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Unraveling the Mechanisms of Light-induced Hemolysis by First Transition Metal Phthalocyanines

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Abstract

Introduction: Phthalocyanines are molecules that possess distinctive properties as a result of their structural composition. They have the capacity to act as catalysts in a variety of chemical reactions and demonstrate potential for use in photodynamic cancer therapy. For this purpose, the phthalocyanine molecule requires a metal ion that is capable of undergoing changes in state and binding to other molecules. Photohemolysis is a widely utilized in vitro test for evaluating the efficacy of phthalocyanines as photosensitizers. Its primary advantages include straightforward detection of the photohemolytic process through UV-VIS spectroscopy and insight into the phototoxic mechanisms that these molecules develop. This study aimed to evaluate how the central metal ion affects the photobiological behavior of phthalocyanines on human erythrocytes and the possible phototoxic mechanisms involved in the hemolysis process.

Materials and Methods: Phthalocyanines with various central metals (Zn, Cu, Ni, Fe, Mn, Co) were synthesized via microwave irradiation. The phototoxicity of the compounds was evaluated through light-induced hemolysis using isolated human erythrocytes. The degree of cell damage was determined by measuring the absorbance of hemoglobin at 545 nm. The traditional spectrophotometric approach was employed to assess the kinetics of photohemolysis under white light irradiation. The rate of hemolysis was employed as a means of determining the extent of phototoxic damage. Radical scavengers were employed to elucidate the phototoxic mechanism (Type I or Type II) of the synthesized phthalocyanines.

Results: The synthesized phthalocyanines demonstrated a range of photohemolytic activities against human erythrocytes. Zinc phthalocyanine (ZnPc) demonstrated the most pronounced effect, followed by iron (FePc), nickel (NiPc), and copper (CuPc) phthalocyanines. Conversely, cobalt (CoPc) and manganese (MnPc) phthalocyanines exhibited the least notable activity. It is likely that photohemolysis occurred via both type I and type II mechanisms, with ZnPc generating the most reactive oxygen species. Moreover, the interaction of ZnPc with erythrocyte membranes may contribute to its pronounced photohemolytic activity. The results of scavenger experiments indicate that ZnPc induces hemolysis primarily via type I radicals.

Conclusion: ZnPc demonstrated the most pronounced hemolytic effect, attributable to its superior membrane interaction and efficient generation of free radicals via a type I mechanism. The role of singlet oxygen was found to be minor. These findings indicate the potential of ZnPc for photodynamic therapy due to its type I phototoxicity.

Keywords: Erythrocytes, Phthalocyanines, Photodynamic therapy, Photohemolysis

1. Introduction

Phthalocyanines display a range of exceptional electronic and physicochemical characteristics. A substantial body of research [1, 2, 3] has demonstrated the potential of these planar aromatic macrocycles, which consist of four isoindole units linked by nitrogen atoms. In various condensed systems, the 42 π electrons are distributed over 32 carbon and 8 nitrogen atoms. The inner ring, comprising 16 atoms and 18 π electrons, represents the primary site of electronic delocalization. It is noteworthy that the outer benzene rings remain unaltered [4].

In a variety of chemical and photochemical processes, metalloporphyrins and metallophthalocyanines have been observed to exhibit enhanced catalytic activity. These photocatalysts have been demonstrated to damage Kraft lignin, oxidize a range of unsaturated hydrocarbons, and degrade a variety of aquatic organic pollutants [5, 6]. Moreover, in organic solutions or when supported on a variety of inorganic materials, metalloporphyrins exhibit exceptional catalytic efficiency. The robust support-complex interaction markedly enhances their photostability and catalytic activity, rendering them highly suitable for a multitude of applications [7,8].

It is important to note that the catalytic ability of the metalloporphyrin is dependent on the redox-active metal ion present within the macrocycle, which must possess two or more oxidation states. Furthermore, the metal ion must possess coordination sites that are available for binding various molecules. The catalyst requires a labile coordinating sphere with high exchange rates, which can be achieved by the use of organic radical substituents and π character. Prior research has indicated that dative π coordination effects exert a considerable influence on the catalytic activities of these compounds [9, 10, 11].

