Current phthalocyanines delivery systems in photodynamic therapy: an updated review

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Abstract

Photodynamic therapy has emerged as an effective therapeutic alternative to treat oncological, cardiovascular, dermatological, infectious, and ophthalmic diseases. Photodynamic therapy combines the action of a photosensitizer with light in the presence of oxygen to generate reactive oxygen species capable of reacting with cellular components resulting in injury and, consequently, inducing cellular death. Phthalocyanines are considered good photosensitizers, although most of them are lipophilic, difficulting their administration for clinical use. A strategy to overcome the lack of solubility of phthalocyanines in aqueous media is to incorporate them into different delivery systems. The present review aimed to summarize the current status of the main drug delivery systems used for Zn and Al phthalocyanines and their effect in photodynamic therapy, reported in the last five years. Liposomes, polymeric micelles, polymeric nanoparticles, and gold-nanoparticles constituted some of the most used carriers and were discussed in this review. The latest studies reported strongly suggests that the application of nanotechnologies as delivery systems allow an increase in photodynamic therapy efficacy and reduce side-effects associated with the phthalocyanine administration, which represents a promise for cancer treatments.

Keywords: Photodynamic therapy, photosensitizers, phthalocyanines, drug delivery systems, liposomes, polymeric micelles, polymeric nanoparticles, gold nanoparticles.

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History of photodynamic therapy

The historical milestones behind the development of photodynamic therapy (PDT) began 3000 years ago. Light has been used as therapy from ancient times to modern days. [1, 2] Phototherapy has been used by ancient Egyptian, Indian and Chinese civilizations to treat skin diseases such as psoriasis, vitiligo, and cancer, but disappeared for many centuries and was rediscovered at the ending of the nineteenth century.[3, 4]

Niels Finsen, a Danish medical scientist, contributed to the foundation of modern phototherapy. In 1893, he observed that the skin of smallpox patients showed the best results after red-light exposure. [5, 6] Later, he also used ultraviolet light to treat cutaneous tuberculosis. In 1899, Finsen published the first modern book about the treatment of diseases using light entitled *La Phototherapie* (in English, The Phototherapy).[7] In 1903, Finsen was awarded the Nobel Prize in Physiology or Medicine for his contribution to the treatment of diseases, especially cutaneous tuberculosis, also called lupus vulgaris.[8]

In 1900, several researchers also observed that a combination of light and certain chemicals could induce cell death.[6] O. Raab, a german medical student, noted the toxicity of acridine dye on *Paramecium spp.* protozoans when sunlight was focused on the sample. This observation led him to conclude that some compounds, now termed "photosensitizers" (PSs), could be used to improve the already known antimicrobial activity of light.[2, 9] In the same year, J.Prime, a French neurologist, observed that epilepsy patients treated with oral eosin developed dermatitis in areas exposed to sunlight.[10] Later, Von Tappeiner investigated the application of eosin and white light for the treatment of skin cancer, lupus vulgaris, and condyloma.[11] At the same time, he found that oxygen was necessary to develop reactions mediated by light and described this phenomenon as a "Photodynamic Action" (*Photodynamische Wirkung*).[12] Regardless of the encouraging results reported, it was also observed that photosensitizers, as acridine, were toxic and highly oncogenic potential.[10]

The most significant advance in PDT was the discovery of hematoporphyrin (Hp).[13] Scherer produced it in 1841, and many years later, Haussman started the porphyrin-based PDT using Hp.[14] Hematoporphyrin was innocuous in the absence of light in contrast to other PSs as acridines. In 1913, Friedrich Meyer-Betz injected himself intravenously with 200 mg of Hp and then exposed himself to sunlight. No drug-related effects were observed previous to light exposure. However, after sun exposure, he suffered edema and hyperpigmentation due to the phototoxic effect of Hp. These reactions were observable for months.[15] Years later, several studies demonstrated that was detected fluorescence emitted from tumors after intravenous administration of porphyrins since these molecules preferentially were accumulated in them.[2, 16, 17, 18] In 1955, Samuel Schwartz and colleagues discovered that the Hp was impure and consisted of a mixture of porphyrins and other impurities. This mixture of oligomers was

optimized and named hematoporphyrin derivative (HpD), which was much more efficient than Hp.[19] Lipson *et al.* demonstrated the preferential accumulation of HpD in tumors. This observation constituted a promising diagnostic tool to detect tumors by endoscopic fluorescence.[20] A few years later, HpD was employed to eliminate recurrent breast carcinoma, marking the beginning of oncologic use of PDT.[6] In the 1970s, T. Dougherty performed clinical trials of oncologic PDT using HpD and red light.[21] Later, it was demonstrated that exogenous application of 5-Aminolevulinic acid (5-ALA) promoted the endogenous synthesis of protoporphyrin IX and was shown to be efficient in combination with red light leading to the destruction of erythroleukemic cells.[22]

These results of PDT allowed several medical regulatory agencies to approve the use of PS for clinical purposes. In 1993, purified HpD marketed as Photofrin® was approved for the treatment of bladder cancer in Canada. The Food and Drug Administration (FDA) in the USA approved Photofrin® in 1995 for treating esophageal cancer, in 1998 for lung cancer, and 2003 for Barrett's esophagus. The treatments with Photofrin® were then extended to the head, neck, abdominal, intestinal, skin, breast, thoracic, brain, and cervical cancer. Photogem® and Photosan-3® are other types of hematoporphyrin derivatives that have been approved for clinical applications in Russia, Brazil, and the European Union, respectively. In 1999, the FDA approved 5-ALA as a topical salve marketed as Levulan Kerastick® to treat precancerous and cancerous skin lesions. In 2000, a verteporfin preparation (another porphyrin) was approved by the FDA as Visudyne® for the treatment of ophthalmological disease, including age-related macular degeneration. Visudyne® is used for the treatment of several diseases characterized by cellular hyperproliferation in more than 50 countries.[6, 23, 24] Currently, the development of more selective and potent PSs for PDT continues being studied, and several are under investigation in clinical trials.[25, 26, 27]

Mechanisms in Photodynamic Therapy

In a clinical setting, PDT involves the intravenous administration or topical application of the PS, followed by irradiation to a specific wavelength. [28, 29] The process involved in PDT is shown in the modified Jablonski diagram (Figure 1). A PS in its ground state PS^0 has two electrons with opposite spins known as singlet state. When PS^0 absorbs a photon, one of these electrons is boosted into a high-energy orbital but keeps its spin, generating an electronic transition to an excited singlet state $^1PS^*$. $^1PS^*$ is a short-lived (nanoseconds) state and can lose its energy by either fluorescence emission or thermal decay. $^1PS^*$may also lose its energy through intersystem crossing (ISC) to generate an excited triplet-state ($^3PS^*$) relatively long-lived (microseconds). The $^3PS^*$ can undergo two kinds of photochemical reactions, type I and type II, to generate

reactive oxygen species (ROS) and singlet oxygen ($^1O_2$), respectively. These species are responsible for injury in cellular components and cell death. [26, 29, 30, 31]

The type I photochemical reaction leads to the production of ROS. ^{3}PS* can react with a substrate, such as a cell membrane or a molecule, to transfers an electron, which leads to the formation of free radicals and radical anions. This process leads to the production of ROS. Frequently occurs the production of superoxide anion ($O_2^{\cdot -}$) by electron transfer from ^{3}PS* to molecular oxygen.[6] Superoxide $O_2^{\cdot -}$ is not particularly reactive in biological systems, but it can react to produce highly reactive species like hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$).[29, 32, 33] In the type II reaction, the ^{3}PS* can transfer its energy directly to molecular oxygen to generate highly reactive $^1O_2$.[25, 26] It is assumed that the mechanism of type II is the most critical process responsible for PDT effects.[28] The $^1O_2$ generated presents a short lifetime and a migration distance of approximately 1 μm.[34] Therefore, the subcellular accumulation and localization of PS are critical for the therapeutical outcome.[34, 35] Both type I and type II photochemical reactions can take place, and the ratio between these processes depends on the PS used, the concentrations of substrate, and oxygen available at the site of reaction.

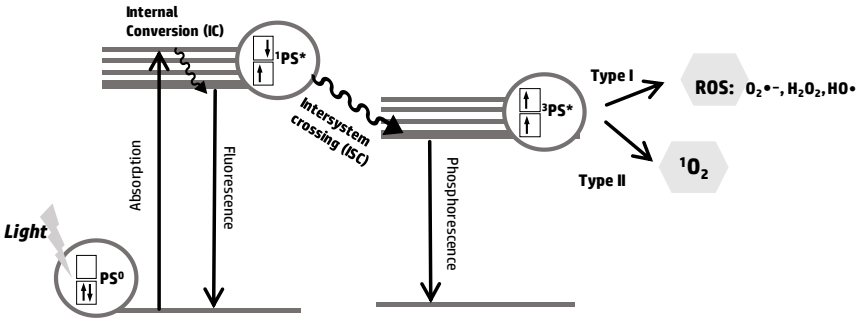

Figure 1. Modified Jablonski energy diagram

Photodynamic therapy

Photodynamic Therapy is a novel and non-invasive therapeutic alternative for the treatment of oncological and non-oncological diseases.[32, 33] In the last decades, PDT has gained increasing attention in treating several cancers, including head and neck, lung, prostate, brain, ovarian carcinomas, bladder, and skin.[31, 36, 37, 38, 39, 40, 41] It has also been successfully used in the treatment of atherosclerosis, age-related macular degeneration, psoriasis, and anti-viral therapies such as for herpes.[31, 32, 42, 43] PDT involves the action of 3 essential components: a photosensitizer, light, and oxygen (Figure 2).[13, 35] They are non-toxic individually, but when the PS absorbs light of the appropriate wavelength in the presence of oxygen, ROS and $^1O_2$ are generated. This phenomena trigger injury and lead to cell death through different mechanisms such as apoptosis or necrosis, among others. [6, 44]

