European Medicines Agency, which are regulatory agencies of the United States and Europe, respectively, have approved approximately 21 nanocarriers that can be administered orally, intravenously, and transdermally. Of these systems, 10 formulations were directed toward cancer therapy, and only 2 nanocarriers were synthesized based on polymers. Approximately 5% of the nanocarriers in the test phase are polymeric and present the opportunity to transport all commercialized antitumor drugs.[4]

Considering the information described in this introduction, polymer-based nanocarriers are believed to be the best alternative for the development of cancer therapy. However, over the years, although the repatterning of systems have occurred with small modifications, there is little news in this regard. Thus, the objective of this work is to update the reader regarding the main synthesized micelle-based nanocarriers so that new systems can be developed without the limitations of previously proposed systems. Our research covers key polymeric antitumor drug delivery systems developed up to 2022, with an in-depth examination of the triggers that allow drugs to be delivered specifically within tumor cells.

POLYMERIC MICELLES

The first publications on the development of polymer-drug systems for cancer therapy were published in 1955, with a proposal to employ conjugates and polymeric micelles for the delivery of antitumor drugs.[22] However, formulations only started to show satisfactory results in 1996 with the synthesis of micelles based on Pluronic, a triblock copolymer formed by poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide), for the delivery of doxorubicin by passive targeting, resulting in 90% tumor inhibition and total tumor disappearance in 33-50% of animal tests.[23,24]

Subsequently, it was discovered that neutral polymers, such as PEG, could prevent opsonization and hinder clearance by the endothelial reticulum system, thus increasing the nanocarrier circulation time. One of the first polymeric micelle systems to enter the clinical trial phase was based on PEG and aspartic acid, with a part of the doxorubicin conjugated to the polymer to increase hydrophobicity inside the micelles, which presented a higher therapeutic index and slow release over a period of 8-24 h.[25]

A micellar system with PEG and aspartate was developed by the introduction of hydrazide and by combining doxorubicin and wortmannin, an antitumor antibiotic and enzymatic inhibitor, respectively, to attack MCF-7 breast cancer cells in two strands.[26] The composition allowed for the reduction of doxorubicin and increased the therapeutic index of treatment by the combined action of the drugs.[26] The development of systems that allow this type of treatment provides, in addition to a safe delivery, greater efficiency of treatment due to the synergistic effect between drugs, which allows the reduction of the doses administered and, consequently, a reduction in the side effects.[26]

PEG-based systems with different modifications continue to be developed; however, studies have revealed that the continuous use of a formulation containing PEG can trigger an immune response, activating anti-immunoglobulin antibodies and increasing the rate of clearance of the formulation, causing a reduction in the uptake of the nanocarrier by tumor cells.[27,28] The need to develop alternative systems for PEG has enabled the creation of new mycelial systems capable of transporting drugs without activating the immune system.

Among them, certain micelles are based on zwitterionic materials. They are characterized by the presence of groups with positive and negative charges that, depending on the proportions, can impart a neutral character to the structure.[29] Zwitterionic micelles can bind water through electrostatic interactions, forming a hydration layer that prevents connections with serum proteins, promoting longer circulation, which facilitates tumor accumulation by EPR. However, because the affinity for tumor cells is due to a cationized micellar surface, which contributes to the deposition of the protein crown, its efficiency in cellular uptake is still in the process of improvement. In 2018, Ou *et al.*[30] developed a zwitterionic micellar system with two block copolymers: poly (ε-caprolactone)-block-poly (2-methacryloyloxyethyl phosphorylcholine) and poly (ε-caprolactone)-poly-block (β-amino ester). Preliminary studies on the 2-methacryloyloxyethyl phosphorylcholine (PMPC) block have shown its efficiency in preventing the adhesion of serum proteins during transport in the circulation. In this study, the combination

with PMPC increased the circulation time by 50%, which helped in the accumulation in the tumor. The rate of cell uptake significantly increased, which was attributed to the protonation of the β-amino ester block due to the acidic environment of the tumor, facilitating cellular internalization of the micelle.[30]

Nanocarriers based on natural polymers have also been studied owing to their superior biocompatibility, biodegradability, low toxicity, and immunogenicity.[31,32] Chitosan is a polymer used for the development of nanomicelles because it is sensitive to pH and allows for several chemical modifications, assisting its application in the synthesis of block copolymers. Recently, in 2019, Niu *et al.*[33] proposed a thermosensitive micellar system formed by poly (N-vinylcaprolactam) and chitosan for the delivery of doxorubicin in triple-negative breast cancer therapy; that is, in the tumor environment, which has a relatively higher temperature, the micelles tend to rupture and release the drug. Chitosan offered the nanocarrier a second trigger for micellar rupture due to amine protonation.[33]

In summary, the primary issue in the use of polymers in the elaboration of a nanocarrier is that its surface is neutral to remain in circulation for a longer period, and at the same time, it can be easily cationized to be directed to the tumor tissue. In practice, this is not an easy task because, despite several studies and all the knowledge acquired in the last 67 years (since 1955), both directly using nanocarriers and based on the behavior of the human organism in the face of these proposals, there are still only a few formulations that are suitable for the safe and effective treatment of cancer with micelle-based therapy.