Figure 1 depicts the phothemolytic process on human erythrocytes induced by phthalocyanines. In the presence of irradiation, the compounds undergo an electronic excitation from a fundamental singlet state (S_o) to an excited singlet state (S_1, S_2, etc.). This transition enables the occurrence of various photophysical processes, including radiative (e.g., fluorescence and phosphorescence) and non-radiative (e.g., intersystem crossing between singlet-triplet systems, ISC) processes [12].

An alternative pathway for the transformation of molecules in the singlet excited state is via intersystem crossing (ISC), which gives rise to two types of phototoxic reactions. The first of these is the type I mechanism, which is produced by an electronic transfer between the photosensitizer (phthalocyanine) and molecular oxygen ($^3O_2$). The second is the type II mechanism, which is an energy transfer between the photosensitizer and $^3O_2$. Both mechanisms result in the production of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$) via reaction I and radicals such as superoxide anion (O_2) and hydroxyl (OH) via mechanism II see in Figure 2. These species are responsible for the generation of photoinduced hemolysis by phthalocyanines, as illustrated in Figure 1. It is crucial to acknowledge that compounds with the capacity to interact with $^3O_2$ and generate ROS are designated as photosensitizers [12, 13].The use of human erythrocytes has been a traditional method for identifying potentially phototoxic agents administered systemically in humans. Furthermore, they are useful for studying phototoxicity mechanisms, including oxygen-dependent processes and those involving free radicals. An alternative method for studying the photoreactive effect of various compounds is the photohemolysis assay. This method determines the potential capacity of various products to damage the erythrocyte membrane or oxidize hemoglobin under UV-VIS light [14].

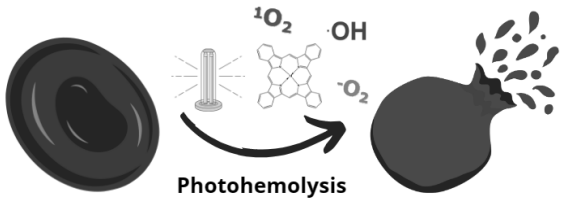

Figure 1. Photohemolysis process.

The experimental measurement of photohemolysis is conducted using a spectrophotometer. This instrument allows for the observation of the impact of photosensitizing compounds, such as phthalocyanines, on red blood cell membranes. The spectrophotometer quantifies the amount of hemoglobin released into the solution, thereby indicating the degree of hemolysis. The quantity of hemoglobin released into the solution is quantified by measuring the variation in absorbance at a wavelength of approximately 540 nm, which is the wavelength at which hemoglobin exhibits its maximum absorbance. It is important to note that a decrease in absorbance at approximately 540 nm indicates the onset of hemolysis, which results in an increase in the amount of free hemoglobin in solution. This subsequently undergoes transformations to become methemoglobin, which can be detected at 630 nm [3,15].

Furthermore, the assessment of photohemolysis can be conducted through spectrophotometry, employing

the specified and selective additive as a quencher and sequestrant of reactive oxygen species. Sodium azide (NaN_3) can be employed for the detection of singlet oxygen, superoxide dismutase for the identification of superoxide anion, and glutathione for the analysis of free radicals. The rate of photoinduced degradation of red blood cells is employed as the standard of comparison in both the absence and presence of these additives. These experiments permit the determination of the phototoxic mechanisms involved in photoinduced hemolysis (type II or type I phototoxic reactions) [16].

Previous studies have demonstrated the potential of phthalocyanines as a new class of photosensitizers for photodynamic cancer therapy. In these studies, the capacity of aluminum phthalocyanine tetrasulfonate (AlPcS) to induce membrane injury under visible light irradiation was evaluated, with the photohemolysis of human erythrocytes serving as the final outcome measure [17]. The results indicated that the efficiency of photohemolysis is not linear with respect to AlPcS concentration. Moreover, the data suggested that the efficiency of the process in question tends to decrease with increasing concentration of AlPcS. Furthermore, the light fluence response curve was observed to have a sigmoidal shape, indicating a sharp increase in hemoglobin release over a limited range of light fluence. Furthermore, it was determined that neither singlet oxygen nor hydroxyl radicals are involved in AlPCS-induced membrane damage. This is evidenced by the observation that the presence of D_2O or glycerol did not affect the rate of photohemolysis.