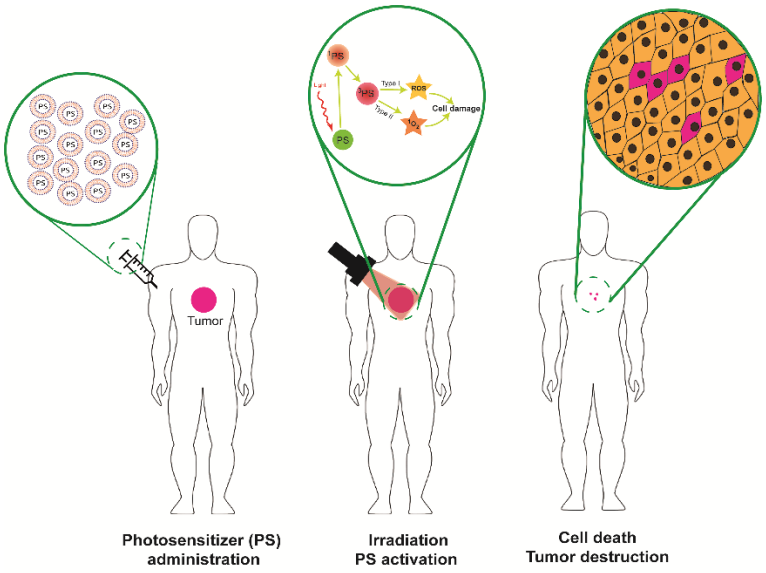

Figure 2. Scheme of photodynamic therapy

Compared to traditional cancer treatment that includes surgery, chemotherapy, and radiotherapy, PDT presents certain benefits. Since PDT induces cellular death followed by tumor regression at the site of irradiation, it minimized the damage to surrounding non-irradiated tissue and diminishing the classical undesirable's side effects. [45, 46, 47]

For an effective PDT, sufficient light must reach the desired tissue. Thus, the tissue-penetration depth of light absorbed by PS is a critical point.[32] It is known that both scattering and light absorption by biological tissues is minimized at longer wavelengths allowing them to reach deeper in the tissue. The absorption of tissue associated compounds such as amino acids, nucleic acids, melanin, myoglobin, hemoglobin, and cytochromes occurs at lower wavelengths, while water absorption increases at wavelengths above 850 nm.[48, 49] Moreover, PSs absorption at shorter wavelengths leads to skin photosensitivity; meanwhile, at wavelengths greater than 800 nm, photons will not have sufficient energy to excite them.[28] Therefore, the optimal light wavelength range to irradiate in PDT is between 650 and 850 nm, which allows an increased penetration with minimal light scatter and maximum PS activation. This wavelength range is known as “therapeutic window.” [30, 32]

For a compound to be considered as a suitable photosensitizer, it should present the following features: simple and readily obtained synthesis, a pure chemical form of known composition, minimum dark toxicity, high extinction coefficient in 650–850 nm, low absorption at 400–600 nm, $^1O_2$ high quantum yield, be stable and remain soluble in biological media.[28, 31, 32, 33, 46] Several PSs are currently commercially available to use in PDT for treatment of several diseases, among these, Photofrin®, Visudyne®, Foscan®, Levulan®, MetVix®, Photochlor®, and Photosens®.[29, 41] Photosensitizers could be classified as porphyrins or non-porphyrins. Although porphyrins structures comprise the majority of PSs, several non-porphyrin PSs exhibit

photodynamic activity. This latter group of PSs has been considerably less studied and involves compounds of a diverse chemical structure as anthraquinones, phenothiazines, xanthenes, cyanines, and curcuminoids. [23, 50, 51]

Porphyrin photosensitizers have been widely and extensively studied for use in PDT. This group of compounds could be classified as first, second, or third-generation photosensitizers. [23, 52] HpD and Photofrin® are known as first-generation photosensitizers and comprise complex mixtures of structures that possess the maximum absorption at a relatively short wavelength (~630 nm), low molar extinction coefficient, long half-life, and high skin accumulation, being responsible for skin phototoxicity. [53, 54]

Second-generation PSs have been developed to improve and minimize drawbacks associated with first-generation PSs. [55, 56, 57] This generation of PSs are chemically pure, presents a maximum absorption in the wavelength range of 650–800 nm, higher yield of $^1O_2$ formation, higher molar extinction coefficient, and they are associated with lower post-treatment skin phototoxicity as compared to the first-generation of PSs. Several compounds are classified as second-generation PSs, and the most extensively studied PSs are porphyrin derivatives, [58, 59] phthalocyanines, [24, 28, 46] chlorins [60, 61] and ALA. [62, 63, 64] Phthalocyanines (Pcs) are aromatic heterocycles that contain four isoindole rings bridged by nitrogen atoms. They have 18π-electron delocalized, which confer their unique physical and optical properties.[65] As second-generation PSs, Pcs have absorption λ_{max} > 670 nm and extinction coefficients (ε_{max}) > 1.10^5 $M^{-1}cm^{-1}$. Furthermore, their chemical structures are readily modified through the introduction of central metals and axial, peripheral, and non-peripheral substituents.[24] The photophysical and photochemical properties of Pcs are influenced by the nature of central metal ions. Diamagnetic ions, such as Zn^{2+} and Al^{3+}, originate Pc complexes with both high triplet yields and long triplet lifetimes. Consequently, these complexes are known as Zinc(II)phthalocyanine (ZnPc) and chloroaluminum(III)phthalocyanine (AlPc), (Figure 3).

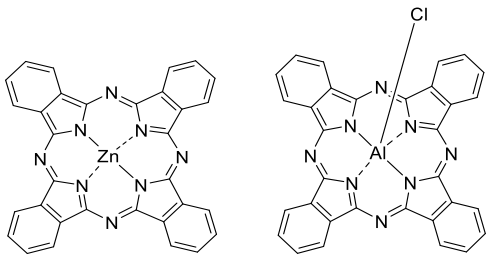

Figure 3. Structure of ZnPc and AlPc

ZnPc and AlPc, usually show a high quantum yield of singlet oxygen, and, in consequence, these Pcs become the main candidates for application in PDT.[28] Al^{3+} is a suitable ion for axial

substitution, which decreases the tendency to aggregate due to sterical hindrance. Also, the nature of the substitute modulates solubility, among other properties.[66] Most second-generation PSs are aromatic and lipophilic with poor solubility in aqueous media, which results in a significantly limiting factor in their intravenous administration. Therefore, under physiological conditions, these PSs tend to aggregate, which affects photochemical properties and bioavailability at the desired site of action. To enhance the solubility as well as the selectivity, second-generation PSs have been incorporated into several delivery systems (Figure 4), such as liposomes,[67, 68, 69, 70] gold nanoparticles (AuNPs),[71, 72, 73, 74] polymeric nanoparticles (PNPs),[75, 76, 77] and polymeric micelles (PM) [78, 79, 80, 81] with or without conjugation to active targeting agents, such as antibodies (Ab), proteins or peptides.[46, 82, 83] Consequently, third-generation PSs comprise second-generation PSs conjugated or encapsulated in carriers that allow the transport of PSs to improve accumulation at the desired site, which significantly reduces damage in surrounding healthy tissues.[41, 50, 82]

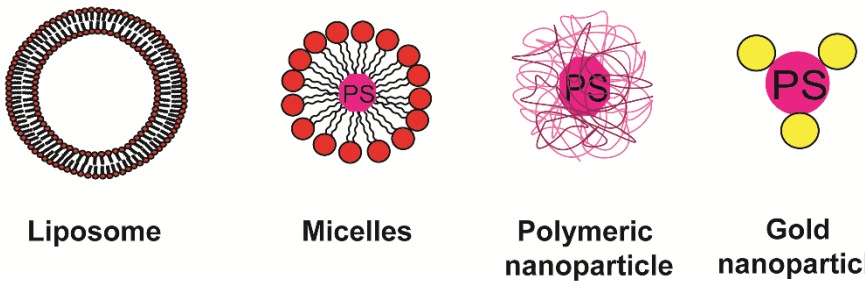

Figure 4. Delivery systems. Liposomes, Polymer micelles (PM), Polymer nanoparticles (PNPs) and Gold nanoparticles (AuNPs), photosensitizer (PS).

Despite the success of PDT, new compounds are still being understudy to enhance their effectiveness in photodynamic therapy. A summary of Pcs presented in this review is shown in Table 1.

Table 1. Spectroscopic data, fluorescence quantum yield (Φ_F) and singlet oxygen quantum yield (Φ_Δ) in DMF of Pcs presented in this review.

Pc	λ_{max} (nm)	λ_{em} (nm)	$\log_\varepsilon$	Φ_F [a]	Φ_Δ [b]	Reference
ZnPc	670	675	5.37	0.17	0.56	[84, 85]
AlPc[c]	672	680	5.31	0.41	0.43	[86, 87]
TAZnPc	702	712	--	0.03	0.45	[84]
DMEZnPc	696[d]	--	5.1[d]	0.05	≤0.01[e]	[88]
$(PhSSO_3Na)_4ZnPc$	669[f]	--	--	--	--	[89]
$AlPcS_4$[g]	679	686	--	0.52	0.30	[90, 91]
PcN	674	683	5.2	0.20	0.69	[92]
TMAESZnPc[h]	688	--	5.17	0.28	0.60	[93, 94]
OMEEZnPc[h]	666	672	5.21	0.22	0.61	[95]
OFMEEZnPc[h]	671	675	5.27	0.20	0.72	[95]
TPPOZnPc[d]	684	--	5.21	<0.01	0.20	[96]
G_1-DPcZn[d]	686	678	5.99	0.86	--	[97]
G_2-DPcZn[d]	684	709	6.09	0.71	--	[97]
TMBZnPc	681 (677) [d]	697	5.20	0.52[d]	0.51[d]	[76]
ZnNPc[d]	766	778	5.0	0.07	0.41	[98]
TPZnPc	672[h]	--	--	--	0.54[d]	[99, 100]
C3Pc[h]	698	--	--	--	--	[101]
C11Pc[h]	701	--	--	--	0.45[i]	[102]
MPZnPc[d]	674	--	--	0.16	0.56	[103]
MPBTrPyZnPc[d]	682	--	5.55	0.15	0.36	[104]
TrBMPZnPc[d]	680	--	5.17	0.1	0.62	[105]

[a] Φ_f = fluorescence quantum yield
[b] Φ_Δ= singlet oxygen quantum yield
[c] Ethanol
[d] DMSO
[e] Determined for Pcs incorporated into liposomes in an aqueous environment.
[f] DMF/H_2O
[g] Methanol
[h] THF
[i] Toluene

Liposomes

Liposomes were the first nanomedicine delivery vehicles applied in the treatment of several pathologies. Liposomes are vesicles composed of one or more concentric phospholipid bilayer. [106, 107] Their size varies from 50 nm to several micrometers. They are non-toxic, highly biocompatible, biodegradable, and suitable for surface modifications allowing to add of targeted molecules. [108, 109, 110] Liposomal formulations can be loaded with both lipophilic and hydrophilic drugs. Lipophilic compounds are associated with the bilayer, while hydrophilic ones may be entrapped inside the liposome aqueous core. [69, 111]