Active targeting link

Cancer cells have specific overexpressed receptors that can be used to target nanocarriers to the tumor cells. Thus, the next generation of nanomicelles is based on the introduction of molecules that function as specific ligands for certain overexpressed receptors in tumor cells. Functionalization of the carrier surface with these binders allows active targeting and, consequently, a higher rate of cellular internalization of the nanosystem by endocytosis.[8,11]

Several studies have reported the efficiency of folic acid, hyaluronic acid, antibodies, transferrin, and aptamers as potential ligands for active targeting of tumors.[34] In 2009, Wang *et al*.[35] developed a micellar system composed of PEG-block-poly (D, L-lactide) functionalized with tripeptide Arg-Gly-Asp (RGD) with a high affinity for integrin receptors overexpressed in cancer cells. The micelles were grafted with a fluorescent probe to monitor the path traveled, resulting in a high rate of targeting of the test cells.[35] Until then, only a few studies had been performed on polymeric micelles, representing a good opportunity to leverage research focusing on nanocarriers that are adjustable to the necessary treatment. A similar study was conducted by Nasongkla *et al*.[36] in 2004 with cyclic RGD coupled to the surface of caprolactone and PEG-based micelles for the delivery of doxorubicin, in which the internalization was 30 times greater than that in the system without the targeting device.[36] Although these ligands presented superior results, as with the PEG-based systems, an immune response in the body was triggered when nanocarriers with the drug were administered for a certain period; therefore, it was necessary to develop new targeting systems.[37]

Polyamines represent a viable and promising alternative to overcome this issue; they are endogenous molecules that participate in various events in the body and are overexpressed in tumors, possessing an exclusive polyamine transport system (PTS) that is also more active in the tumor environment, and as such can be used for active targeting. Formulations using polyamines as ligands in phase I/II clinical trials have been tested against canine lymph node cells.[38] Chen *et al*.,[34] in 2019, proposed micelles formed by using poly(lactic acid) and poly(2-ethyl-2-oxazolin) (PEOZ) di-block polymers functionalized with spermine, which is a polyamine that is abundant in living organisms and binds with the PTS; spermine is overexpressed in cancer cells and prevents the connection of other polyamines, thereby promoting tumor apoptosis. The micelles carried paclitaxel, and the drug delivery was stimulated by the tumor pH by dissociating the PEOZ block that was protonated at the endosomal pH.[34]

Research has also focused on the development of new acceptors, such as the binding of DUP-1, a peptide composed of 12 amino acids, which binds specifically to cells present in prostate tumors that are negative for prostate specific membrane antigen.[39] In 2016, Jing *et al*. used the linker to develop a dual-directional system,[40] and in 2019, Wang *et al*.[41] used it for the synthesis of polymeric micelles responsive to reactive oxygen species based on methoxy-PEG-b-poly (l-lysine) and vitamin E; both systems were used for the delivery of doxorubicin.[41]

Tumor receptors often agglomerate and disturb ligand–receptor interactions. Therefore, double functionalization with different ligands has been applied to promote the targeting of different overexpressed receptors in tumor cells.[42]

Several types of cancer cells exist, all with their peculiarities and different mechanisms of action, generating cancer with variations in strength and speed of progression. Therefore, when designing a polymer-based micellar system, one should consider the types of cancer cells to be targeted as well as their key characteristics to ensure the efficiency of the formulation. Another point to be considered is the human organism itself, which has different defense mechanisms and reactions to the same drug, depending on the individual.

Stimulated release

Although micellar nanocarriers are a promising alternative in cancer therapy, several biological barriers remain that interfere with their performance, such as interactions with proteins and lipoproteins, in addition to changes in the critical concentration of micelles when introduced into the body, which can cause early disassembly and release at nonspecific sites in the body.[9] The introduction of certain molecules called crosslinks that establish covalent bonds with micelles can reduce this premature rupture and allows disassembly at strategic sites, such as in tumor tissue. The cleavage of the bond between the micelle and the crosslinks can be triggered by specific characteristics of the tumor environment, such as pH, temperature, redox potential, enzymes, and even exogenous molecules.[43]

Tumor cells exhibit variations in temperature (40-42 °C), lipid metabolism, amino acids, increased glycolysis, changes in redox homeostasis, and overexpression of certain enzymes. Tumor environments also present slightly acidic pH because of lactic acid accumulation (pH of 6.2-7.2), with some organelles exhibiting a higher acidity, such as endosomes (pH of 5-6) and lysosomes (pH of 4-5), owing to increased aerobic glycolysis.[7,44]

The pH-triggered release can be programmed by the introduction of polymers with donor groups and electron receptors, such as weak acids and bases, that can destabilize the nanocarrier.[45] Polymers containing carboxylic acids such as poly (glutamic acid)[46] and poly (lactic acid-co-glycolic acid)[11] are appropriate because carboxylate protonation reduces electrostatic interactions, thereby promoting leaks in the structure of the nanocarrier. The use of polymers containing imidazole or amine has also presented superior results in the controlled release of medications; when protonated, they promote micelle rupture, in addition to assisting in the active targeting of tumors because cationized groups have a greater affinity for cancerous tissues.[47]

Ma *et al.* (2018) proposed a micellar system composed of PMPC and poly (L-lysine) linked to doxorubicin and 4-carboxy benzaldehyde by the terminal nitrogen of an amino acid. Drug delivery was triggered by the breakdown of the imine bond an acidic pH, promoting the release of 80% of the drug in a period of 48 h.[48]