Furthermore, zinc phthalocyanine (ZnPc) has been demonstrated to possess a notable capacity to induce photosensitized hemolysis in human erythrocytes. Two distinct types of hemolysis were identified: light hemolysis, which occurred during irradiation, and dark hemolysis, which manifested subsequent to irradiation. The occurrence of light hemolysis was preceded by a series of changes in the erythrocyte membrane, including hyperpolarization, membrane fluidization, and cell swelling. Moreover, the rate of hemolysis was found to be dependent on the erythrocyte membrane potential. A reduction in membrane potential resulted in the inhibition of both types of hemolysis [14].

It is of particular significance to note that the interaction of ZnPc with the erythrocyte membrane gives rise to a multitude of predominant membrane structural transformations. These include the formation of transient and short-lived pores resulting from photodenaturation and aggregation of membrane proteins, such as band 3 protein, which plays a significant role in erythrocyte photostability. Furthermore, the erythrocyte membrane undergoes fluidization and a notable expansion of its surface area, which is consistent with the phenomenon of photosensitized cell swelling. These structural and functional alterations of the membrane contribute to both "light" hemolysis during irradiation and "dark" hemolysis post-irradiation [14,18].

In the present study, phthalocyanine molecules were synthesized by linking them to metals of the first transition series (Zn, Ni, Cu, Fe, Co, and Mn) with the objective of evaluating their phototoxic activity in human erythrocytes. To this end, a conventional spectrophotometric approach was employed, whereby the differences in absorbance at 545 nm following erythrocyte irradiation were quantified as a measure of phthalocyanine-induced photohemolysis. The objective of this study was to ascertain the phototoxic mechanisms (type I or type II) exhibited by phthalocyanine compounds. This was achieved by employing the use of ROS trapping reagents, including NaN_3, GSH, and superoxide dismutase.

2. Materials and Methods

Synthesis of phthalocyanines

The synthesis of phthalocyanines was conducted in accordance with the methodology outlined by Staicu et al. [19]. The starting materials, including 0.01 mole of phthalic anhydride, 0.05 mole of urea, $1x10^{-5}$ mole of ammonium molybdate as catalyst and 0.0026 mole of the metallic salts (Zn, Cu, Ni, Co, Fe, Mn), were homogenized in a mortar, then placed in a vessel and irradiated with microwaves for 10 minutes. The resulting product was purified through a series of solvent extractions, including methanol and chloroform, before undergoing recrystallization in a solution of sulfuric acid and water.

Isolation and preparation of human erythrocytes solutions

In order to evaluate the phototoxic mechanisms of the synthesized phthalocyanines, photoinduced hemolysis experiments were conducted on human erythrocytes. To this end, fresh human blood samples were subjected to centrifugation in order to separate the plasma from the erythrocytes (see Figure 3.a). Subsequently, human erythrocyte solutions were prepared in phosphate-buffered saline (PBS) at a pH of 7.4. The final solutions were prepared as a mixture of the phthalocyanine (dissolved in DMF) and 3-4 ml of the human erythrocyte buffer solution (Figure 3.b).

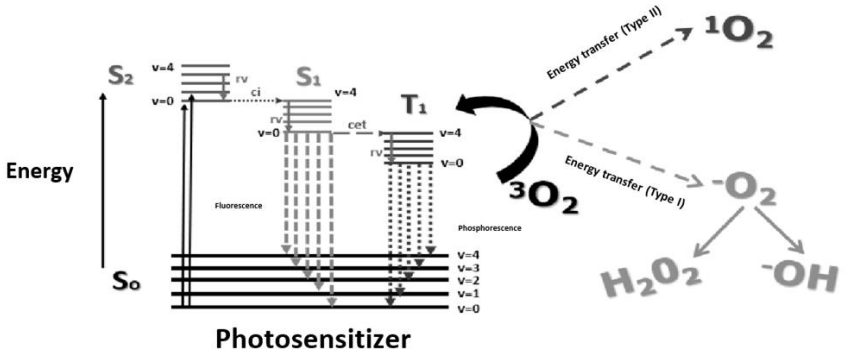

Figure 2. Jablonski diagram. Adapted from reference [12].