The efficiency of liposomal formulation as drug delivery depends on their pharmacokinetics, which is strongly related to the size, surface charge, steric stabilization, dose, and route of administration.[112] Features, as mentioned above, could be easily modified and are influenced

by the type of phospholipid and components from which liposomes are prepared (Figure 5). It's well known that liposomes have a short lifetime in blood circulation since they are prone to clearance by the reticuloendothelial system (RES). The clearance of liposomes by the RES increases with increasing particle size; therefore, smaller liposomes (≈ 100nm) tend to have longer circulation half-life than larger liposomes of the same membrane composition and rapidly localize in tumor tissue due to their permeation through tumor microvessels.[113, 114] Drug release and clearance by the RES could also be reduced if high phase-transition phospholipids such as distearoyl or hydrogenated phosphatidylcholines (PC) and cholesterol (chol) are incorporated in the liposome formulation.[115] Another widely used strategy consists in the use of polyethylene glycol (PEG), the most commonly used polymeric stabilizing agent for liposomes, since it reduces the identification by macrophages, increasing their circulation time.[110, 116] The use of charged phospholipids generates liposomes with charge capable of interacting with different surfaces [117]; for example, cationic liposomes can attach to vascular endothelium and sites of inflammation. It was reported previously that negatively charged liposomes are taken up more efficiently by cells than neutral liposomes.[118] Ultradeformable liposomes (UDLs) are vesicles composed of phospholipids combined with a molecule known as an edge activator, such as surfactants or co-solvents, which allows the UDLs to undergo significant fluctuations in their structure. UDLs can deform sufficiently to penetrate pores 1/5th of their size and use the transepithelial water gradient as a driving force to infiltrate across the skin.[119]

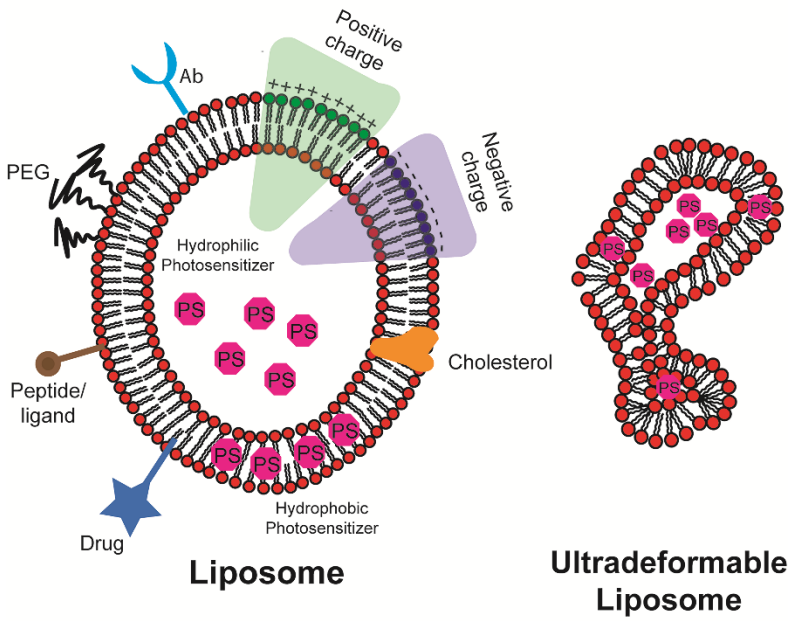

Figure 5. Different type of liposomes.

Another remarkable advantage of liposomes as carriers is that it's can be modified for a specific purpose, such as specific targeting. These functionalized liposomes have one or more molecules with a high affinity for particular membrane markers on malignant cells bounded to its surface, resulting in increased and specific interaction with target cells. A wide range of molecules has been used for targeting, including glycolipids, peptides, glycoproteins, growth factors, and monoclonal antibodies. [120, 121, 122]

Liposomes appeared particularly attractive for the delivery of lipophilic PSs. These molecules can be entrapped in their phospholipid bilayer, decreasing aggregation and, in consequence, increasing the photodynamic efficiency of them.[69, 111, 113, 123, 124] Liposomes as drug delivery systems for PSs have been studied since the late '80.[125] The incorporation and spectroscopic studies of ZnPc into dipalmitoylphosphatidylcholine (DPPC) liposomes were reported in 1987.[126] In the same year, Redi *et al*. reported the pharmacokinetic behavior of ZnPc into DPPC liposomes in BALB/c mice with a transplanted fibrosarcoma.[127] In the last five years, several studies using liposomes as drug delivery systems for Zn and Al Pcs were reported (Table 2).

Conventional liposomes

In 2016, Silva *et al.* [128] studied *in vitro* PDT using liposomes as delivery systems for AlPc on glioblastoma cell line U87MG. They have loaded AlPc into liposomes (LipAlPc) composed of soybean phosphatidylcholine (SPC) and chol, and determined the ID_{50} (irradiation dose responsible for killing 50% of cells). The results obtained using free AlPc exhibit an ID_{50} = 1.17 J/cm^2; meanwhile, when LipAlPc was applied, the effect of the light doses was higher compared with free AlPc showing an ID_{50} = 0.47 J/cm^2. Furthermore, a recent report from Miretti *et al.* also studied PDT on glioblastoma cells (T98G) using ZnPc and Tetraamine-substituted ZnPc (TAZnPc), both free and incorporated into DPPC/chol liposomes.[67] In this work, two liposomal formulations were analyzed for each Pc, where lipids concentration remains constant in both formulations, although Pc concentration was different. Liposomal formulations didn't affect the behavior of ZnPc-liposomes in PDT; nevertheless, TAZnPc-liposomes showed a slight difference in the effectiveness of both formulations. *In vitro* cytotoxicity showed that TAZnPc both free and into liposomes at 0.5 μM exhibited similar behavior showing cell viability of ~15% in combination with higher light dose supplied. Interestedly, enhanced performance of TAZnPc-liposomes was observed at lower concentrations. The application of free ZnPc at 0.5 μM after irradiation reduced cell viability to ~10% in both light doses applied, while for ZnPc-liposomes, comparable results were obtained with a concentration of 0.05 μM. These results allowed to reduce 10 times ZnPc concentration when it is administrated into liposomes to achieve the same cytotoxic effect.

Table 2. Liposomal formulations for PDT.

Phthalocyanine	Liposomes composition	Cell line	Reference
AlPc	SPC/chol SPC/chol/SDC	U87MG (Glioblastoma)	[128]
ZnPc TAZnPc	DPPC/chol	T98G (Glioblastoma)	[67]
AlPc	DSPC/DOPC/chol DSPC/DOPC/chol/CHCA	MCF-7 (Female mammary adenocarcinoma)	[129]
ZnPc AlPc	POPC/PG	HeLa (Cervical adenocarcinoma) HSC-3 (Oral squamous carcinoma)	[68]
DMEZnPc	POPC/PG POPC/PG/chol POPC/DOTAP POPC/DOTAP/chol	HSC-3, H413 (Oral squamous carcinoma)	[88]
ZnPc	DOPC/DMPC DOPC/DMPC/Tween20	B16-F10 (Murine melanoma)	[130]
$(PhS.SO_3Na)_4$ ZnPc	SPC/chol SPC/SDC	HEPG2 (Liver hepatocellular carcinoma)	[89]
$AlPcS_4$	DPPC/DOTAP/chol/ DSPE-PEG	SGC-7901(Gastric cáncer)	[131]
PcN	DPPC/chol/DSPE-PEG DPPC/chol/FA-DSPE-PEG	HeLa (Cervical adenocarcinoma) MCF-7 (Female mammary adenocarcinoma	[132]
ZnPc	DPPC/DC-chol/chol/DSPE-PEG DPPC/DC-chol/chol/DSPE-PEG/ACF* DPPC/DC-chol/chol/DSPE-PEG/TPZ	A-431 (Epidermal squamous carcinoma) Sk-Cha1 (Perihilar cholangiocarcinoma)	[133, 134]

*only A-431

Calori *et al*.[129] analyzed the liposomal delivery of PSs for PDT on breast cancer cells (MCF-7). The experiments were carried out using AlPc incorporated into liposomes composed of DSPC (34.5 mol%), DOPC (34.5 mol%), chol (31.0 mol%). Photocytotoxicity studies on MCF-7 cells in the presence of AlPc-liposomes showed a light dose-dependent reduction of cell viability. Individual molecules could be added to liposomes in order to evaluate their effect in PDT. In this case, alpha-cyano-4-hydroxycinnamic acid (CHCA), besides its antioxidant activity, is an efficient substrate for some monocarboxylate transporters (MCTs). MCTs are integral

membrane proteins responsible for the transport of lactic acid across the cell membrane and can be employed as potential targets in the development of novel therapeutic agents to treat some types of cancers.[135] However, the *in vitro* photocytotoxicity assay of CHCA-AlPc-liposomes showed a lower capacity to reduce MCF-7 viability with respect AlPc-liposomes, indicating a protective effect of CHCA in the oxidative process involved in PDT.[129]

Charged liposomes

Several lipids can be used for liposome formulation. Since the liposomal surface charge plays an important role in the interaction with cell membranes, it can vary from anionic to cationic using phospholipids such as PG and DOTAP, respectively. Different authors studied the applications of PDT *in vitro* on human squamous carcinoma cells, HSC-3, using modified liposomes. Young *et al*.[68] used ZnPc loaded into POPC/PG (1:1) liposomes and observed that the phototoxicity of ZnPc loaded in negatively charged liposomes was enhanced compared to the free compound. Also, Skupin-Mrugalska *et al.* [88] assayed disubstituted ZnPc (DMeEZnPc) both in free form and loaded into four liposomal formulations: POPC/PG, POPC/PG/chol, POPC/DOTAP, and POPC/DOTAP/chol. The lower half-maximal inhibitory concentration (IC_{50}) values obtained were 22nM and 29 nM for free Pc and Pc into POPC/PG, respectively. Higher IC_{50} values were found for Pc into POPC/PG/chol, POPC/DOTAP, and POPC/DOTAP/ chol. These results indicate that liposomes containing cholesterol and a cationic agent (DOTAP) were less efficient than free Pc and Pc incorporated into POPC/PG liposomes, which results were comparable.