The tumor environment has glutathione (GSH) concentrations of approximately 2-10 mM in relation to a healthy environment with only 2-20 μM. This difference in GSH concentration alters the redox potential of the tumor medium, functioning as a stimulus for redox reactions that are responsible for drug release. The use of disulfide bonds in nanocarriers is an attractive strategy in certain studies because, by the action of GSH, these bonds are reduced to sulfhydryl groups and the encapsulated drug is released. The insertion of disulfide bonds in the polymeric nanocarrier can be incorporated directly with a binder containing them or indirectly by the oxidation of sulfhydryl groups.[44,49]

In 2019, Zhang *et al.*[50] investigated a system with pH-conditioned delivery and a tumor-like redox potential. The micelles were designed with a poly(caprolactone)-based core and cystine surrounded by PEG to increase permeability by circulation. The nanocarrier delivered paclitaxel to 4T1 cells by reducing disulfide binding through the action of GSH mediated by a GSH-transferase enzyme (GST), which promoted the breakage and release of 98% paclitaxel in 144 h. Micellar rupture was intensified with the value of the endosome pH through cleavage of the imine bond, reducing the delivery time of the same percentage of the drug to 70 h.[50] A similar study was conducted by Li *et al*.[51] in 2019, wherein prodrug systems were developed using micelles with a PEG hydrophilic cover and a hydrophobic center of camptothecin coupled with poly (N-propargyldiethanolamine 3,3′-dithiopropionate) (PPD), which also responded to the pH and GSH of the tumor environment for the delivery of doxorubicin.[51] Multi-stimulated release is another factor that is being increasingly considered in this field, as it allows a higher rate of delivery and endosomal escape, which may increase the therapeutic index and contribute to a reduction in treatment time. Some recent proposals for nanocarrier systems based on polymeric micelles have been compiled and are listed in Table 1S in the supplementary material.

The proposed nanocarriers with an efficient polymeric system and ligands can contribute ideally as an additional type of treatment; however, attention should also be focused on choosing the right resource; although a quick release for certain patients can offer more resource options, its benefit for the body must also be considered. However, if long-term release for tumor containment can result in death, a shorter release period would be beneficial.

SMART DELIVERY

Over the years, several studies have been conducted in this discipline, but only a few formulations based on polymeric micelles have shown a higher therapeutic index *in vivo* when compared to free drugs. Polymeric nanocarriers still act only as a palliative treatment because their targeted delivery action exhibits a small but significant increase in terms of efficiency with respect to the side effects owing to the biological mechanisms of capture and degradation. Therefore, it is necessary to develop nanocarriers that modulate the biological environment and target cells by adapting their geometry, size, and coating to obtain a therapeutic index that is higher than the existing one.[52] This line of research is currently underway. Recently, polymeric nanocarriers have been used to deliver more efficient but more toxic assets, such as monomethyl auristatin E, a derivative of the antimitotic peptide dolastin extracted from the mollusk *Dolabella auricularia*, which achieve greater tumor inhibition without the toxicity.[53,54]

Currently, the biggest challenge in this field is combining technologies developed in a single nanocarrier and maintaining their stability in the bloodstream. Nanocarriers are associated with two or more discoveries in the area to increase system performance.

pH-stimulated release

In 2020, Palanikumar *et al*.[11] proposed a biodegradable and biocompatible micellar nanosystem formed by the hydrophobic center of polylactic-co-glycolic acid (PLGA) and a bovine serum albumin (BSA) hydrophilic cover functionalized with acidity-triggered rational membrane (ATRAM) peptide for the transport of doxorubicin-triphenyl phosphonium (Dox-TPP) (Figure 4).[11] This chemotherapeutic agent is derived from doxorubicin, which acts on enzymes and binds to deoxyribonucleic acid to prevent cancer cells from reproducing. Dox-TPP, developed in 2014 by Han *et al*.,[55] exhibits approximately four times higher cytotoxicity than free doxorubicin and superior effectiveness against breast cancer, acting preferably in the mitochondria.[55]

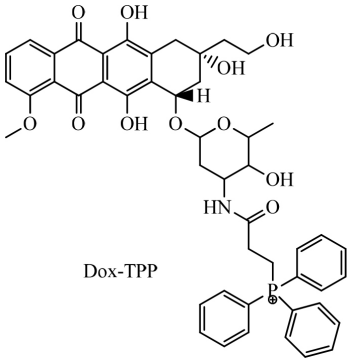

Figure 4. *Doxorubicin-triphenyl phosphonium structure[11] (adapted)*

ATRAM is a pH-sensitive synthetic peptide formed by a 34 amino acid sequence (GLAGLAGLGLLEGGLGLGLGLLEGLWLGLELEGN), developed in 2015 by Nguyen *et al*.[56] It can direct and assist in the internalization of the nano-conjugated system because, in acidic environments such as tumors, glutamic acid is protonated and hydrophobicity is increased, promoting greater affinity for lipid bilayers of cells. Its incorporation into the lipid membrane, followed by endocytosis, occurs with a change in the conformation of the unstructured peptide to a helical conformation at physiological pH.

