

CHITOSAN-DERIVATIVES IN COMBINATIONS WITH SELECTED PORPHYRINOIDS AS NOVEL HYBRID MATERIALS FOR MEDICINE AND PHARMACY

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Abstract

Chitosan and its derivatives are renewable biopolymers characterized by high biocompatibility; therefore, they are harmless to humans and allow immune tolerance and improved hydrophilicity. Moreover, chitosan has been the most studied of all polysaccharides used in biomedical applications during the last decade. Combinations of chitosan and porphyrinoid compounds in hybrid materials have revealed many potential applications for biomedical sciences. The main advantage of such materials is an increase in the solubility of porphyrinoids in body fluids and therefore greater release of singlet oxygen to the treated tissue. Chitosan-based drug delivery systems can improve the targeting of porphyrinoids and their release at predetermined locations and finally achieve desired therapeutic effects with minimal side effects. Hence, porphyrinoid-chitosan materials can be applied in drug delivery systems, cancer theranostics and magnetic resonance imaging. The combination of chitosan and porphyrinoids also appears useful in the healing and repairing of damaged organs, tissue engineering, regenerative medicine, as well as dressing materials. Huge benefits are related to the treatment of wounds, which has been presented for self-healing hydrogels based on chitosan and porphyrinoids. Furthermore, the chitosan/porphyrinoid combinations have revealed enormous benefits for antimicrobial photodynamic therapy.

Keywords: chitin, chitosan, hybrid materials, imaging, photodynamic therapy, porphyrinoids

Received: 11.05.2020

Accepted: 21.06.2020

1. Introduction

Chitin (poly- β -1,4-*N*-acetylglucosamine (**1**)) is the second most common polysaccharide in the environment after cellulose (Fig. 1). It was first isolated by the scientist Henri Braconnot in 1811. This polysaccharide can be found in the exoskeleton of insects, the cell wall of fungi and sponges and corals [1–3].

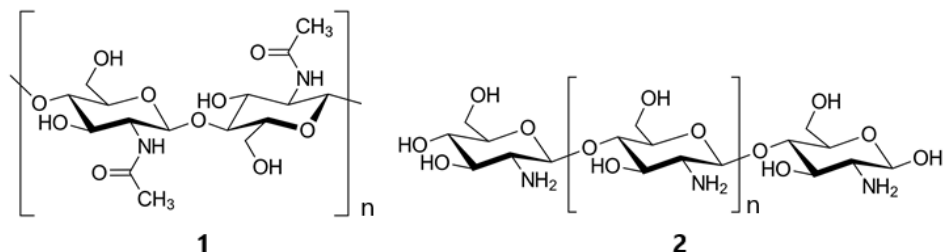


Figure 1. Chemical structures of chitin (**1**) and chitosan (**2**).

Chitin is a polymorphous substance, i.e. it occurs in crystalline modifications, such as α , β and γ . These forms are differentiated by the arrangement of individual polymer chains, the degree of hydration, the size of the unit cell and the number of chitin chains that make up the unit cell [1, 4–6].

Chitin has been widely applied in pharmacy and medicine. Biocompatibility, as well as biodegradability, make this polysaccharide very useful for biomedical applications. It is usually transformed into a gel and applied for the preparation of membranes, fibres, polymer films and as a blend component. Chitin has also been used in chromatography, for biosensors, as well as in the food industry, for cosmetics and even the removal of impurities from water [6]. Nevertheless, most interest has been focused on the use of chitin – in particular, its derivatives like chitosan and oligosaccharides – in biomedical sciences. The field of interest has especially been in dressing materials, drug carriers, tissue engineering, to form cell scaffolds, as well as in regenerative medicine to differentiate stem cells [7]. Chitin is mainly used as a source for glucosamine production, commonly used in dietary supplements to relieve arthritis pain. Chitin has also proved important in the treatment of wounds in the form of dressings because it accelerates the healing process by impacting angiogenesis, granulation, epidermis and scarring processes. During the biodegradation of chitin in the wound environment, its oligomers and -mers are released. What is more, macrophages are activated, fibroblast proliferation is stimulated and there are influences on vascularization [1, 6, 8].

The main limitation that hinders a more extensive biomedical application of chitin is its limited solubility in water. In order to overcome this issue, chitin is transformed into its derivatives, which improves its ability towards medical applications. Many scientific studies have been focused on new biocompatible chitin derivatives with excellent processing properties. The main methods of chemical modification of chitin are based on the transformation of its three functional groups, namely the *N*-acetylamine group at position 2, as well as two hydroxyl at positions 3 and 6. In practice, chitin derivatives can be obtained via hydroxylation, carboxylation, alkylation, acylation and esterification reactions. As a result of partial deacetylation, which can be carried out by chemical hydrolysis of amide groups in alkaline conditions, the formation of strongly alkaline amine groups towards chitosan is possible [9].