Besides, Young *et al*. [68] compared the photodynamic effects of ZnPc and AlPc both free and incorporated into POPC/PG liposomes on cervical cancer (Hela) and oral squamous cell carcinoma (HSC-3) cells. ZnPc incorporated into liposomes of POPC/PG results more effective than free ZnPc in reducing cell viability in both cell lines after irradiation. The viability of HeLa and HSC-3 cells was reduced by free ZnPc (1 μM) to 52 and 22%, respectively, and by ZnPc-liposomes (0.1 μM) to 68 and 7%, respectively. Similarly, AlPc delivered into liposomes was much more effective than free AlPc in the photoinactivation. However, for free AlPc Hela cells were susceptible to PDT, while in HSC-3 cells, no reduction of cell viability was observed. AlPc liposomes 0.1 μM, diminished to 25% and 72% HeLa and HSC-3 cells viability, respectively. HeLa cells were more sensitive to AlPc, whereas HSC-3 cells were more sensitive to ZnPc. The results reported suggest that the phototoxicity of free and liposomal ZnPc and AlPc was dependent on cell type.

In addition to loading lipophilic Pcs, liposomes can be loaded with hydrophilic Pcs.[136] Xin *et al*.[131] evaluated the incorporation of hydrophilic sulfonated AlPc ($AlPcS_4$) into cationic liposomes (Clip) composed of DPPC/ DOTAP/ chol /DSPE-PEG. The photodynamic activity was evaluated on a human gastric cancer cell line (SGC-7901). Clip–$AlPcS_4$ generated a significant decrease in SGC-7901 cell viability. The reduction of cell survival was a concentration-dependent manner resulting in a decrease of cell viability to 20% with 8 μg/mL.

Ultradeformable liposomes

The ultradeformable liposomes have been used in different studies.[137] As described before, Silva *et al.* [128] evaluated PDT *in vitro* on glioblastoma cell line U87MG and observed that LipAlPc showed higher activity than free AlPc. Besides, the authors used UDLs composed of SPC and edge activators: Tween 80, Span 80, and sodium deoxycholate (SDC). In PDT assays performed with AlPc loaded into UDLs composed of SPC with SDC (UDLCSAlPc) comparable results were observed (ID_{50} = 0.40 J/cm^2) respect LipAlPc. The photocytotoxic effect of both liposomal formulations, LipAlPc, and UDLCSAlPc, did not differ significantly.

UDLs with different lipid compositions were used to load different Pcs. In 2017, Lima *et al*. [130] studied the transdermal application of ZnPc loaded into UDLs composed of DOPC/DMPC and the non-ionic surfactant Tween 20 on murine melanoma cells (B16-F10). In the dark, B16-F10 cell viability remains unaffected by the presence of empty UDLs, even containing a high concentration of Tween 20. However, after irradiation, a substantial reduction of cell viability in the presence of ZnPc-UDLs was observed. Also, the authors compared the results obtained in this study with those previously reported in which the same compounds were incorporated into DPPC:chol liposomes,[138] and they concluded that the administration of ZnPc using UDLs allow decreasing 10 times the concentration of PS used to obtain the same reduction of cell viability. Moreover, the skin permeation observed after topical application suggested that ZnPc-UDLs containing Tween 20 could be used in the transdermal delivery of PSs. [130]

Fadeel *et al*.[89] evaluated the PDT efficacy of a new hydrophilic thiophenyl sulfonated ZnPc ($(PhS.SO_3Na)_4ZnPc$) loaded into liposomes and UDLs on a hepatocellular carcinoma cell line (HEPG2). Liposomes were formulated using SPC and chol, whereas UDLs were composed of SPC and SDC. The results indicated that UDLs exhibited higher encapsulation efficiency, higher deformability, and a higher rate of release compared to liposomes. After irradiation, a higher reduction of cell viability was attained using Pc encapsulated (both in liposomes or UDLs) compared with aqueous Pc solution. In the case of aqueous $(PhS.SO_3Na)_4ZnPc$ solution, an increase in the concentration didn't improve the photodynamic activity. However, the phototoxicity was enhanced by increasing the Pc concentration in the case of liposomes and UDLs.

Targeted liposomes

As described before, liposomes can also be modified by adding ligands that target them to tumor surface markers, e.g., growth factor receptors, transferrin, integrin, insulin, and folic acid.[139] The folate receptor (FR) represents a selective tumor marker and is overexpressed in a variety of epithelial cancer cells, including ovarian, breast, kidney, lung, and colon cancer cells. FR binds extracellular folate with high affinity and delivers it into cells via endocytosis. An increase in phototoxicity of several photosensitizers conjugated with folate or encapsulated in folate-targeted liposomes has been reported.[140] In a recent study, Lin *et al*.[132] also used a

folate-modified liposome loading a monosubstituted ZnPc (PcN). Liposomes were composed of chol/DPPC/distearoyl phosphoethanolamine-PEG (DSPE-PEG) modified with or without folic acid and were denominated Lip-FA and Lip, respectively. In this work, it was investigated the photodynamic activity against FR-positive human cervical cancer cells (HeLa) and FR-negative human breast cancer cells (MCF-7). After PDT on HeLa cells, PcN-Lip-FA shown higher phototoxicity than PcN-Lip and free PcN, displaying an IC_{50} value 28-fold lower than free PcN. However, in FR-negative MCF-7 cells, PcN-Lip-FA and PcN-Lip have comparable photodynamic activity, whereas free PcN showed lower efficiency. PcN-Lip and free PcN exhibited similar behavior against both cell lines; nevertheless, the photocytotoxicity of PcN-Lip-FA against HeLa cells was higher than against MCF-7 cells. *In vivo* studies suggested that PcN was unable to efficiently inhibit tumor growth, while the tumor growth of mice treated with PcN-Lip could be retarded, showing a 48.6% inhibition rate. PcN-Lip-FA showed a tumor inhibition rate of 98.0%, exhibiting a highly efficient PDT.

Tumor cells can survive in hypoxic conditions, which dramatically reduce the PDT efficacy.[141] Pre-existing tumor hypoxia and activation of the hypoxia-inducible transcription factor 1 (HIF-1) occur when the tumor growth exceeds the rate of neoangiogenesis.[142, 143] HIF-1 has been targeted for pharmacological intervention in cancer therapy. Acriflavine (ACF) is a specific inhibitor of HIF-1. Broekgaarden *et al*. [133] studied the inhibition of HIF-1 with ACF in PDT application using cationic ZnPc-liposomes composed of DPPC/DC-chol/chol/DSPE-PEG (66:25:5:4 molar ratio). They evaluated the cytotoxic effects *in vitro* on human epidermal squamous cell carcinoma (A431) at normoxic and hypoxic conditions. Experiments performed with cationic ZnPc-liposomes showed that under hypoxic conditions, A431 cells were more susceptible to PDT compared to normoxic conditions. Also, when the cytotoxic effect on cells preconditioned with ACF at hypoxic conditions was evaluated, it was found that the reduction in cell viability was higher compared with PDT alone, indicating that ACF enhances PDT efficacy. The authors also reported the liposomal co-encapsulation of both ACF and ZnPc. This formulation also increases PDT efficacy, but this effect only was observed after a long incubation time (24 h) and under hypoxic conditions. A year later, Broekgaarden *et al*. [134] studied the potential of tirapazamine (TPZ), a hypoxic cytotoxin capable of inducing oxidative DNA damage at low intracellular oxygen tensions, in PDT. Two PDT protocols were evaluated: TPZ pre-treatment followed by the same cationic ZnPc-liposomes used previously [133] or liposomal co-encapsulation of TPZ and ZnPc. This study was carried out on two cell lines, human A431 cells and human perihilar cholangiocarcinoma cells (Sk-Cha1). The cell viability determination post-PDT was performed under both normoxic and hypoxic conditions. The results have shown that PDT using only cationic ZnPc-liposomes decreased the viability of A431 cells up to ~52% under both normoxia and hypoxia conditions. For Sk-Cha1 cells, the cell viability was reduced to 42% and 70%, respectively. In contrast, the combination of TPZ pre-

incubation with cationic ZnPc-liposomes PDT did not further reduce cell viability under normoxic conditions on A431 cells. Still, the viability was significantly decreased to 39% under hypoxic conditions. The application of cationic liposomes containing both ZnPc and TPZ showed higher therapeutic efficacy of PDT compared with the combination of PDT and TPZ pre-incubation in both cell lines, showing higher efficiency under hypoxic conditions.

Polymeric micelles

Micelles are self-assembled of surfactant molecules with a hydrophobic core and a polar surface. The particle size varies from 5 to 100 nm. They can be classified into polymeric and lipid micelles if they are composed of copolymers or micelles, respectively. [110, 112] Polymeric micelles are nanocarriers composed of both hydrophobic and hydrophilic block copolymers. [110, 144] PM are formed spontaneously when amphiphilic block copolymers are dispersed above the critical micelle concentration (CMC) in water. The self-assembly is based on nonpolar and hydrophobic interactions between lipophilic polymer chains, which form the core of micelles.[145] Most amphiphilic copolymers employed contain either a polyester or a poly(amino acid) derivative as the hydrophobic segment. Poly (lactic acid) (PLA), poly(caprolactone) (PCL), and poly(glycolic acid) (PGA) are biocompatible and biodegradable polyesters approved by the FDA. While, poly(l-amino acid) commonly used in drug delivery include poly(aspartic acid) (PAsp), poly(glutamic acid) (PGlu), poly(l-lysine) (PLys) and poly(histidine) (PHis). However, to form self-assembled micelles, the poly (l-amino acid) segment must either be electrostatically neutral or conjugated to hydrophobic moieties.[146] Compared to lipid micelles, PM have relatively high stability for their low CMC.[147] The CMC seems to be not susceptible to the length of the hydrophilic block. Nevertheless, an increase in the hydrophobic portion decreases the CMC. Furthermore, the addition of hydrophobic drugs or nonpolar solvents decreases the CMC.[144] Polymeric micelles can be loaded by either physical drug entrapment or covalent drug conjugation. The covalent drug conjugation implies different linkers to bind the drug to the micelle. In contrast, physical drug entrapment incorporates the drug of interest by the hydrophobic interaction with the micelle core.[148]

The inner core of PM is suitable for hydrophobic PS accommodation. At the same time, the hydrophilic segments stabilize the micelle interface with the aqueous environment and allow the PM circulation in the bloodstream for prolonged periods. [111, 149] It was reported that PM made with poly (ethylene oxide) blocks are sterically stabilized and undergo less opsonization and clearance by RES. [148]

The inefficient drug release in the tumor cells is associated with a decrease in therapeutic efficacy. Therefore, to improve the above mention pitfall, PMs with controlled micellar dissociation and triggered drug release has been developed.[150] The use of stimuli-responsive