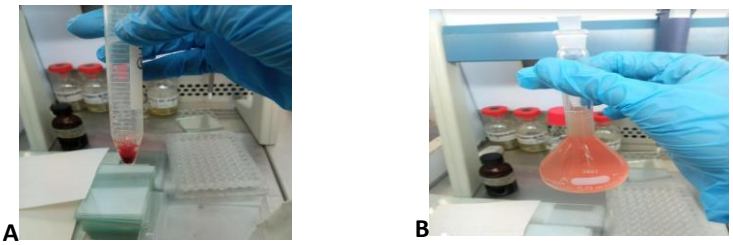

Figure 3. Isolation and preparation of human erythrocytes solutions: (a) human erythrocytes after centrifugation; (b) human erythrocytes solution.

Measurement and determination of photohemolysis

Prior to conducting the photohemolysis experiment, the UV-VIS spectra of the human erythrocyte solutions were obtained to ascertain the specific wavelength to be investigated. One of the less pronounced bands was selected at 545 nm, which aligns with the wavelength utilized in existing literature to quantify the extent of photoinduced hemolysis by photosensitizers and correlates with the absorbance of unoxidized hemoglobin (Figure 4).

Subsequently, the traditional spectrophotometric method was employed to investigate the photolytic effects of phthalocyanine on red blood cells [14, 15, 16, 17]. To this end, the solutions were placed in quartz cells and subsequently irradiated for 40 minutes in a LuzChem reactor using white light lamps with an irradiance of 3840 LUX (see Figure 5).

Figure 4. UV-VIS spectra of human erythrocytes solutions (without phtalocyanines).

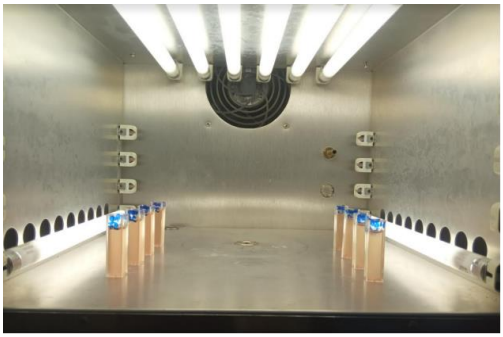

Figure 5. Irradiation of human erythrocytes solutions (with phtalocyanines).

Absorbance measurements were conducted at 10-minute intervals in a Lambda UV-VIS spectrophotometer at a wavelength of 545 nm. The corresponding absorbance values were recorded in order to construct a graph (curve) and calculate the equation of the line. The degree of photohemolytic damage to human erythrocytes caused by phthalocyanines was determined by comparing the values of the constant provided by the equation of the line.

Determination of phototoxic mechanisms

To ascertain the phototoxic mechanisms elicited by phthalocyanines (types I and II), identical irradiation experiments were conducted in the presence of radical scavenging agents, including NaN_3, GSH, and superoxide dismutase. To this end, 10 µL of each

scavenger reagent solution was added to the mixture of human erythrocytes and phthalocyanines. The absorbance measurement was continued at 545 nm, and the percentage of photohemolysis was compared in each case to determine the mechanism of action of the phthalocyanines under investigation.

3. Results

The photohemolytic capacity of phthalocyanines was determined by means of an equation that contains a constant value that correlates with the aforementioned activity. A higher value of this parameter indicates a more pronounced effect on human erythrocytes.

It is evident that a clear trend is observable in the photohemolytic capacity of the phthalocyanine molecules synthesized. Figure 6 illustrates that the equation of the straight line has a constant value of 0.0596, which represents the highest value observed across all curves. This suggests that the absorbance decreased considerably for zinc phthalocyanine (ZnPc), which correlates with its high capacity to induce hemolysis. This finding is consistent with the existing literature [14].

Figures 7 and 8 illustrate the values of the equation of the straight line for copper (CuPc) and nickel (NiPc) phthalocyanines, with a value of 0.0136. It is evident that the photohemolytic activity of these phthalocyanines is more pronounced than that of the cobalt (CoPc) and manganese (MnPc) phthalocyanines, as evidenced by the values of the constant of the equation of the straight line, which were 0.0133 and 0.0097, respectively (see Figures 9 and 11). It is noteworthy that iron phthalocyanine (FePc) exhibits a higher value of the equation constant of the line (0.0282, Figure 10), which is to be expected, particularly when considering the structural similarity of this molecule with the porphyrin of the heme group. In consideration of the data presented in Figures 6 to 11, the photohemolytic trend may be summarized as follows: The order of the photohemolytic activity of the phthalocyanines is as follows: ZnPc > FePc > NiPc ~ CuPc > CoPc > MnPc.