The most known and commonly used derivative of chitin is chitosan (**2**), which is a linear, semi-crystalline polysaccharide composed of (1→4)-2-acetamido-2-deoxy-β-D-glucan (N-acetyl D-glucosamine) and (1→4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) units. This macromolecule is a natural sugar obtained by deacetylation of chitin. Chitosan has a positive charge due to the presence of amino groups in its structure, which allows for its combination with other molecules. Chitosan is soluble in water with an acidic pH. This compound easily creates self-aggregates through electrostatic and hydrophobic interactions, hydrogen bonds and van der Waals forces. This polymer tends to adhere to the mucosa and penetrates between endothelial cells, which allows its use as a biomaterial for drug delivery systems or use in the production of dressings that accelerate wound healing. It is essential that chitosan can be used in combination with other compounds in various biomedical processes. The properties of **2**, like adhesion abilities, non-toxicity and biocompatibility, make it an excellent material for film forming and potentially as a carrier in modern drug delivery. For example, chitosan combined with porphyrins has been proposed for applications in photodynamic therapy (PDT). Moreover, chitosan is also considered to be a very interesting material for the preparation of dispersion solutions with multi-walled carbon nanotubes. The possibility to use chitosan in two-dimensional (2D) and three-dimensional (3D) scaffolds in various forms, including gels, sponges, films and fibres, makes it an excellent material for wound healing applications [9–13]. Chitosan-based hydrogels can be a solution to many problems relevant to delivery systems. They enable the increase of solubility of porphyrins due to encapsulation. Hydrogels prevent leakage of photosensitizers and enhance the effectiveness of the transfer. Hydrogels are designed to be biocompatible and biodegradable. Self-healable hydrogels have recently become a replacement for ordinary, fragile gels due to their stability and long-term lifespan [14]. Chitosan is also very often used as a polymer coating covering the core of magnetic nanoparticles, which are used in medicine and pharmacy for several applications such as catalysis [15], drug and gene delivery [16], magnetic resonance imaging and magnetic hyperthermia [17, 18], tissue engineering [19], ligand fishing and protein immobilization [20, 21].

The use of materials based on chitin and chitosan is very promising in modern anticancer therapies such as PDT, because they can create composites with porphyrinoid compounds. The biopolymer plays the role of a matrix or carrier for the drug with a porphyrinoid included in the composite. Modern delivery systems are necessary to overcome the limitations of currently applied photosensitizers and for the widespread use of PDT. The problems related to photosensitizers are their low solubility in various organic solvents and water, which leads to their aggregation in a biological environment. Another problem is related to the non-specific action of photosensitizers in PDT against cancer and healthy tissues. However, many options can be applied as a solution to these issues, e.g. a conjugation of photosensitizers with targeting molecules, application of photosensitizers in connection with nanoparticles, various formulations and new drug delivery systems. Materials chemistry and nanotechnology allow photosensitizers to be combined, e.g. porphyrinoids and their derivatives with many targeting molecules, excipients and drug delivery systems that can increase their selectivity and effectiveness, as well as reduce the dose of the administered photosensitizer. One of the materials meeting many of these requirements is chitin and its derivatives. Biocompatibility, as well as biodegradability, make chitin and its derivatives very useful for biomedical applications, including pharmacy and medicine. Various cancers affect more and more people worldwide, often as a result of hereditary tendencies. Many other issues pose a direct risk of cancer, such as environmental pollution or radiation exposure as well as lifestyle-related aspects including, but not exclusively being overweight, a sedentary lifestyle, diet and stimulants such as alcohol and cigarettes

[22]. Over the years, there has been a growing trend in cancer incidence among both men and women. Cancer is statistically more common among older people, but it also occurs in children [23]. Hence, there is interest in new therapies that can help to reduce the development of this disease. Chemotherapy, radiation therapy and surgery are the most common anticancer approaches that are currently available. There is also growing interest in novel therapeutic approaches, including immunotherapy, specially designed nanomaterials and PDT, which are coming to the attention of scientists and physicians [24].

PDT has been applied in cancer therapy because of its non-invasive nature and exceptional therapeutic effects. The base for PDT is a photodynamic reaction in which a molecule, a photosensitizer, is activated by light of a specific wavelength. An excited photosensitizer generates reactive oxygen species (ROS), including singlet oxygen, which causes the death of tumour cells through necrosis or apoptosis when the cancer tissue is exposed to an appropriate wavelength of light. Moreover, during the photodynamic reaction, the destruction of blood vessels, the result of angiogenesis, is possible. An immunological response against cancer cells is also observed [25].