PM constitutes an interesting programmable drug delivery system that releases drugs in response to specific stimuli, such as temperature, pH, ultrasound, or enzymes.[151] The pH-sensitive PM can be designed to carry, deliver, and control the release of hydrophobic agents in the tumoral acidic pH microenvironment. [150] Regarding PDT, several studies indicate that PM encapsulated PSs improved phototoxicity compared to free PS.[113]

Covalent Pc conjugation

A few works reported the covalent PS conjugation to polymeric micelles. [152, 153, 154] Di gao *et al.*[155] reported the covalent Pc conjugation to PM (Table 3). In this work, the authors reported different PM, which were composed of different ratios of ZnPc and doxorubicin (DOX), a common chemotherapeutic drug used for the treatment of various kinds of cancer. These PMs were formed by self-assembly of amphiphilic block copolymers of methoxy-PEG and poly(β-benzyl-L-aspartate) (PBLA), in which DOX and ZnPc were conjugated to the aspartate (Asp) side chains through an acid-labile hydrazone linker and a redox-responsive disulfide linker, respectively. Dark toxicity in HepG2 cells was observed for different DOX-ZnPc-micelles due to the presence of DOX, showing a direct relationship between the toxicity and the DOX concentration in the micelles. Upon irradiation, the DOX-ZnPc-micelles exhibited enhanced cytotoxicity, demonstrating the dual therapeutic effects. The DOX-ZnPc-micelles-2, which has a DOX/ZnPc molar ratio of 3.8, triggered synergistic cytotoxicity as calculated by a combination index.[156] For the DOX-ZnPc-micelles-1 with a molar ratio of DOX/ZnPc of 0.7, the antagonistic effect was observed, which was attributed to the low amount of DOX present in the micelles.

Pc entrapment: diblock polymers

Regarding micelles with physical Pc entrapment, several examples in recent years have been reported in the literature (Table 3). Obata *et al.* [157] synthesized polystyrene-block-poly(polyethylene glycol monomethyl ether acrylate) (PSt-b-PPEGA) polymers loaded with ZnPc for PDT. The *in vitro* photocytotoxicity of ZnPc transported in PSt-b-PPEGA micelles was examined in HeLa human cervical cell line and found to be innocuous in the absence of irradiation. The cell viability decreased with the increasing light dose delivered for the ZnPc-loaded micelles, but a non-significative photocytotoxicity (~60% of cell viability) was observed.

A wide variety of copolymers have been used and reported in the literature. The self-assembled diblock copolymer methoxypoly (ethylene oxide)-b-poly(L-lactide) (mPEG-b-PLLA) micelles were loaded with ZnPc, and the effectiveness for PDT was assessed on metastatic melanoma cells (Me45) and normal human keratinocytes (HaCaT) by Lamch *et al*. [158] This work, reports that the photosensitizer loaded in PMs was more effective compared to free form. Free ZnPc like empty PMs were non-cytotoxic at the tested concentration range, even after irradiation. The results showed suggests that metastatic melanoma was more sensitive than

normal keratinocyte to the PDT when the ZnPc was delivered using PMs, and cytotoxicity after irradiation was dependent on the ZnPc concentration as well as for the light dose delivered.

Recently it was reported ZnPc loaded in folate-functionalized micelles of mPEG-b-PLLA with folate (FA) attached to the end of the PEG chain (FA-PEG-b-PLLA).[159] The potentiality of the resulting micelles was evaluated in photocytotoxicity studies on ovarian carcinoma (SKOV- 3) and metastatic melanoma (Me45) cell lines. The results reported indicate that ZnPc incorporated in micelles was not cytotoxic in the absence of irradiation in both cell lines. On the other hand, after irradiation, ZnPc encapsulated in PMs was more effective in comparison to the free ZnPc both on SKOV-3 and Me45 cells. The photodynamic activity was enhanced in both cell lines with the increase of folic acid percentage and light dose supplied.

ZnPc in PMs was also applied for photodynamic therapy in lung carcinoma. Lu *et al*.,[160] used ZnPc incorporated into self-assembled mixed micelles synthesized from SDC and D-alpha tocopheryl polyethylene glycol succinate (TPGS), a an FDA-approved pharmaceutical excipient derivative of natural Vitamin E and formed by the esterification of Vitamin E and succinate with polyethylene glycol (PEG) 1000 having amphiphilic nature.[161] Three micelles formulations were synthesized with different SDC ratios (micelles-1, -2 and, -3 having 15, 25, and 30 mg of SDC, respectively). The *in vitro* photodynamic activity was evaluated on A549 lung cancer cells. Free ZnPc was not cytotoxic, even after irradiation. However, cytotoxicity was observed for three mixed micelles formulations at the highest ZnPc concentration after irradiation. The viability decreased to 38.0%, 45.49%, and 35.91% after the application of micelles-1, micelles-2, and micelles-3, respectively. The IC_{50} values attained were 0.5, 0.65 and 0.57 μg/mL for micelles-1, micelles-2 and micelles-3, respectively.[160]

Micelles composed by an amphiphilic block copolymer poly (ethylene glycol)-poly[2-(methylacryloyl)ethylnicotinate] (PEG-PMAN) with aromatic nicotinate was loaded with ZnPc.[162] The photocytotoxicity assay of free ZnPc and PEG-PMAN/ZnPc (PPZ) was performed in four osteosarcoma (OS) cell lines *in vitro:* MNNG/Hos; U2OS; Saos-2 and MG-63 cells. The cell viability decreased both in free-ZnPc and PPZ in a concentration-dependent manner. However, PPZ had higher cytotoxicity compared to free ZnPc and chemotherapy using drug cisplatin in all four OS cell lines. The IC_{50} obtained for MNNG/Hos, Hos, Saos-2, and MG-63 were 0.36, 1.46, 0.64, and 0.97 μM/ml, respectively. These values were 15- to 100-fold and 15- to 25-fold lower with respect to free ZnPc and cisplatin, respectively, in four OS cell lines. In the *in vivo* study, PPZ inhibited tumor growth 65.8 times more compared to free ZnPc. [162]

Additionally, diblock copolymers PMs were used to load AlPc. Vilsinski *et al*.[86] studied distinct formulations of Poly(styrene)-block-poly(acrylic acid) (PS-b-PAA) diblock copolymer nanostructures containing different AlPc concentrations *in vitro* on Caco-2 cell-line. The cell viability using free AlPc under illumination was unaffected. However, the cellular viability after

irradiation of AlPc-PS-b-PAA was strictly dependent on AlPc concentration; thus, higher AlPc concentrations showed lower cellular viability.

Table 3. Polymeric micelles formulations for PDT.

Phthalocyanine	Polymeric micelles composition	Cell line	Reference
ZnPc	PEG-P(Asp-ADH-DOX)-P(Asp-Ca-ZnPc)	HepG2 (Hepatocellular carcinoma)	[155]
ZnPc	PSt-b-PPEGA	HeLa (Cervical adenocarcinoma)	[157]
ZnPc	mPEG-b-PLLA	Me45 (Metastatic melanoma) HaCaT (Normal Keratinocytes)	[158]
ZnPc	mPEG-b-PLLA FA-PEG-b-PLLA	SKOV- 3 (Ovarian carcinoma) Me45 (Metastatic melanoma)	[159]
ZnPc	SDC-TPGS	A549 (Adenocarcinoma)	[160]
ZnPc	PEG-PMAN	MNNG/Hos, U2OS, Saos-2, MG-63 (Osteosarcoma)	[162]
AlPc	PS-b-PAA	Caco-2 (Colorectal adenocarcinoma)	[86]
TMAESZnPc	Tetronic® (T1107)	CT26 (Murine colon carcinoma) Caco-2, HT-29, SW480 (Colorectal adenocarcinoma)	[93]
ZnPc OMEEZnPc OFMEEZnPc	Pluronic® (L121, F127, P123)	A549 (Adenocarcinoma) CT26 (Murine colon adenocarcinoma) 2H11 (Murine endothelial)	[95]
TPPOZnPc	Pluronic® (F-127)	MCF-7 (Breast carcinoma)	[96]
AlPc	Pluronic® (F-127)	A549 (Lung carcinoma)	[163]
$AlPcS_4$	Pluronic® F127	SGC-7901 (Gastric cáncer)	[131]
$G_{1\text{-}2}$-DPcZn	PLL-b-PEG-b-PLL	HeLa (Cervical adenocarcinoma)	[164]
ZnPc	HDH	HeLa (Cervical adenocarcinoma)	[165]
ZnPc	PBA−PEG−PCL/Gal−PEG−PCL	HepG2 (Hepatocellular carcinoma)	[166]

Pc entrapment: poloxamers and poloxamines

Poloxamer (Pluronic®) and poloxamine (Tetronic®) block copolymers are constituted by poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), and they are commercially available.[167] Poloxamer (Pluronic®) is composed of triblock PEO–PPO–PEO copolymers. Their physical and chemical properties can be modified by altering the molar mass ratio between the PEO and PPO blocks, which changes the interactions with cells and membranes.[168] Poloxamines (Tetronic®) are formed by four PEO-PPO blocks linked to a central ethylenediamine group, with pH-responsive micellization properties.[167]

Several Pcs have been loaded into these commercial micelles. The photocytotoxic activity of tetrasubstituted ZnPc (TMAESZnPc), encapsulated into Tetronic® 1107 on human nasopharynx KB carcinoma cell line, was reported. The results showed a higher phototoxic activity after encapsulation of TMAESZnPc into two different Tetronic®: T1107 or T1307, increasing its photocytotoxicity by 10 times in comparison with the free form in water–DMSO.[169] Later, the authors evaluated the effectiveness of TMAESZnPc -T1107 micelles on murine colon adenocarcinoma cell line (CT26).[93] PMs did not show a cytotoxic effect in the dark. However, TMAESZnPc -T1107-loaded exhibited a cytotoxic effect after irradiation on CT26 cells, revealing an IC_{50} value of 10 nM. Similar results were attained on other cancer cell lines (Caco-2, HT-29, and SW480) evaluated under the same experimental conditions. Then, the photocytotoxicity of TMAESZnPc - T1107 on CT26 cells was assessed using different light doses showing a light dose-dependent decrease of cell viability. The effectiveness of TMAESZnPc - T1107 was also demonstrated in 3D culture models since spheroids models are an excellent option for a closer approach to the tumor structure developed *in vivo*.