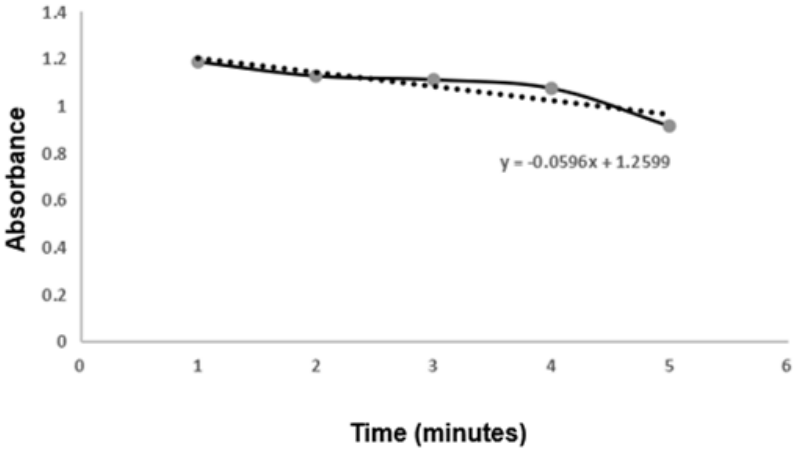

Figure 6. Photohemolysis induced by ZnPc.

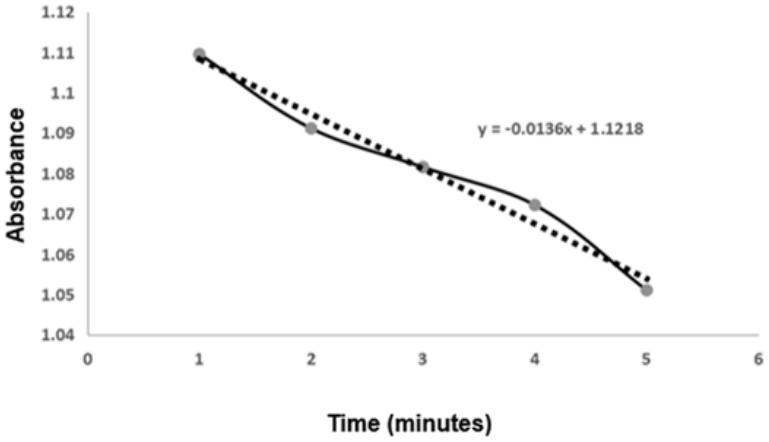

Figure 7. Photohemolysis induced by CuPc.

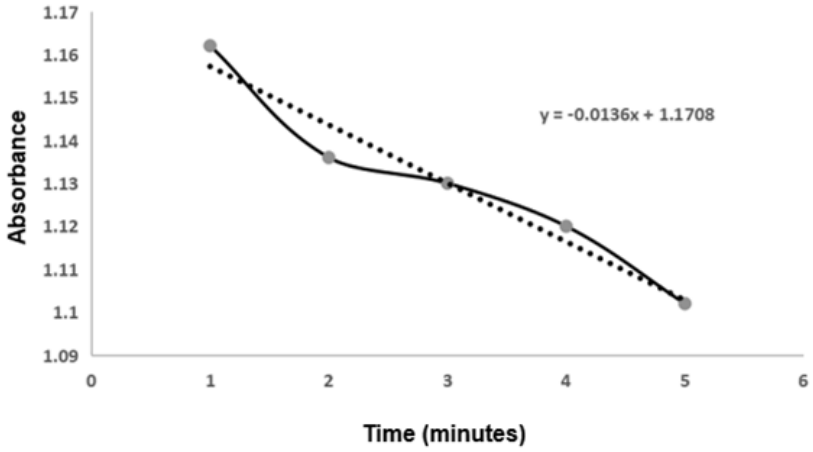

Figure 8. Photohemolysis induced by NiPc.