The importance of such a novel approach for medicine seems to be beyond dispute, but the broad application of this method is limited by the delivery of photosensitizers to cancer-affected cells. As the consequences of systemic administration of photosensitizers are difficult to predict, it is essential to combine them with various drug delivery systems or equip them with unique functionalities that can selectively deliver them to cancer tissues [26]. The delivery systems that can transfer photosensitizers to the target tissues help overcome some limitations that are usually associated with these molecules. They concern their low solubility in an aquatic environment, which causes aggregation. Another problem is usually related to the lack of selectivity of photosensitizers towards cancer cells. However, there are many methods that allow for the improvement of currently applied photosensitizers, e.g. their functionalization, combining them with various targeting molecules and excipients, application in modern formulations, as well as binding to new drug delivery systems. These approaches increase selectivity and effectiveness and reduce the dose of the administered photosensitizer. Materials meeting these requirements are chitin and its derivatives. Herein, we present a state of knowledge on the use of porphyrinoids in combination with chitin and its derivatives in medicine and pharmacy.

2. Porphyrinoids and Their Combinations With Chitin, Chitosan, and Its Derivatives

2.1. Porphyrins in Combinations

Porphyrins are macrocyclic aromatic compounds consisting of four pyrrole rings bonded through methane bridges. This highly conjugated system contains nine conjugated π bonds. Strong electron interactions influence the appearance of porphyrins' interesting spectroscopic properties. Porphyrins are ubiquitous in nature. For example, heme constitutes a cofactor group of hemoglobin, cytochromes and other redox-active enzymes. Synthetic porphyrins can be modified in the core with various metal ions as well as in their periphery in β and meso positions. Drug delivery and cancer theranostics represent some of the medical uses of porphyrins and porphyrin-based nanocomposites [27, 28].

Chitosan-drug delivery systems have been presented in many *in vitro* and *in vivo* studies for various pharmaceutically active ingredients. As previously mentioned, chitosan stabilizes the built-in drug molecules and maintains their pharmacological activity. This is particularly important in the study on anticancer drugs: a controlled and prolonged release of pharmaceutically active ingredients allows adequate diffusion and absorption into cancer cells for many cycles of their division. The release of water-insoluble therapeutic agents

is facilitated, allowing their direct delivery to the site of the disease [29]. One of the essential applications of porphyrins in medicine is PDT. There are many problems associated with the broader application of porphyrins in PDT. One of the issues is related to an inadequate accumulation of porphyrins in tumour tissue, while the other is the limited solubility of the photosensitizer [30]. Ferreira et al. [30] studied a porphyrin dye (**3**) in biopolymer chitosan films consisting of chitosan, polyethylene glycol (PEG) and gelatin for prospective application in localized PDT of cancer (Fig. 2) [30]. They noted the stabilization of porphyrin molecules embedded in chitosan. It was also possible to control and prolong porphyrin release from the films to ensure adequate diffusion and uptake into cancer cells over many cycles of HeLa cell division (a cancer cell line). The advantage of such system is the possibility to load and release porphyrins directly to the localized tumour. An additional advantage of such a polymer-based drug delivery system is the avoidance of systemic circulation of drugs.

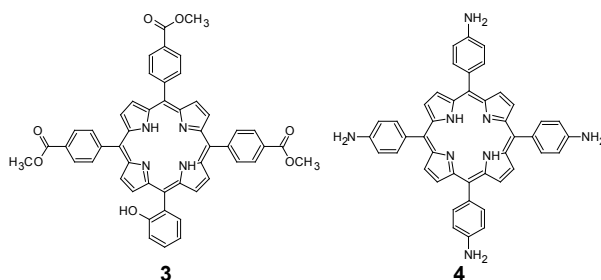


Figure 2. Chemical structures of selected porphyrins **3** [30] and **4** [32].