Three Pluronic® (L121, F127, P123) copolymer micelles were exploited to incorporate a fluorinated, and its non-fluorinated ethers substituted zinc phthalocyanine (OFMEEZnPc and OMEEZnPc).[95] Their photodynamic activity on human A549 cells and murine CT26 and 2H11 cells was evaluated using unmodified ZnPc as a reference. The authors reported that fluorinated derivative was more efficient than a non-fluorinated analog to photoinactivate cells. Still, ZnPc was less effective than fluorinated and non-fluorinated analogs in the PDT protocol evaluated. Moreover, the incorporation of Pcs into pluronic micelles increased the photodynamic activity in all cases. Besides, *in vivo* studies indicate that tumor volume were significantly reduced in CT26 xenograft-bearing mice treated with fluorinated and non-fluorinated ZnPc and a light dose of 120 J/cm^2 compared to untreated control mice.

The encapsulation of tetrasubstituted zinc phthalocyanines (TPPOZnPc) into Pluronic F-127 and their photodynamic activity on human breast carcinoma MCF-7 cell was studied by Motloung *et al.* [96] The comparison of photodynamic activity among TPPOZnPc both free and encapsulated in pluronic F-127 shows a higher effect of Pc delivered into micelles.

Pluronic F127 micelles incorporated with chloroaluminum phthalocyanine (F127/AlPc) were studied by Py-Daniel *et al.*[163] PDT *in vitro* tests were performed on A549 human lung carcinoma cells at different AlPc concentration, followed by light irradiation. The F127/AlPc formulation, even at the lower AlPc loading of 0.1 μg/mL, decreased the cell viability after irradiation.

Xin *et al.*[131] incorporated hydrophilic $AlPcS_4$ into pluronic F-127, Clip, and gold nanorods (AuNR), and their photodynamic activity on SGC-7901 cells was evaluated. F127–$AlPcS_4$ exhibited inferior PDT activity with respect to other formulations; however, a significant increase in the photocytotoxicity at a higher incubation time was observed.

Pc entrapments: triblock polymers

Triblock copolymers based on PEG and PLL (PLL-b-PEG-b-PLL) showed to be more effective than the diblock (PEG-b-PLL) for PDT.[170] Huang *et al.*[164] reported micelles formed via electrostatic interaction between the periphery of negatively charged 1 and–2 generation dendrimer zinc phthalocyanines (G_{1-2}-DPcZn) and positively charged poly(L-lysin) segment of triblock copolymer poly(L-lysin)-block-poly(ethylene glycol)-block-poly(L-lysin) PLL-b-PEG-b-PLL (G_{1-2}-DPcZn/m). Photodynamic activity *in vitro* was evaluated on HeLa cells. The cell photoinactivation using G_{1-2}-DPcZn incorporated into the nanocarrier (G_{1-2}-DPcZn /m) was higher compared to free G_{1-2}-DPcZn. Still, second-generation dendrimer G_2-DPcZn showed higher photocytotoxicity over the first generation of G_1-DPcZn both free and into micelles. G_{1-2}-DPcZn or G_{1-2}-DPcZn/m exhibited an increase in photocytotoxicity in a concentration-dependent manner. IC_{50} attained for G_1-DPcZn, G_2-DPcZn, G_1-DPcZn/m, and G_2-DPcZn/m was 5.95 μM, 3.96 μM, 1.59 μM, and 0.883 μM, respectively.

Pc entrapment: stimuli-responsive PM

As described before, stimuli-responsive PM represents an attractive drug delivery system to be used in PDT. The synthesis of pH-sensitive micelle composed of Heparin, DSPE, and l-Histidine (HDH micelles) loaded with ZnPc, and their further evaluation in photocitotoxicity experiments on HeLa cells was performed by Debele *et al.*[165] The ZnPc release assays from HDH micelles indicate that under acidic conditions (pH 5.0), it was reached 91% in comparison to 63% under physiological conditions (pH 7.4). The authors reported that ZnPc-loaded HDH micelles were more cytotoxic than free ZnPc after irradiation at the same concentration. Also, the decrease in cell viability was concentration-dependent.

A pH-responsive polymer with mutual shielding of dual ligands was developed and reported by Cao *et al.*[166] As a dual-ligand mutually shielding strategy, phenylboronic acid-functionalized poly- (ethylene glycol)-b-poly(ε-caprolactone) (PBA−PEG−PCL) and galactose-functionalized poly(ethylene glycol)-b-poly(ε- caprolactone) (Gal−PEG−PCL) were mixed to prepare dual ligand micelles, PBA/Gal. PBA and Gal can be employed as a targeting ligand to recognize biologically relevant sialic acid residues (e.g., 2-O-methyl-α-D-N-acetylneuraminic

acid, Me-SA) and asialoglycoprotein receptor (ASGPR)- reported to be overexpressed on tumor cells.[171] At physiological pH (7.4), PBA groups can form borates with Gal groups at the micellar surface, [171] leading to the mutual shielding of their targeting abilities. At the tumoral acidic pH (6.8), the binding affinity between PBA and Gal became unstable, and the unbound PBA and Gal regained their targeting abilities toward Me-SA and ASGPR. This dual-ligand mutual shielding and reshieldable targeting system exhibited prolonged blood circulation, reduced RES capture, and enhanced tumor accumulation. The pH-response was studied on a human hepatocellular carcinoma cell line (HepG2) with overexpressed biological sialic acid derivatives and asialoglycoprotein receptors. After irradiation, HepG2 cells treated with ZnPc-loaded PBA/Gal micelles exhibited a decrease of cell viability dose-dependent. Moreover, the IC_{50} at pH 6.8 was lower with respect to that observed at pH 7.4. *In vivo* experiments indicate that ZnPc-encapsulated reversible-shielding micelles improved the tumor inhibition efficacy compared with irreversible micelles.

Polymeric nanoparticles

Besides micelles, amphiphilic block copolymers can also form nanoparticles. In contrast to micellization of block copolymer based on the self-assembly around of CMC, the formation of nanoparticles is kinetically regulable with several factors, such as temperature, pH, electrolytes, solvent contents, etc.[144] The morphological transition from the micelle to the nanoparticle also relies on the molecular weight or the hydrophobic unit length in the amphiphilic block copolymer. The amphiphilic copolymers having longer hydrophilic blocks tend to form micelles; however, the copolymers having longer hydrophobic blocks tend to form nanoparticles. The morphological transition for chain length influence from micelle to nanoparticle has been studied previously.[172, 173]

Hence, polymeric nanoparticles are structures of solid colloidal particles with a size dimension ranging from 10 to 1000 nm (1 μm).[110] The size, properties, and morphology of these nanoparticles depend on the polymer composition and preparation method.[174] Biodegradable synthetic polymers, such as polyacrylamide (PAA), PLA, PGA, PCL, and also copolymers like poly (lactic-co-glycolic acid) (PLGA), have been largely employed for the formulation of nanoparticles for PSs delivery due to their high physical stability, biocompatibility, high drug loading capacity, and controllable drug release.[111, 113] A small size and PEG coating on PNPs reduce the recognition by macrophages and increase their time of circulation in the bloodstream. The surface of PNPs also can be modified by specific ligands for active targeting.[112, 175] As polymeric micelles, polymeric nanoparticles could be stimuli-responsive vehicles capable of reacting to temperature, ionic strength, pH, light, and pressure. Most of the stimuli-responsive block copolymers are temperature or pH-sensitive systems.[176]

Conventional PNPs

In the last five years, only a few works reported the use of PNPs to load ZnPc and their potentiality in PDT (Table 4). Ping *et al.*[177] reported ZnPc-loaded into three types of biocompatible PNPs. PSty-NPs were composed of polystyrene (PSty), dodecyltrimethoxysilane (DTS), and poly-L-lysine hydrobromide (PLL). The replacement of PSty for poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO- a conjugated polymer with a rigid backbone) or poly(N-vinylcarbazole) (PVK- linear polymer with a rigid side chain) generated PFO-NPs and PVK-NPs, respectively. The photocytotoxicity of the three different NPs loaded with ZnPc was tested on HepG2 cells. The results of *in vitro* PDT showed that ZnPc-loaded PNPs are able to reduce cell viability after irradiation as following PSty-NPs<PVK-NPs<PFO-NPs. Consequently, PFO-NPs showed the highest efficacy; therefore, i*n vivo* PDT efficacy of PFO-NPs in H22 solid tumor-bearing mice was evaluated. The volume of PDT-treated tumor was reduced by 60% compared with the control.

Mehraban *et al.*[76] reported the use of PLGA-b-PEG nanoparticles to encapsulate a zinc phthalocyanine derivative TMBZnPc and evaluated its photodynamic efficacy on A549 cells. The results of this report indicate that encapsulated dye showed a nearly 500-fold increase in phototoxicity in A549 cancer cells compared to free dye. *In vitro* assays were performed comparing TMBZnPc into both free and loaded into PNPs with unsubstituted free ZnPc. It was observed that PDT cytotoxicity was not significant in cells treated with free Pcs. However, viability was significantly reduced after the treatment using TMBZnPc loaded into PNPs, since TMBZnPc into PNPs concentration was 500 times lower than free TMBZnPc, which did not exhibit photocytotoxicity.

Zn-naphthalocyanine (ZnNPc) and ZnPc loaded into PLGA nanoparticles were evaluated *in vitro* on MCF-7 cells.[178] The results showed a higher photodynamic activity after the treatment with ZnPc-PLGA-NPs, since an inhibition of 60% MCF-7 cell viability was observed. The photocytotoxic results of free ZnNPc, free ZnPc, and ZnNPc-PLGA-NPs showed inhibition in cell viability of 25, 38, and 32%, respectively. *In vivo* results highlight a significant antitumor effect of both PLGA-NPs of ZnNPc and ZnPc compared to control.

Table 4. Polymeric nanoparticle formulations for PDT.