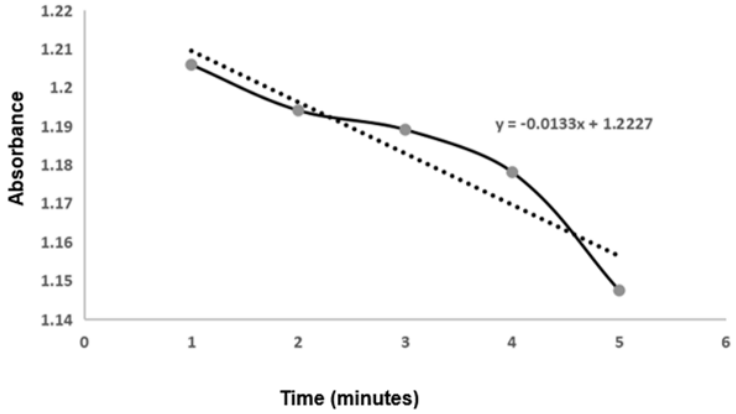

Figure 9. Photohemolysis induced by CoPc.

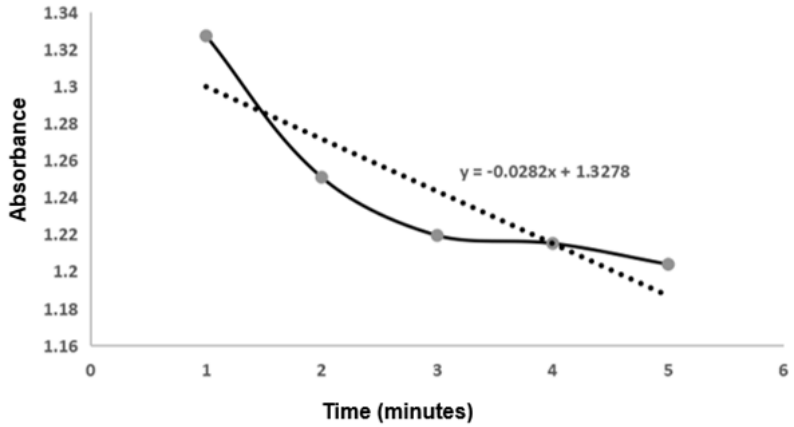

Figure 10. Photohemolysis induced by FePc.

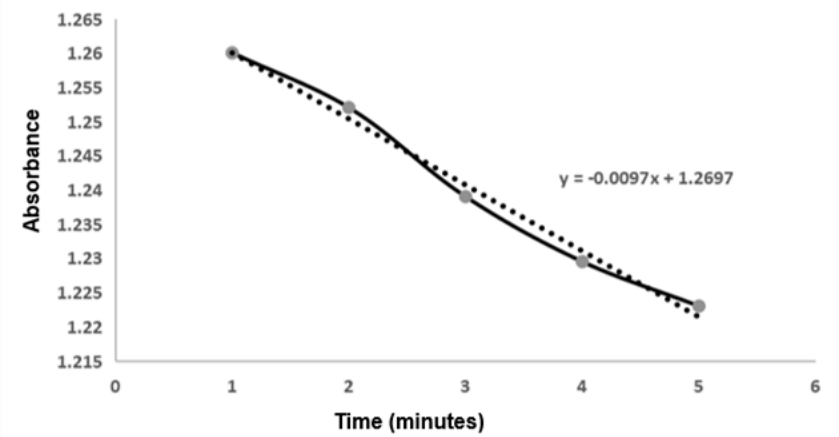

Figure 11. Photohemolysis induced by MnPc.

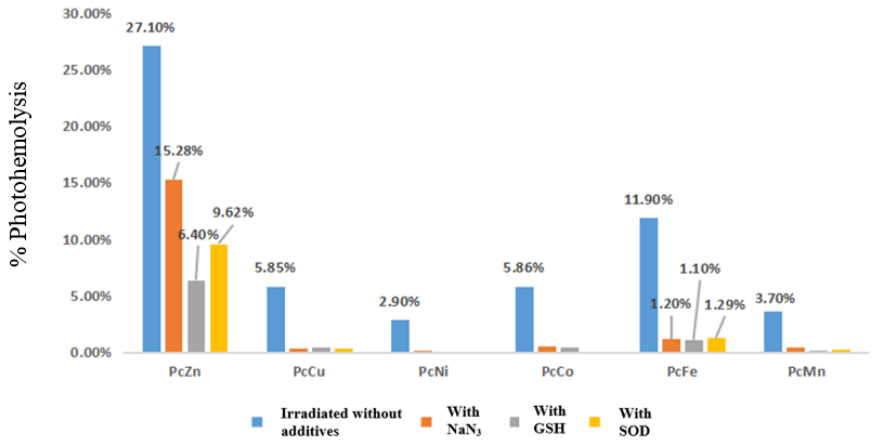

Figure 12. Photohemolysis induced by Zn, Cu, Ni, Co, Fe and Mn phthalocyanines in the presence of NaN_3, GSH and SOD additives.