Nanoparticle-based drug delivery systems can improve the targeting of photosensitizers and their release with controllable photoactivity at predetermined locations, to achieve desired therapeutic effects with minimal side effects [31]. Chitosan can be cross-linked with a Schiff-based bond, which is considered very good for hydrogel synthesis in terms of new and specific features. A cell-specific and pH-sensitive nanostructure hydrogel based on chitosan and photosensitizer (**4**) has been proposed for selective PDT by Belali et al. [32]. The homogeneous distribution of porphyrin and the ability to restore the integral gel after injection is a unique feature of this system. A hydrogel consisting of conjugated porphyrin-triazine-chitosan system functionalized with folate was targeted to cancer cells possessing overexpressed folic acid receptors. The structure of the hydrogel is affected by pH, which is related to the strength of the imine bond. At acidic pH, the imine bonds is cleaved, followed by the release of porphyrins. At pH 7.4, the release of porphyrin **4** from the cross-linked hydrogel is less than 5% after 12 days. The lower the pH, the faster the release rate of **4** at pH 6.8 – the release after 12 days is 30% – and at pH 5, it is around 90%. Furthermore, the production of singlet oxygen from porphyrin alone is lower and reaches 0.50, whereas for the hydrogel porphyrin system it is 0.64, which improves its applicability for PDT. Of note, the system functionalized with folates is more specific and more effective in killing of immortalized human HepG2 cells (a liver cancer cell line) and MCF-7 cells (a breast cancer cell line) compared with the hydrogel containing only porphyrin and chitosan [32].

Applications of chitosan-porphyrinoid systems go far beyond cancer treatment. A very interesting area for modern PDT applications is microbiology and the possibility to control bacterial and fungal infections. The problem of antimicrobial resistance is

a growing threat to humans, so it is necessary to look for new ways to fight microorganisms. The photodynamic reaction, representing the joint action of light, photosensitizer and oxygen in anticancer PDT, also applies to pathogenic microbial organisms. Porphyrinoids in their free form and their conjugates can be used in bacterial and fungal pathogens to produce singlet oxygen that lead to their death, which may be attributed to novel ways of coping with the ever-growing issue of drug resistance. One way is to use photodynamic inactivation (PDI) of bacteria or fungi, where the microorganisms are exposed to ROS [33]. Control of *Listeria innocua* biofilms is possible by biocompatible photodynamic antifouling chitosan-based materials [34]. One of the PDI applications is the control of microbiological biofilms. *Listeria monocytogenes* can form dense biofilms that are resistant to biocides. This bacterium causes listeriosis, which is transmitted through food and can lead to gastrointestinal and neurological disorders. *L. monocytogenes* infection has a high mortality rate, and it can survive adverse conditions when present in a biofilm, so new methods are being tried, including PDI, in the fight against this microorganism. In another study, four porphyrins (**5a–d**) were combined with chitosan and the obtained activities of porphyrin-chitosan films were researched against *L. innocua* (Fig. 3). The films made of chitosan and porphyrins revealed potential as antifouling coating materials for the food industry [34].

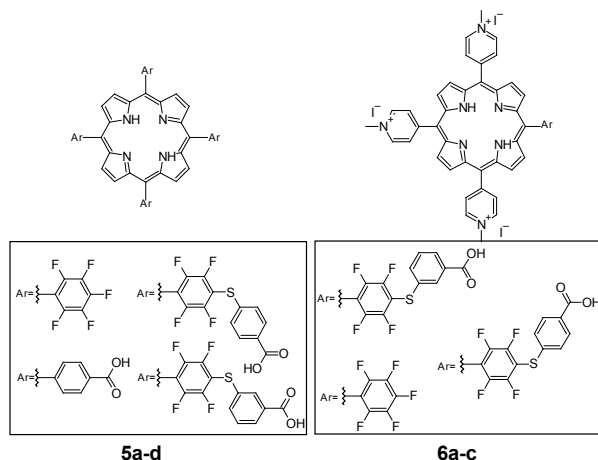


Figure 3. Chemical structures of porphyrins **5a–d** [34] and **6a–c** [35].

The next examples constitute materials based on cationic porphyrins conjugated to chitosan or titanium dioxide [35]. The authors studied Gram-positive and Gram-negative bacteria in terms of their applicability to PDI. Gram-positive bacteria were more sensitive to cationic, neutral or anionic porphyrins, while Gram-negative bacteria were sensitive only to cationic porphyrins. This dependence of Gram-negative bacteria results from the physiology of these microorganisms. The Gram-negative cell wall is made of a thin layer of peptidoglycan surrounded by an asymmetrical, impermeable inner layer of phospholipids and outer lipopolysaccharides. The lipopolysaccharides give a strong negative charge to the bacterial surface. The most common human pathogen *Escherichia coli* was chosen for the study. The light output correlates with its metabolism and makes *E. coli* a very suitable model to monitor the effectiveness of the photoinactivation. Of note, tricationic porphyrins with carboxyl groups in its periphery (**6**) were applied for the preparation of films with chitosan-based support material. The obtained films revealed a promising antimicrobial potential [35].