Although the mechanism of internalization of ATRAM is still in the elucidation phase, its high therapeutic index in preliminary tests makes it beneficial for cancer cell death.[57]

ATRAM coupling to the surface of BSA occurs through the formation of a peptide bond between lysine residues present in albumin and the ATRAM peptide terminal nitrogen by a reaction with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Carbodiimides are well known for their application in peptide synthesis, aiding in the activation of carbonyl to couple the amines and generate amides. The choice of carbodiimide depends on the medium and its solubility in water.[58]

As shown in Figure 5, the mechanism of this coupling reaction of the ATRAM peptide with a lysine albumin residue (1) begins with the protonation of EDC (2), increasing the electrophilicity of the carbonylated carbon. The carboxylate oxygen (3) attacks the electrophilic carbon of the carbodiimide (4), forming the *O*-acylisourea intermediate (5), which, despite being highly reactive, has a short half-life. Thus, the reaction proceeds with the help of *N*-hydroxysuccinimide (NHS) (6), which leads to the formation of the NHS ester (7), a soluble intermediate that is reactive with amines

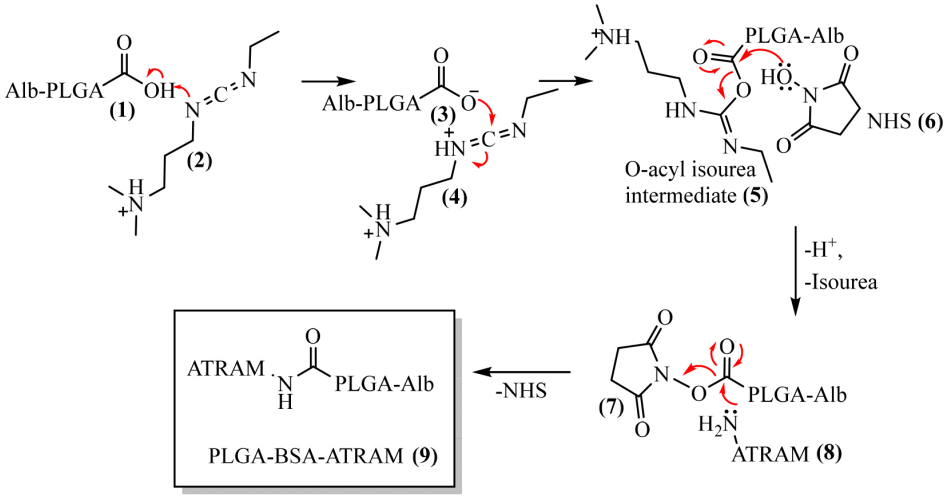

Figure 5. *Coupling mechanism of ATRAM to PLGA-BSA via carbodiimide reaction*[57,58]

and has a longer half-life, allowing the reaction to achieve higher yields.[58] Upon activation of the carbonyl group, the amine group (8) donates a pair of electrons to the carboxylic groups of the carbon, establishing a bond with the amine. Simultaneously, the ester bond breaks, producing isourea and an amide (9), which is a polymeric conjugate for drug transport.[58,59]

The influence of ATRAM on cell uptake was evaluated via fluorescence confocal microscopy and flow cytometry in MCF-7 cells for breast cancer, and the results indicated approximately seven times greater internalization at a pH of 6.5, when compared to that of the nanocarrier without the ligand, within 4 h.

The PLGA-BSA-ATRAM NP exhibited good solubility and stability, retaining the drug in a healthy cellular environment and releasing it only at low pH through the acid hydrolysis of the ester bond present in PLGA. The acid hydrolysis of ester bonds is well known and has been exploited. As shown in Figure 6, it initially occurs by the activation of the carboxylic carbon through the protonation of the carbonyl oxygen present in PLGA (10). The electrophilic carbon undergoes a nucleophilic attack from water, and consecutively, the electron pair of the C=O bond migrates to the protonated oxygen (11). Subsequently, the carbonyl is restored in

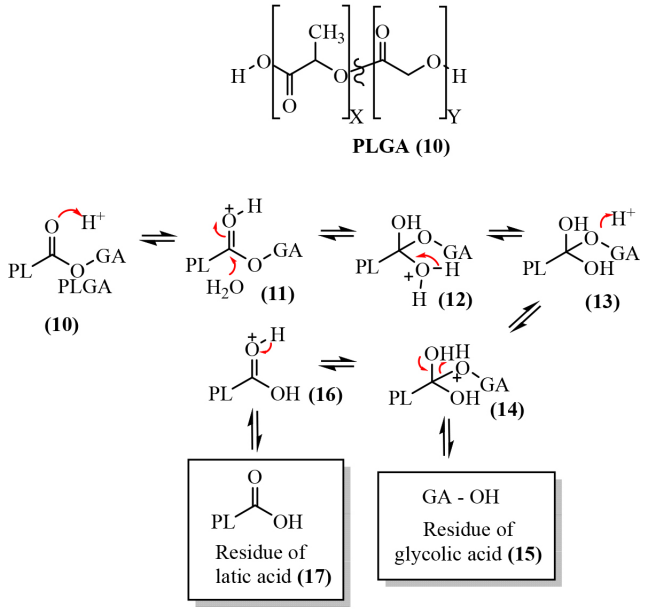

Figure 6. *Mechanism of PLGA cleavage in acid medium for drug release*[11,60]

the posterior intermediate (12), releasing the H^+ ion, which receives a pair of electrons from the oxygen attached to the glycolic acid (GA) moiety (13). The next intermediate (14) undergoes cleavage of the C-GAOH bond, releasing the GA residue (15). Finally, the intermediate (16) is deprotonated and the carbonyl is restored, resulting in lactic acid residues (17).[11,60]

The use of PLGA NPs as carriers of antitumor drugs is well established.[61] However, in 2015, Esfandyari-Manesh *et al.* proposed the functionalization of the PLGA surface by albumin through a peptide bond to avoid opsonization of the system.[62] In this study, the characterization of the nanocarrier was performed via transmission electron microscopy (TEM), zeta potential analysis, and infrared spectroscopy, and micelles with a diameter between 105 and 130 nm with a 10 nm thick layer of albumin were observed. The quantification of the corona protein on the surface of the nanocarrier was verified, presenting a significantly lower concentration in relation to the system without albumin, which prevented its formation. The pH-triggered release was simulated, yielding a detachment of 45% of the drug at a pH of 5.8 and 79% over 24 h at a pH of 5, which are the values observed in the initial and late endosomes, respectively.