Phthalocyanine	Polymeric nanoparticle composition	Cell line	Reference
ZnPc	Sty/DTS/PLL PFO/DTS/PLL PVK/DTS/PLL	HepG2 (Hepatocellular carcinoma)	[177]
TMBZnPc	PLGA-b-PEG	A549 (Adenocarcinoma)	[76]
ZnPc ZnNPc	PLGA	MCF-7 (Female mammary adenocarcinoma)	[178]
TPZnPc	PCPN: TPZnPc/Zn/DOPA PCPNsLip: PCPN/DSPC/chol PCPNsLip/DLC	MCF-7 (Female mammary adenocarcinoma)	[99]

Stimuli-responsive PNPs

A coordination polymer (CP) is constructed from polydentate bridging ligands and metal ions or clusters via coordination bonds through self-assembly processes. It possesses several promising features as a drug delivery vehicle, including chemical diversity, nanoscale sizing, functional modification, and intrinsic biodegradability.[179] Huang *et al*.[99] reported the synthesis of tetra carboxy-phenoxy substituted ZnPc (TPZnPc). TPZnPc is coordinated with the zinc ion to form the core of phthalocyanine coordination polymeric nanoparticles (PCPN) and coated with a lipid (Lip) bilayer, composed by DSPC and chol, to form lipid-coated PCPN (PCPNsLip). To enhance the tumor internalization of PCPN, an intelligent cholesterol derivative, 1,2-dicarboxylic-cyclohexane anhydride-modified lysyl-cholesterol (DLC), was synthesized, and then it was functionalized on the surface of the nanoparticles (PCPNsLip/DLC). DLC is degraded in the presence of a mildly acidic environment. Thus, it was obtained a PCPN with a pH-sensitive material for enhanced cellular uptake. *In vitro* PDT assays evaluated the activity of free TPZnPc, PCPNsLip, and PCPNsLip/DLC on MCF-7 cells at pH 7.4 or 6.5. The results showed that PCPNsLip and PCPNsLip/DLC have a higher photocytotoxicity than free TPZnPc. Likewise, the photocytotoxicity of PCPNsLip/DLC was more efficient at pH 6.5 than that at pH 7.4. At a relatively low concentration (2.5 μM), the cell Inhibition of PCPNsLip/DLC at pH 6.5 was approximately 2.3 times higher than that observed at pH 7.4. Also, in *vivo* assays showed that free TPZnPc only inhibited the tumor growth to some extent; meanwhile, with PCPN, the tumor growth was remarkably suppressed.

Gold nanoparticles

Gold nanoparticles (AuNPs) have been largely investigated for several applications in diagnostic, bioimaging, and drug delivery.[110] The nanoparticle size usually ranged between 1

and 100 nm.[123] Additionally, AuNPs possess various geometries, including nanospheres,[180] nanorods,[181] nanoshells,[182] nanocages,[183] nanorings,[184] and nanostars.[185] AuNPs can be functionalized using several ligands, including proteins, nucleic acids, and carbohydrates, that allow selective targeting to the cancer tissue.[186, 187]

AuNPs have been widely used as drug delivery systems for their low toxicity and their capacity to extend the body circulation time.[188, 189] Besides, AuNPs have photothermal properties.[190] Photothermal therapy (PTT) is based on achieving heating of the local environment to killing cancer cells. When localized surface plasmon resonance (LSPR) on AuNP is excited by irradiation, the PTT effect is achieved.[191] PDT combined with PTT has been used to accomplish a more effective tumor treatment.[111, 185] AuNPs linked with a PS can be excited by two different lasers to optimizes the individual effects.[192]

AuNPs in PDT

In the last five years, some works in the literature reported the effectiveness of AuNPs in photodynamic therapy (Table 5). AuNPs were synthesized and functionalized with a mixed monolayer of polyethylene glycol (PEG) and zinc phthalocyanines.[193] The Pcs consisting of two octa-alkyl substituted zinc (II) phthalocyanines, differing in the length of the carbon chain that connects the Pc to the sulfur atom that interacts with the surface of the gold core. The carbon chain is composed of either three carbon atoms (C3Pc) or eleven carbon atoms (C11Pc). The purpose of the sPEG ligand was to facilitate the solubility of the Pc-sPEG-AuNPs in aqueous solutions. PDT *in vitro* was evaluated on SK-BR-3 adenocarcinoma cell line. The results of C11Pc–PEG–AuNPs application showed minimal cell death. However, cells treated with the C3Pc–PEG–AuNPs increasing cell death as the concentration of C3Pc was increased.

In further studies, gold nanoparticles functionalized with a mixed monolayer of C3Pc and C11Pc and a lactose derivative were reported.[101] Lactose was used to stabilize AuNPs in aqueous solutions and as the targeting agent for the galectin-1 receptor, which is overexpressed on the surface of breast cancer cells. The AuNPS used in this study were lactose-C3Pc-AuNPs and lactose-C11Pc-AuNPs and compared with C3Pc-sPEG-AuNPs, and C11Pc-sPEG-AuNPs reported previously.[193] *In vitro* assays were carried out on two breast adenocarcinoma cell lines SK-BR-3 and MDA-MB-231 cells, and non-cancerous mammary epithelial cells, MCF-10A cells. No changes in cell viability of MCF-10A by either lactose-C3Pc-AuNPs or C3Pc-sPEGAuNPs before or after irradiation were observed. These results indicate that the presence of lactose in AuNPs does not increase the uptake by non-cancerous cells. Also, the results post-irradiation showed that MDA-MB-231 cell viability was reduced using both lactose-C3Pc-AuNPs and C3Pc-sPEG-AuNPs with similar levels of cell death. The observation mentioned above suggests that lactose in AuNPs does not increase the photocytotoxicity in MDA-MB-231 cells. The cell viability was unaffected in MDA-MB-231 cells treated with lactose-C11Pc-AuNPs and C11Pc-sPEG-AuNPs with or without irradiation. However, the treatment of SK-BR-3 cells, using both

lactose-C11Pc-AuNPs and C11Pc-sPEG-AuNPs induce cell death following irradiation without dark toxicity; being lactose-C11Pc-AuNPs the most effective. Furthermore, after irradiation and at low concentration, lactose-C3Pc-AuNPs showed more photodynamic activity than C3Pc-sPEG-AuNPs in SK-BR-3 cell death. Consequently, SK-BR-3 cells were more susceptible to PDT in all cases, and C3Pc induces higher levels of photocytotoxicity in both cell lines.

The effectiveness of free zinc monosubstituted phthalocyanine (MPZnPc) and MPZnPc conjugated to Au and gold-silver (AuAg) nanoparticles on A375 human skin melanoma cells was reported.[194, 195] After irradiation, MPZnPc, MPZnPc-Au and MPZnPc-AuAg showed a significant dose-dependent decrease in cellular viability. MPZnPc-Au and MPZnPc-AuAg were more effective than free MPZnPc, indicating that the conjugation to AuNPs and Au-AgNPs improves the output of PDT using MPZnPc.

On the other hand, dendrimer-entrapped gold nanoparticles (AuDENPs) are powerful targeted agents and confers improved therapeutic activity and water solubility. [196, 197] The synthesis of multiple particle delivery complexes (MPDC), which consists of a sulfonated zinc-phthalocyanine mix ($ZnPcS_{mix}$) conjugated to AuDENPs was reported by Mfuo-Tynga *et al*. [198] PDT *in vitro* was evaluated on MCF-7 breast cancer cells, and WS1 fibroblast cells were used as control. Cell viability of WS1 cells remained unchanged after all treatments. Free $ZnPcS_{mix}$ and AuDENPs alone were previously evaluated and reported to not causes substantial changes in MCF-7 cell viability.[199, 200] However, after PDT on MCF-7, MPDC led to a significant decrease in cell viability at concentrations greater than 0.1 μM. Consequently, 0.3 μM of MPDC was sufficient to induce an approximately 50% decrease in cell viability, and the concentration was 1.6 times lower than $ZnPcS_{mix}$ to obtain the same viability decrease (50%).[198]

Table 5. Gold nanoparticles formulations for PDT.

Phthalocyanine	Gold nanoparticles	Cell line	Reference
C3Pc C11Pc	sPEG–AuNP lactose-AuNP	SK-BR-3, MDA-MB-231 (Adenocarcinoma) MCF-10A (Mammary epithelial)	[101, 193]
MPZnPc	AuNP AuAgNP	A375 (Human skin melanoma)	[194, 195]
ZnPcSmix	AuDENPs	MCF-7(Female mammary adenocarcinoma)	[198]
MPTrPyZnPc	AuNS AuNR	MCF-7(Female mammary adenocarcinoma)	[104]
$AlPcS_4$	AuNRI	SAS (Human oral cancer)	[201]
$AlPcS_4$	AuNR	SGC-7901 (Gastric cáncer)	[131]
TrBMPZnPc	GQDs-MnO_2 -AuNPs	MCF-7(Female mammary adenocarcinoma)	[105]

AuNPs in PTT and PDT

The combination of PTT and PDT using Pcs coupled to AuNPs was studied. Dube *et al*.[104] reported the synthesis of asymmetric substituted ZnPc (MPTrPyZnPc) and its linkage to gold nanoparticles (AuNPs) of different shapes: gold nanospheres (AuNS) and gold nanorods (AuNR). MPTrPyZnPc was linked to gold nanoparticles (AuNPs) through the S\\Au or N\\Au bond. PDT activity was analyzed on MCF-7 breast cancer cells. MPTrPyZnPc showed low photodynamic therapy activity. MPTrPyZnPc-AuNR afforded superior PDT activity in comparison to MPTrPyZnPc-AuNS. MPTrPyZnPc alone showed cell viability >50% at maximum concentration probed (160μg/mL). The AuNPs alone displayed phototoxicity attributed to the photothermal activity of gold.[202] The conjugate, MPTrPyZnPc-AuNR showed a capability to reduce >50% the cell viability at concentrations ≥40 μg/mL compared to ≥80 μg/mL of MPTrPyZnPc-AuNS needed to obtain the same cell viability reduction after irradiation. The higher activity of MPTrPyZnPc-AuNR was attributed to the photothermal effect since for the PDT studies, excitation was performed at 680 nm, in which AuNR absorbs more light compared to AuNS.

Chu *et al*. [201] synthesized gold nanorings (AuNRI) linked with sulfonated aluminum phthalocyanines ($AlPcS_4$), and their effect on human oral cancer cells SAS was assessed. The PTT effect was associated with the localized surface plasmon resonance (LSPR)-enhanced absorption of the AuNRI. The results observed *in vitro* showed an effective killing of oral cancer cells. Still, the laser intensity needed to trigger cancer cells photocytotoxicity using AuNRI linked with $AlPcS_4$ was significantly lower compared to AuNRI not linked with $AlPcS_4$.

Xin *et al*. [131] loaded AuNR with hydrophilic $AlPcS_4$, as described above. AuNR-$AlPcS_4$ required an additional stimulation to release $AlPcS_4$. For that, the SGC-7901 cells treated with AuNR–$AlPcS_4$ were first irradiated with 808 nm to release the Pc; then, they were irradiated with 635 nm to excite the Pc. After that, cell viability was significantly decreased in a dose-dependent manner. Besides, the experiments revealed a significant photothermal effect on cells in addition to the PDT effect of $AlPcS_4$.