Applications of porphyrinoids in medicine are also directed at diagnosis, including magnetic resonance imaging (MRI), where they can be applied as contrast agents. While MRI is a powerful tool for non-invasive mapping of biological structures, it is essential to remember that the method has ubiquitous limitations, such as a low signal-to-noise ratio, which can be improved by using exogenous contrasting agents. MRI has many advantages, such as high spatial resolution, non-invasiveness, lack of ionizing radiation and the possibility to receive both anatomical and physiological information at the same time. One of the problems with MRI is its lack of sensitivity, which can be improved by using contrast agents. The development of nanostructures with well-defined homogeneous particle size and shape is highly demanding but results in novel nanoparticles of improved physicochemical characteristics. In diagnostic imaging and drug delivery, particles in the nanometre range of uniform size and spherical shape increase their ability to penetrate cell membranes and reduce the risk of rapid removal from the body. Chitosan nanoparticles have been extensively studied for the administration of the delivered drug. The main obstacle to use neutral porphyrins in this method is their low water solubility. The application of chitosan as a carrier or polymer matrix in the composite leads to systems with greater hydrophilicity, a feature demonstrated for close analogues of porphyrins, which are porphyrazines [36]. By encapsulation or absorption of the therapeutic agents into hydrophilic polymer nanoparticles, it is possible to increase their solubility, which effectively reduces early release and minimizes the necessary dose of therapeutic agents. Chitosan is known to act as a drug delivery medium and contrast carrier for imaging. The core of chitosan nanoparticles can capture various therapeutic agents while its surface tends to adhere to a negative charge on the cell surface, allowing for the transfer of nanoparticles through cell membranes in subsequent steps. It is also a perfect material for encapsulation, which increases solubility for its 'cargo', e.g. porphyrins [37–39]. Jahanbin et al. [37] obtained chitosan nanoparticles by the ionic gelation method and loaded them with *meso*-tetrakis(4-pyridyl)porphyrin gadolinium (**7**) by passive adsorption (Fig. 4). Optimization resulted in the preparation of chitosan nanoparticles with a 45–65 nm diameter and a hydrodynamic radius of 412 nm. These nanoparticles were studied by MRI *in vitro*; the results revealed their potential utility in cancer theranostics. What is essential is that nanoparticles with **7** applied in the MRI study improved contrast at lower concentrations much better than porphyrin gadolinium complex **7** alone.

Another diagnostic application of porphyrin-chitin material in medicine is an ultrasensitive chemiluminescence aptasensor for thrombin detection based on iron porphyrin (hemin, **8**), proposed by Sun et al. [40]. They obtained chitosan modified magnetic oxide graphene composite and immobilized aptasensor for thrombin and **8** to its surface. The authors then applied the composite in the chemiluminescence study and observed an improvement in sensitivity and selectivity for thrombin detection [40].

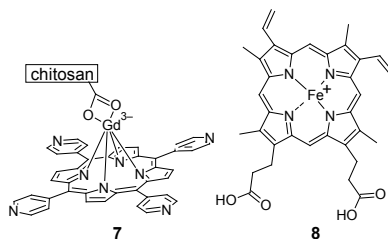


Figure 4. Chemical structures of porphyrins **7** [37] and **8** [40].

2.2. Chlorins and 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) in Combinations

Chlorins constitute a group of macrocyclic compounds – derivatives of porphyrins, which have found wide application in PDT due to their photosensitizing properties. Chlorin e6 (**9**) has been researched for both diagnostic and therapeutic purposes (Fig. 5) [41]. Compound **9** was also used as a bactericide to fight methicillin-resistant *Staphylococcus aureus* (MRSA) strains. For this purpose, **9**, chitin and magnetic (Fe_3O_4) polydopamine were applied for the synthesis of novel photodynamic antibacterial nanoparticles. The nanoparticles exhibited activity against bacteria after excitation with near-infrared light. The resulting complex produced ROS more efficiently than chlorin e6 alone [42]. Chlorin e6, in combination with chitosan, may also find application in oncology. In studies on tissue cultures and model organisms, it has been shown that the chitosan complex with chlorin e6 is characterized by an increased uptake by tumour cells, greater efficiency in the production of reactive oxygen species and higher cytotoxicity [43, 44].

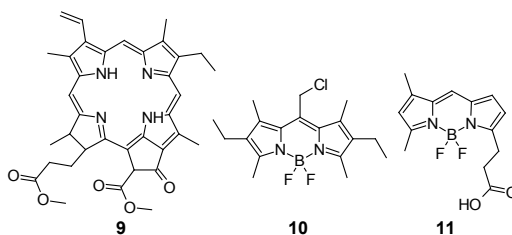


Figure 5. Chemical structures of chlorin e6 (**9**) [41] and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPYs) (**10**, **11**) [45, 49].