The research group performed an additional evaluation correlating the release of the drug with the concentration of GSH, obtaining a detachment of 47% of Dox-TPP. This can be explained by the reported affinity between albumin and GSH.[63,64]

Release stimulated by redox potential

In 2020, Yang *et al.*[42] developed amphiphilic micelles for the first time with a hydrophobic center of vitamin E succinate (VES) functionalized by folic acid (FA) and hyaluronic acid (HA), which act as specific ligands for the folate receptor (FR) and CD44 receptor, respectively, with delivery triggered by GSH. VES is harmless to healthy cells and is of great importance to the body. However, it is significantly toxic to cancer cells and aids in the containment of tumor growth by inducing apoptosis and inhibiting metastasis.[65] The system accommodated paclitaxel, which is a chemotherapy drug with low solubility and high toxicity, which is derived from *Taxus brevifolia*. It is an alkaloid that inhibits mitosis and the performance of enzymes in the production of proteins necessary for cell reproduction.

As shown in Figure 7, first, the VES (18) undergoes amidation with cystamine (CYS) (23). This process occurs with the activation of the VES (18) carbonyl by EDC (19) in the presence of NHS (21) via the carbodiimide reaction, generating VES-CYS conjugate (24). Triethylamine was used to increase the pH and assist in the NHS exit;

at an acidic pH, the amino group of the amino acids is protonated and becomes less reactive, while the hydrolysis of the NHS ester is also slower.[58]

Then, as shown in Figure 8, the HA (25) is activated by EDC and it receives the VES-CYS (24) conjugate in one of the carbonyls bound to the CYS terminal nitrogen coupled to VES, which forms the HA-CYS-VES (HSV) conjugate (27).[42]

Additionally, as shown in Figure 9, the terminal carbonyl of FA (28) is activated with the aid of N,N'-dicyclohexylcarbodiimide, and the ethylene diamine nitrogen donates a pair of electrons to the carbonyl carbon, generating FA-NH_2 (31), which reacts with the HSV conjugate (27) to form the amphiphilic polymer FA-HA-SS-VES (FHSV) (32).[42]

The polymer reached a critical micelle concentration of 15.78 mg L^{-1}, a relatively low value that contributed to the stability of the micelles in the blood circulation, providing safe transport for the drug.[42] The currently marketed drug Taxol® was used as a comparative standard, releasing approximately 80% paclitaxel at pH 7.4 (pH of healthy blood circulation) in a period of 12 h. This indicates that only 20% of paclitaxel will reach the tumor, with higher doses required to achieve the desired cytotoxic effect. The FHSV micelles exhibited a release of approximately 41% at the same pH and period, causing the amount of drug delivered to tumor cells to be tripled.[42]

Despite the favorable results of nanocarriers, their effectiveness is compromised by the differences in the body of each patient, which may develop into a means to eliminate the system before the target is reached. In this study, targeting through the affinity of more than one linker for different receptors helped in circumventing biological processes, performing what is called intelligent delivery.

Tests with 10 mmol L^{-1} GSH were also performed in a medium similar to that of the tumor, with a cumulative release of 92.4% for the nanosystem when compared to the 76.8% release of free paclitaxel in

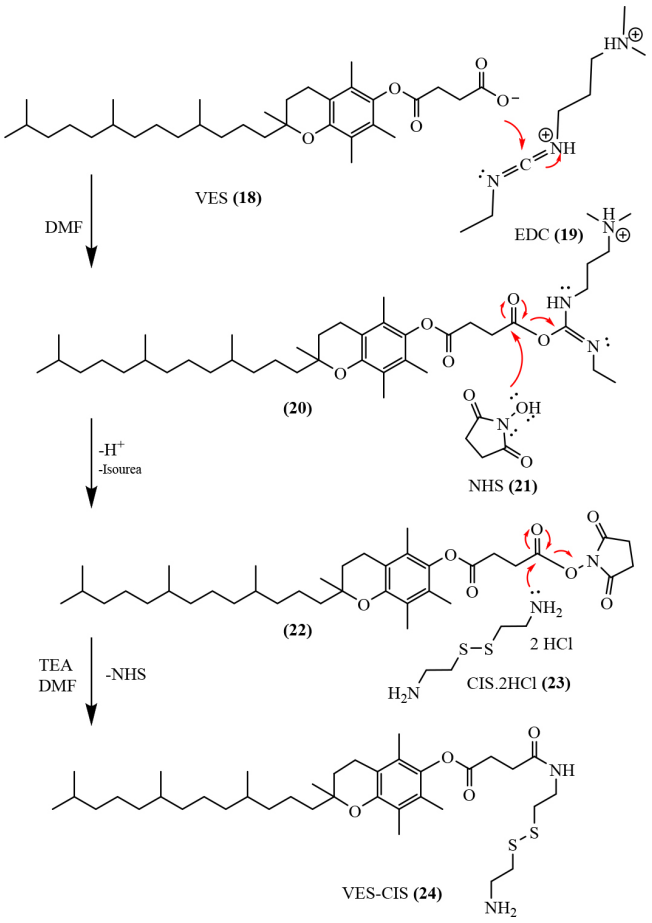

Figure 7. VES-CYS synthesis[42]