AuNPs in hypoxic PDT

In a recent study, the development of oxygen-independent Pc-nanoparticle for hypoxic PDT was reported by Nwahara *et al*. [105] The inclusion of Pcs into hypoxia-active materials could combine the singlet oxygen-generating ability of Pcs with the high catalytic activity of manganese dioxide nanoparticles towards the oxygen generation from H_2O_2. The Pc utilized was asymmetric substituted ZnPc (TrBMPZnPc), and it was covalently conjugated to graphene quantum dots (GQDs) to form TrBMPZnPc@GQDs. The GQDs surface was used as a support for MnO_2 nanoparticles alone or in the presence of AuNPs (MnO_2-AuNPs) to create TrBMPZnPc@GQDs-MnO_2, or TrBMPZnPc@GQDs-MnO_2-AuNPs respectively. The NPs were then further encapsulated into liposomes to improve their water solubility and physiological stability. PDT *in vitro* assays of the liposome-loaded composites under normoxic and hypoxic conditions was performed on MCF-7 cells. Under both normoxia and hypoxia, the photocytotoxicity was concentration-dependent. PDT activity was more effective under normoxia in all cases; since under hypoxia, the results showed a lower photodynamic activity of TrBMPZnPc alone or the TrBMPZnPc@GQDs without MnO_2. Also, cell viability decrease was higher for TrBMPZnPc@GQDs-MnO_2-AuNPs with respect to TrBMPZnPc@GQDs-MnO_2.

Conclusions

Photodynamic therapy is considered a therapeutic alternative to treat several cancers. Throughout the history of PDT, many PSs have been evaluated, and Pcs are considered good second-generation PSs for their unique characteristics. The incorporation of hydrophobic Pcs into delivery systems result in third-generation PSs, which allow its intravenous administration.

In this review, the works of the last five years regarding the main drug delivery systems for both, Zn and Al Pcs, and their application in PDT were analysed and summarized. Zn and Al Pcs demonstrated to be effectives for PDT application, however, their incorporation into a suitable carrier, in most cases, evidenced an enhanced PDT efficiency. Among the nanocarriers of Pc, liposomes with different lipids combinations and decorated with targeting molecules that improve the cellular uptake, and consequently, the photodynamic effect were studied and the PDT outcome was significantly improved. Furthermore, all liposomal formulations assessed allow for incorporation of Pcs and enhanced the photodynamic activity. In most cases, liposomes allows a

reduction in the amount of Phthalocyanine employed to obtain the same photocytotoxicity compared to Phthalocyanine free form. Different co-polymers blocks have been used for the design of delivery systems; their application as PSs carriers for PDT has not yet been much explored and needed future studies. However, the reports in the last years demonstrated that polymeric micelles and nanoparticles are promising nanocarriers for the delivery of anticancer drugs and photosensitizers for PDT. Gold nanoparticles are attractive PSs carriers since they are suitable for the application of both, PDT and PTT, increasing the cellular damage. In the last years, these nanocarriers showed their effectiveness in numerous studies.

Finally, the results reported by several authors strongly suggested that the incorporation of Pcs into delivery systems such as liposomes, polymeric micelles, polymeric nanoparticles, and gold nanoparticles substantially improved PSs solubility and their pharmacokinetics, enhancing the effectiveness of the PDT. These observations constitute a promise for the treatment of several cancers in which conventional therapies are not effective enough. Still, the development and design of nanocarriers with active-targeting or stimuli-responsive represent an exciting area for future studies. Furthermore, it is crucial to continue the evaluation of these nanocarriers on PDT both *in vitro* and *in vivo*.

List of abbreviations

5-ALA: 5-aminolevulinic acid
Ab: antibody
ACF: Acriflavine
ASGPR: asialoglycoprotein receptor
AuAgNP: gold-silver () nanoparticles
AuDENPs: dendrimer-entrapped gold nanoparticles
AuNP: Gold nanoparticle
AuNR: gold nanorods
AuNRI: gold nanorings
AuNS: gold nanospheres
C3Pc–sPEG–AuNP: PEG–AuNP funcionalized with C3Pc
C11Pc–sPEG–AuNP: PEG–AuNP funcionalized with C11Pc
CHCA: alpha-cyano-4-hydroxycinnamic acid
chol: cholesterol
CMC: critical micelle concentration
CSAlPc: AlPc into SPC/SDC liposome
DC-chol : 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol
DLC: 1,2-dicarboxylic-cyclohexane anhydride-modified lysyl-cholesterol
DMPC: dimyristoylphosphatidylcholine
DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine
DOTAP: N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride
DOX: doxorubicin
DPPC : 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine
DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine
DSPE-PEG: 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-N-amino(polyethyleneglycol)]
DTS: Dodecyltrimethoxysilane

FR: folate receptor
Gal−PEG−PCL: galactose-functionalized poly(ethylene glycol)-b-poly(ε- caprolactone)
GQDs-MnO2 –AuNPs: Graphene quantum dots - MnO2 –AuNPs
HDH: heparin/DSPE/l-histidine
HIF-1: hypoxia-inducible transcription factor 1
HpD: hematoporphyrin derivative
lactose-C3Pc-AuNPs: lactose-AuNPs funcionalized with C3Pc
lactose-C11Pc-AuNPs: lactose-AuNPs funcionalized with C11Pc
LipAlPc: AlPc into SPC/chol liposome
Lip–AlPcS4: ALPcS4 into DPPC/DOTAP/chol/DSPE-PEG liposome
IC_{50}: half-maximal inhibitory concentration
ID_{50}: Irradiation dose for killing 50% cells
MCTs: monocarboxylate transporters
Me-SA: 2-O-methyl-α-D-N-acetylneuraminic acid
MPDC: multiple particle delivery complexes
mPEG-b-PLLA: methoxypoly(ethylene oxide)-b-poly(L-lactide)
$^1O_2$: singlet oxygen
PAA: Poly(l-aminoacid)s
PAsp: poly(aspartic acid)
PBA−PEG−PCL: poly- (ethylene glycol)-b-poly(ε-caprolactone)Pluronic®: triblock PEO–PPO–PEO
PBLA: methoxy-PEG and poly(β-benzyl-L-aspartate)
Pc: Phthalocyanine
PC: hydrogenated phosphatidylcholines
PCL: poly(caprolactone)
PcN-Lip: DPPC/chol/DSPE-PEG liposome
PCPN: phthalocyanine coordination polymer nanoparticles (TPZnPc/Zn/DOPA)
PCPNsLip: PCPN into liposome of DSPC/chol
PDT: Photodynamic therapy
PEG: polyethylene glycol
PEG-P(Asp-ADH-DOX)-P(Asp-Ca-ZnPc): DOX-ZnPc-micelles
PEG-PMAN: poly(ethylene glycol)-poly[2-(methylacryloyl)ethylnicotinate]
PEO: poly(ethylene oxide)
PFO: poly(9,9-dioctylfluorenyl-2,7-diyl)
PFO-NPs: PFO/DTS/PLL nanoparticle
PG: L-α-phosphatidyl-DL-glycerol
PGA: poly(glycolic acid)
PGlu: poly(glutamic acid)
Phis: poly(histidine)
PLA: poly(lactic acid)
PLGA: poly(lactic-co-glycolic acid)
PLL: poly-L-lysine hydrobromide
PLys: poly(l-lysine)
PM: Polymeric micelles
PNP: polymeric nanoparticles
POPC: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
PPO: poly(propylene oxide)
PPZ: ZnPc into PEG-PMAN micelle
PS: photosensitizer
PS-b-PAA: Poly(styrene)-block-poly(acrylic acid)
PSt-b-PPEGA: polystyrene-block-poly(polyethylene glycol monomethyl ether acrylate)
PSty: Polystyrene
PSty-NPs: PSty/DTS/PLL nanoparticle
PTT: Photothermal therapy
PVK: poly(N-vinylcarbazole)

PVK-NPs: PVK/DTS/PLL nanoparticle
RES: reticuloendothelial system
ROS: reactive oxygen species
SDC: sodium deoxycholate
SPC: soybean phosphatidylcholine
TPGS: D-alpha tocopheryl polyethylene glycol succinate
TPZ: tirapazamine
UDLs: Ultradeformable liposomes

Phthalocyanines abbreviations:
AlClPc: chloroaluminum(III)phthalocyanine
$AlPcS_4$: chloroaluminum(III)phthalocyanine tetrasulfonic Acid
C3Pc: 1,4,8,11,15,18-Hexahexyl-22-methyl-25-(3-mercaptopropyl) phthalocyaninato zinc
C11Pc: 1,4,8,11,15,18-Hexahexyl-22-methyl-25-(11-mercaptoundecyl) phthalocyaninato zinc
DMEZnPc: 1,4-bis[2-(morpholin-4-yl)ethoxy]phthalocyaninato zinc
$G_{1\text{-}2}$-DPcZn: poly (aryl benzylether) dendrimer zinc phthalocyanines first or second generation
MPTrPyZnPc: tris-[(2,2,7,7-tetramethyltetrahydro-3aH-bis([1,3]dioxolo)[4,5-b:4′,5′-d]pyran-5-yl)methoxy)-2-(4-benzo[d]thiazol-2-ylphenoxyphthalocyaninato] zinc
MPZnPc: mono(4-carboxyphenoxy)phthalocyaninato zinc
OFMEEZnPc: Octakis[2-(2- trifluormethoxytetrafluorethoxy)tetrafluorethoxy]phthalocyaninato zinc
OMEEZnPc: Octakis[2-(2-methoxyethoxy)ethoxy]phthalocyaninato zinc
PcN: 2-(4-(1-ethylamine)phenoxy)phthalocyaninato zinc
$(PhS.SO_3Na)_4$ZnPc: tetra(4–thiophenyl)phthalocyaninato zinc sulfonated
TAZnPc: tetraaminephthalocyaninato zinc
TMAESZnPc: 2,9(10),16(17),23(24)-tetrakis[(2-dimethylamino)ethylsulfanyl]phthalocyaninato zinc
TMBZnPc: 2(3),9(10),16(17),23(24)-tetrakis-(4'-methyl-benzyloxy)phthalocyaninato zinc
TPPOZnPc: Tetra[(4-phenyldiazenyl)phenoxy]phthalocyaninato zinc
TPZnPc: tetra(4-carboxyphenoxy)phthalocyaninato zinc
TrBMPZnPc: tris(4-benzo[d]thiazol-2-ylphenoxy)-2-carboxyphenoxyphthalocyaninato zinc
ZnNPc: Zinc(II)naphthalocyanine
ZnPc: Zinc(II)phthalocyanine
$ZnPcS_{mix}$: sulfonated zinc-phthalocyanine mix

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