BODIPY (**10**) is composed of dipyrromethene complexed with a disubstituted boron center. BODIPY dyes are molecules with high fluorescence capability. Due to this property, they can be used as imaging agents. Their fluorescent properties can be easily modified by slight changes in their structure [45]. They reveal a significant decrease in fluorescence in the presence of oxygen; therefore, they can be used to determine various analytes *in vitro*. They are currently widely used in analytical chemistry, enzymology and molecular biology as fluorescent probes [46]. The main problem with the clinical use of BODIPY is its high toxicity. Cell culture studies have shown that complexing them with chitosan reduces their cytotoxic properties, but not enough for them to be safely applied to patients [47, 48]. Taki and Ardestani [49] recently conjugated the anti-nucleolin aptamer AS1411 with BODIPY-labelled chitosan nanoparticles. The vital part of this BODIPY-chitosan-aptamer system is BODIPY FL carboxylic acid functionalized dye (**11**). After mixing chitosan-aptamer with fluorescent dye **11**, the resulting material was purified and lyophilized, characterized and subjected to biological study. Cytotoxicity and *in vitro* cellular uptake of BODIPY-labelled chitosan–AS1411 aptamer conjugates were studied using the XTT (cell proliferation) assay on breast cancer cells (T47D) and normal kidney cells (HEK-293) and flow cytometry on T47D cells. The scientists did not observe any statistically significant cellular toxicity of this system on normal cells and concluded that this conjugate could be used as a potential diagnostic agent for fluorescent imaging of cancer cells.

2.3. Phthalocyanines in Combinations

Phthalocyanines are synthetic porphyrinoids that structurally resemble naturally occurring porphyrins. The key difference is that the methine groups between pyrroles in porphyrins are replaced in phthalocyanines with nitrogen bridges. The phthalocyanine structure shares similar physical and chemical properties to natural porphyrins. Phthalocyanine complexes containing various metal ions, create colourful compounds ranging from red to navy blue. Phthalocyanine complexes with copper and cobalt have been used as dyes for the production of paints. These macrocycles have found substantial use in the modern dye and polygraphic industry. They possess the ability to absorb light from the red region of the visible spectrum and have a photosensitizing potential; thus, they have been applied in anticancer and antimicrobial PDT. Unfortunately, the π - π aromatic stacking, which induces phthalocyanines aggregation in solution, contributes largely to their low solubility in water and leads to the formation of clusters impeding their singlet oxygen generation and tumour cell uptake. The targeting of phthalocyanines towards specific cells is another limiting factor. Therefore, combining them with transporting agents could not only help to solubilize and restrain their aggregation in water media but also accumulate them specifically in tumour cells [50–55].

Much research has aimed to solve issues related to high aggregation and low biological uptake of phthalocyanines by tumour cells. For example, de Souza et al. [56] presented results on zinc(II) phthalocyanine loaded into nanocapsules containing chitosan, with a detailed assessment of their physicochemical properties, photodynamic activity, photostability and drug release profile. All of the nanocapsule types allowed high encapsulation of zinc(II) phthalocyanine with particle sizes ranging from 182 to 512 nm. The nanocapsules also remained stable during prolonged storage at 25 and 37°C. Zinc(II) phthalocyanine loaded conventional core chitosan nanocapsules also showed the highest singlet oxygen yields (ϕ_{Δ} 0.61) compared with the standard (zinc(II) phthalocyanine - ϕ_{Δ} 0.67) and other studied nanoparticles presenting lower yields (ϕ_{Δ} 0.36 and 0.41) [56]. Another study showed the linkage of phthalocyanines with chitosan. Such an approach is reliable for overcoming the aggregation issue. Due to high biocompatibility, biodegradability and the presence of functional groups allowing for easy linking of chitosan with bioactive compounds, it has been under the spotlight as a potential carrier for phthalocyanines in anticancer and antimicrobial PDT. Human serum albumin, as well as chitosan conjugates with zinc mono-carboxyphenoxy phthalocyanines, were synthesized and their photophysical behavior and cytotoxicity against MCF-7 human breast adenocarcinoma cell line were assessed. Mono substituted zinc(II) phthalocyanine (**12**) was chosen so that only a single bond between human serum albumin or chitosan and the phthalocyanine was created, which allowed avoiding aggregation and multi-bonding of phthalocyanine to more than one NH_2 groups in the same molecule (Fig. 6). All conjugates also showed virtually unchanged ground state absorbance compared to zinc(II) phthalocyanine, and slight improvements in fluorescence quantum yields (probably due to lower aggregation of conjugates) and lifetimes, as well as a lack of self-quenching within the conjugates. Triplet quantum yields were slightly higher in the chitosan conjugate and stayed unchanged for human serum albumin; however, there was significant elongation in triplet lifetimes and a slight increase in singlet oxygen yields. Dark cytotoxicity was also lowered in monosubstituted zinc(II) phthalocyanine conjugates, and PDT viability assessed after illumination with a quartz lamp showed slightly less cytotoxicity of the conjugates compared to zinc(II) phthalocyanine which might be attributed to its higher dark cytotoxicity. PDT activity of monosubstituted zinc(II) phthalocyanine conjugated with human serum albumin was slightly higher than that of chitosan [57].