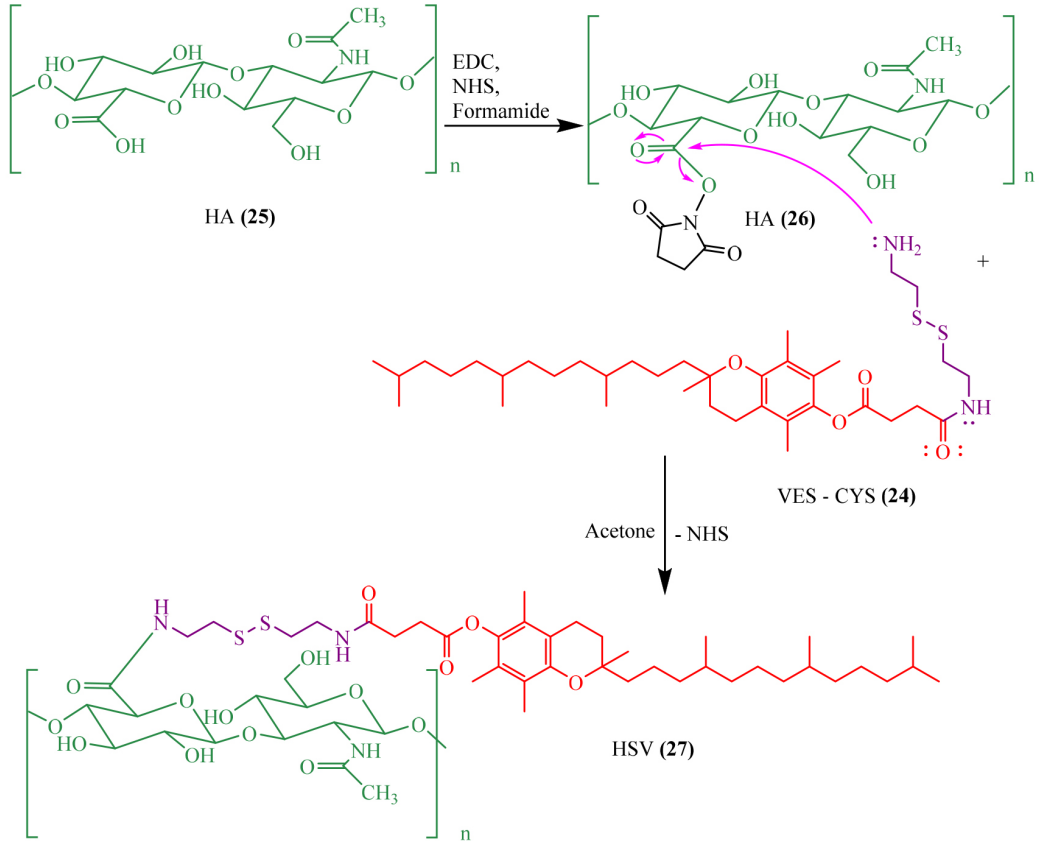

Figure 8. HSV synthesis[42]

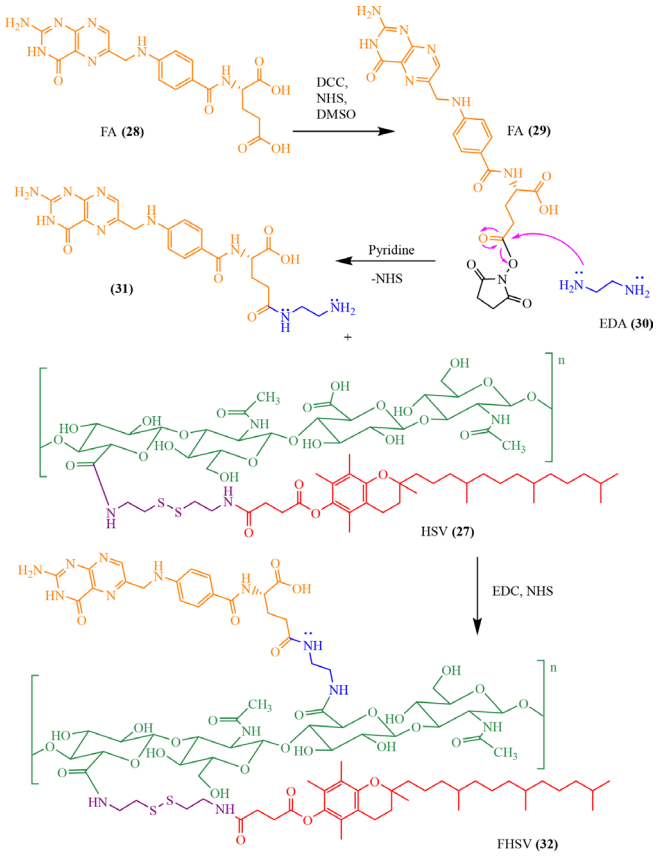

Figure 9. *FHSV polymer synthesis*[42]