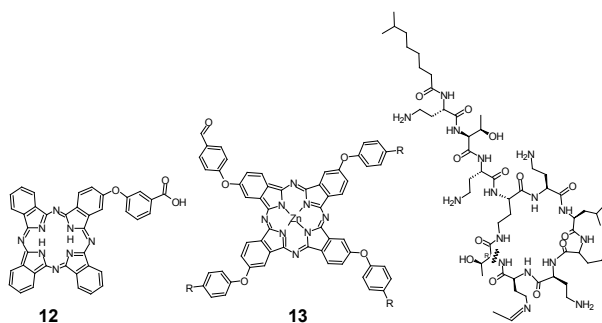


Figure 6. Chemical structures of phthalocyanines **12** [57] and **13** [52].

Zinc(II)phthalocyanine is receiving substantial interest thanks to its ability to produce singlet oxygen. This macrocycle absorbs light in the red part of the electromagnetic spectrum, which penetrates tissues very well. An example of such a formulation is a hydrogel consisting of zinc(II) phthalocyanine conjugated with colistin (**13**); its antibacterial properties were assessed on colistin-vulnerable *Pseudomonas aeruginosa*. Colistin served as both a bacterial membrane disrupting and the linking agent. Singlet oxygen produced by zinc(II) phthalocyanine passed through the cell membrane and damaged the bacteria. A hydrogel consisting of zinc(II) phthalocyanine exhibited unremarkable antibacterial properties, probably due to the absence of cationic units, which would link it to the Gram-negative cell membrane. The performed tests using the disk method revealed that colistin antibacterial properties in zinc(II)-colistin conjugate were preserved, as well as an increase in antibacterial properties of mentioned gels was noted after laser light illumination at ca. 660 nm for 15 min. This leads to the belief that chitosan hydrogel does not impede the release of the zinc(II)-colistin conjugate into the environment and that the chitosan hydrogel bearing phthalocyanine-colistin conjugate holds significant potential as hydrating dressings [52].

Chitosan in combination with porphyrinoids may also be important for antifungal therapy. For some time now, PDI has been researched as a potential antifungal tool in the face of a fungal drug resistance issue. Tang et al. [58] studied a series of conjugates consisting of chitosan oligosaccharides with 1-[4-(2-carboxyethyl)phenoxy]phthalocyanine zinc(II) with regard to their photochemical and photophysical properties as well as biological uptake and photoinactivation *in vivo* in *Candida albicans*. All the conjugates revealed lower aggregation in aqueous media than phthalocyanine alone. However, they presented an increase in singlet oxygen generation, higher biological uptake, and inactivation of *C. albicans*. The quaternized derivatives expressed the best antifungal properties towards *C. albicans* mitochondria, as shown by fluorescence microscopy. Higher biological uptake of quaternized chitosan oligosaccharides was associated with their a more positive charge, allowing better interaction with the negatively charged *C. albicans* cell membrane. All of the compounds exhibited cytotoxic properties on *C. albicans* only after irradiation with red light, with none exhibiting them in dark conditions. Quaternized conjugates showed lower aggregation in an aqueous medium, higher biological uptake in fungal cells and higher affinity towards the fungal mitochondria, which significantly increased their antifungal cytotoxicity [58]. In a recently performed study, Tang et al. [59] employed two molecular weights of carboxymethyl chitosan (50 and 170 kDa) for conjugation with zinc(II) 1-[4-(aminoethyl)phenoxy]phthalocyanine to assess the antifungal properties of both conjugates against *C. albicans*. Carboxymethyl

chitosan is another chitosan derivative that has shown promising properties, such as increased water solubility over a broader pH range, high availability and antimicrobial activity. Its antifungal capabilities include the disruption of fungal cell wall functions. All of the prepared conjugates revealed decreased aggregation and increased solubility in water compared with macrocycle. ROS generation of the conjugates was also much lower than that of macrocycle due to the modified chitosan backbones hindering the transmission of ROS. Some of the compounds showed higher antifungal properties than phthalocyanine alone due to higher uptake of conjugates by *C. albicans* and their particular affinity for *C. albicans* mitochondria, as shown by fluorescence microscopy. Carboxymethyl chitosan (50 kDa) conjugated to phthalocyanine presented the best cytotoxic results due to its high uptake and medium ROS generation. Of note, this conjugate revealed even better antifungal properties than the methylene blue, a known antifungal photosensitizer, which served as a positive control [59]. In another study, Hsieh et al. [60] researched cationic chitosan/tripolyphosphate nanoparticles containing phthalocyanine, iron(III)phthalocyanine-4,4,4,4-tetrasulfonic acid, for antifungal PDT against *C. albicans* and *C. tropicalis*. The use of adherent colonies was dictated by the fact that biofilm-creating *Candida* spp. show higher resistance than their planktonic counterparts. The nanoparticles revealed excellent antifungal properties against the used strains compared with fluconazole and flucytosine. The effectiveness was lower against the adherent biofilm-producing colonies. However, the use of flucytosine after PDT with phthalocyanine encapsulated chitosan/tripolyphosphate nanoparticles yielded greater yeast cell death in these adherent colonies. These data confirmed the greater effectiveness of combined phthalocyanine encapsulated chitosan/tripolyphosphate-PDT and flucytosine therapy compared with PDT or flucytosine treatment alone [60].

3. Conclusions

Chitin and its derivatives have been transformed into gels and applied for the preparation of membranes, fibres and polymer films. Moreover, they have also been applied in chromatography and biosensors, as well as the food industry, in cosmetics and even for removing various pollutants from water. Much interest in this polysaccharide and its derivatives is related to the increasing use of chitin in biomedical sciences, especially in dressing materials, drug carriers, tissue engineering for the preparation of cell scaffolds and also in regenerative medicine to differentiate stem cells. Drug delivery systems based on chitin and chitosan have been presented in many *in vitro* and *in vivo* studies for various pharmaceutically active ingredients, including porphyrinoids. These studies have shown that chitosan stabilizes the built-in drug molecules and maintains their pharmacological activity. Porphyrin dyes in biopolymer chitosan films reveal prospective applications in localized PDT for cancer. It is also possible to control and prolong porphyrin release from the films to ensure adequate diffusion and uptake into cancer cells over many cycles of cancer cell divisions. The advantage of such system is the ability to load and release porphyrins directly into the tumour. An additional benefit of such a polymer-based drug delivery system is the ability to avoid the systemic circulation of drugs. Nanoparticle-based drug delivery systems have improved the targeting of photosensitizers and their release with controllable photoactivity at predetermined locations, to achieve desired therapeutic effects with minimal side-effects. A cell-specific and pH-sensitive nanostructure hydrogel based on chitosan and porphyrin photosensitizer has been proposed for selective PDT. The chitosan-porphyrin system functionalized with folates is also very useful for killing various cancer cell lines. Similar important applications of chitosan and its derivatives have been found for their

combinations with chlorins, BODIPYs and phthalocyanines. Many studies performed for combinations of phthalocyanines with chitin or chitosan have demonstrated the effectiveness of this approach. It is possible to overcome various limitations of phthalocyanines, such as aggregation and limited biological uptake by tumour cells. Notably, many studies have been performed for zinc(II) phthalocyanine, which has been loaded into nanocapsules containing chitosan. Porphyrinoid combinations with chitosan and its derivatives have also revealed antibacterial and antifungal potential for PDT. Porphyrinoids can be used for diagnostics as contrast agents in MRI. The development of nanostructures with well-defined homogeneous particle size and shape is highly demanding for these particular applications but results in novel nanoparticles of improved physicochemical characteristics. It is worth noting that chitin nanoparticles loaded with gadolinium porphyrin demonstrate an improved contrast at lower concentrations compared with the porphyrin gadolinium complex alone. Another diagnostic application of porphyrin-chitin material in medicine is an ultrasensitive chemiluminescence aptasensor for thrombin detection based on iron porphyrin.

4. Acknowledgments

The authors acknowledge the funding provided by the National Science Centre, Poland, under grant no. 2016/21/B/NZ9/00783. M. Z.-B. is a member of the Center of Excellence 'Towards Personalized Medicine' operating under the Excellence Initiative – Research University.

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