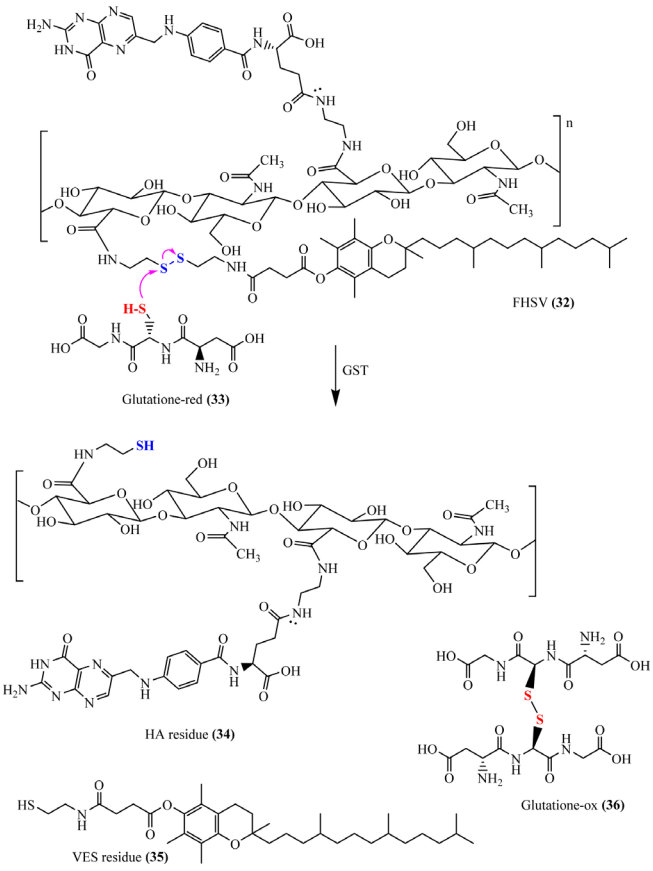

Figure 10. *Mechanism for reducing disulfide binding*[65,67]

a 60 h period. The cytotoxicity against MCF-7 cells (a type of tumor cell with overexpressed CD44 receptor and FR of the nanocarrier with paclitaxel, when compared to that of the commercial drug, was considerably higher, allowing for a reduction of approximately 77% of the administered dose. This can be explained by the presence of GST, which promotes nucleophilic attack of reduced GSH on substrates containing electrophilic sulfur atoms, such as the FHSV polymer. In this reaction, as shown in Figure 10, the sulfur of the sulfhydryl group present in the reduced GSH (33) donates an electron pair to the disulfide bond of the polymer (32), promoting the rupture of this bond and forming sulfhydryl groups in the polymer. This causes the rupture of this polymer and, consequently, of the micelle, releasing the drug, polymeric residues (34 and 35), and oxidized GSH (36) into the intracellular medium.[66,67]

Multi-stimulated release

The development of nanocarriers with disassembly triggered by more than one stimulus represents one of the most promising strategies in this field, as these nanocarriers allow for greater efficiency of therapy through both accelerated and prolonged delivery.

Li *et al.*[51] synthesized a novel prodrug nanocarrier based on a camptothecin derivative, PEG2000-N3, and PPD for the transport of doxorubicin with pH-responsive release and a high concentration of GSH.[51]

To prepare this nanocarrier, three synthetic precursors were obtained and synthesized separately: PEG2000-N3 (41), camptothecin derivative (47), and PPD (50). As shown in Figure 11, to synthesize the first precursor, two reactions are performed: tosyl chloride (OTS) (37) is reacted with PEG2000 (38) to produce PEG2000-OTS (39), which is reacted with sodium azide (40) in the second step to form PEG2000-N3 (41). Subsequently, in the second multistep reaction, the camptothecin derivative (47) containing a disulfide bond and an azide group is obtained. The third precursor is PPD (50), a polymer produced through a chemoenzymatic reaction, which was developed in 2018 by the same research group. Finally, in the fourth reaction, PEG2000-N3 (41) and the camptothecin derivative (47) were coupled to PPD (50) through a click reaction to produce the nanocarrier (51). PEG was added to provide a hydrophilic-hydrophobic balance to the system, which was positioned in the outer layer of the micelle.[51]

The preparation of prodrug nanocarriers has been gaining ground in recent years, and their use in combined chemotherapy allows the reduction of the administered doses, thus attenuating the side effects of the treatment and improving their efficiency owing to the synergistic effect of drug therapy that presents different action mechanisms while hindering the resistance to medication.[68]

In this study, the authors characterized the degree of polymerization and the occurrence of the click reaction via H^1 nuclear magnetic resonance spectroscopy, which demonstrated a ratio of 1:0.56:0.38 for PPD, camptothecin, and $mPEG_{2000}$, respectively. The micelles self-assembled at a critical concentration of 6.08 mg L^{-1} and were characterized by fluorescence spectrometry, dynamic light scattering, zeta potential, and TEM, resulting in an average diameter of 31.7 ± 1.8 nm and low polydispersity with a load of 20% doxorubicin. The measured zeta potential value was −1.15 ± 0.23 mV, which is close to neutrality, and therefore may corroborate the prolonged circulation and higher rate of accumulation by EPR.

The release of drugs triggered by GSH in the intracellular tumor medium was programmed to occur in two parts of the nanosystem. First, the disruption of the S–S bond was introduced into camptothecin by the previous reaction in various stages with 2,2'-dithiodiethanol, allowing the drug to be decoupled from the polymer. Then, in the PPD structure, the rupture of the micelle and release of both drugs were promoted. The

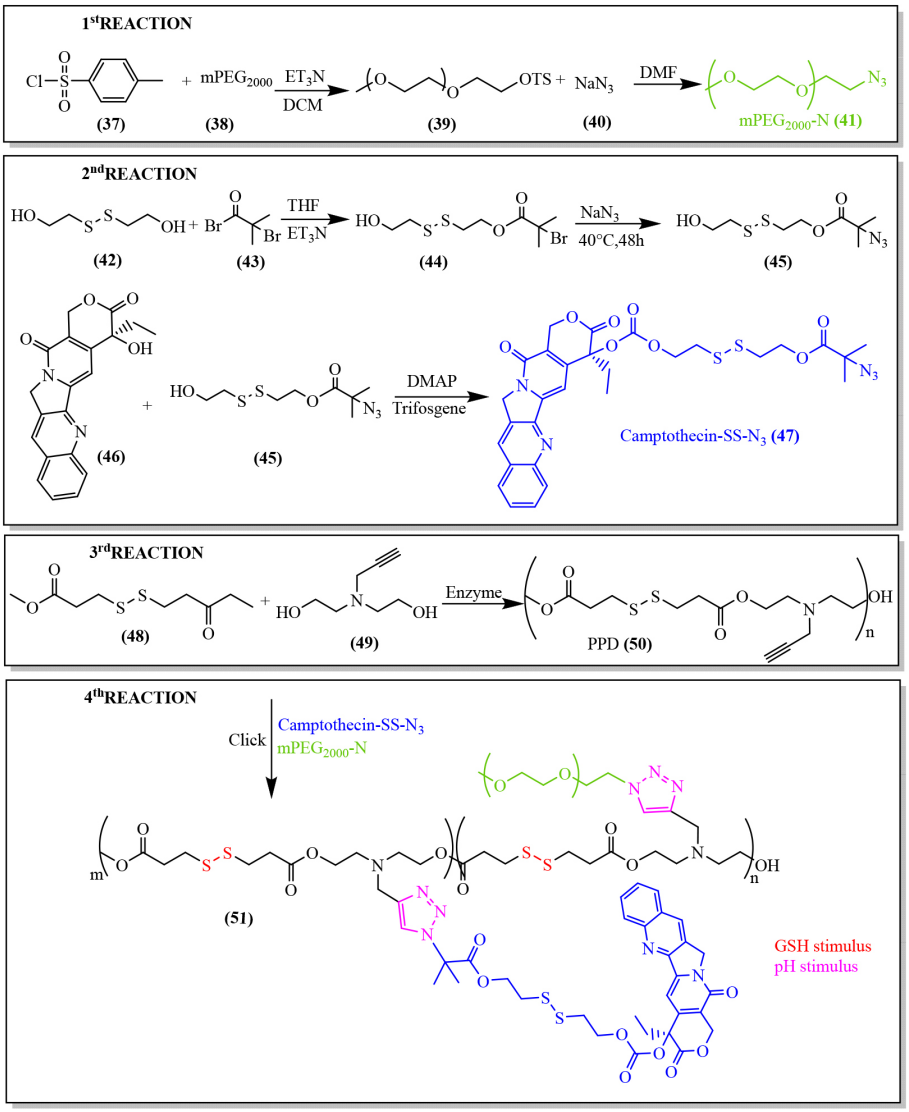

Figure 11. *Multi-stimulated delivery nanocarrier reaction*[51]

number of drugs dispensed was simulated *in vitro*, yielding 17 and 10%, respectively, for doxorubicin and camptothecin under physiological conditions over a period of 72 h. Under tumor conditions, the release rates were approximately 73 and 72%, respectively, in the same period. In cytotoxic tests performed on healthy and HepG2 cancer cells, the prodrug nanocarriers loaded with doxorubicin exhibited a 123.9% greater synergistic effect, 127% more selectivity, and 36.8% reduction in IC_{50} when compared to those of free drugs. Cell uptake was also higher, with a 30% higher internalization rate in cancer cells owing to endocytosis-mediated intake.

In these works, it is possible to verify that a system already known as the nanocarrier up to the PLGA base and even the PEG can present superior performance when performing specific changes that fulfill a limitation. The merging of approaches is also a feature that continues to produce satisfactory results and further research on this should be encouraged.

CONCLUSIONS AND PERSPECTIVES

The studies conducted in recent years offer ample opportunities to continue advancing research in the field of nanomedicine, focusing on the limiting factors of each system and the reproduction of its positive points.

It is known that an ideal nanocarrier must have a neutral and easily cationized surface, and that surface ligands can increase the rate of internalization; however, even with this prior knowledge, there are still formulations that are easily detected by the immune system, which are then cleared out by the organism in a short time. This implies that important information about previous research and the functioning of the human organism may not be reaching researchers as it should.

New trends have recently emerged in the synthesis of polymeric nanocarriers employing supramolecular chemistry to develop micelles with a simpler disruption process and moderate drug entrapment, resulting in the requirement of less effort for intracellular delivery. However, satisfactory results have not yet been obtained and further studies using this approach are required.

The application of nanotechnology to medicine is a multidisciplinary area, and this may be one of the barriers to obtaining an ideal nanocarrier. One of the biggest obstacles to the development of an ideal nanocarrier is the difficulty in predicting the behavior of this nanotechnological product in terms of their interaction and reception in a human organism.

To design an efficient antitumor drug delivery system, in-depth knowledge is required of not just the synthesis processes, polymers, human metabolism, or medicine, but of all these areas. The fusion of

all of these aspects is the basis for the construction of a drug that can, in fact, not only contain cancer, but also cause its regression and cure.

SUPPLEMENTARY MATERIAL

Table 1S with the caption "The main systems used in the fabrication of the polymeric nanocarriers" and Table 2S with a small glossary are freely available at http://quimicanova.sbq.org.br, in PDF format.

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