The phothemolytic activity of the phthalocyanines under investigation can be attributed to two main factors. From a scientific standpoint, the capacity of photosensitive molecules to generate photoinduced free radicals is fundamental to understanding how damage to the membrane of human erythrocytes can lead to the induction of hemolysis. This is evidenced by the following references [17, 18, 20]. It is noteworthy that both type II and type I mechanisms, such as the generation of $^1O_2$ and H_2O_2, demonstrate the greatest capacity to photogenerate reactive oxygen species, with ZnPc exhibiting the most convincing photohemolytic activity. It is postulated that the photohemolytic damage occurs preferentially through the generation of radicals such as $\cdot O_2$ and $\cdot OH$, given that the phthalocyanine molecules of FePc, NiPc, and CuPc are well-established type I photosensitizers [20, 21, 22].

It is also crucial to examine the interaction between phthalocyanine molecules and the membrane of human erythrocytes. Although this is not the primary focus of the current study, it can be postulated that the photohemolysis induced by ZnPc is also attributable to its capacity to generate components of the blood cell membranes. In consideration of the aforementioned details, it can be stated that the photohemolytic activity is contingent on not only the type I and type II phototoxic mechanisms, but also on their behavior in the presence of biological targets [20, 23, 24] since it establishes the catalitic activity of porphyrin-based polymers on biomimetic systems.

Furthermore, it is crucial to understand the underlying mechanisms that facilitate the photohemolytic processes initiated by phthalocyanines. Figure 12 illustrates the extent of photohemolysis induced by phthalocyanines in the presence of additives, namely NaN_3, GSH, and SOD. It is also noteworthy that ZnPc exhibited a higher photohemolytic percentage in the absence of additives, with 27% photoinduced hemolysis.

Moreover, the bar graphs illustrate that ZnPc generated 15.28% photohemolysis in the presence of NaN_3 (an oxygen trapper), and 6.4% and 9.62% photohemolysis in the presence of GSH and SOD (free radical trappers), respectively. These findings suggest that ZnPc induces photoinduced hemolytic damage via the action of type I radicals in a preferential manner. In contrast, it is observed that for CuPc, CoPc, NiPc, and MnPc, the percentages of photohemolysis in the presence of the corresponding additives are markedly low. This indicates that the effects of these molecules on human erythrocytes are likely due to mechanisms that are not covered in this study.

4. Discussion

The findings of this study indicate a clear trend in the photohemolytic capacity of the synthesized phthalocyanine molecules, with ZnPc displaying the most significant potential to induce hemolysis. This is demonstrated by a straight line equation constant of 0.0596. This finding is consistent with previous research, which also indicates that ZnPc has a significant ability to induce photosensitized hemolysis in human erythrocytes. The process of hemolysis is initiated by erythrocyte membrane hyperpolarization, membrane fluidization, and cell swelling, indicating that ZnPc effectively interacts with the erythrocyte membrane, inducing structural transformations that facilitate hemolysis [14].

Moreover, scientific literature indicates that compounds such as porphyrins and other photosensitizers can cause hemolysis by generating reactive oxygen species [3, 15, 18]. This finding corroborates the high activity observed for ZnPc. This phenomenon is caused by reactive oxygen species, such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), which can damage erythrocyte cell membranes, causing them to rupture and release hemoglobin. In particular, porphyrins have been the subject of extensive study with regard to their capacity to induce hemolysis under conditions of irradiation, thereby underscoring the significance of phototoxic mechanisms in the context of photohemolytic activity. The high efficacy of ZnPc in inducing hemolysis can be attributed to its ability to generate reactive oxygen species with high efficiency, which positions it as one of the most potent photosensitizers in this context.

The literature also indicates that phthalocyanines, like porphyrins, can integrate into the lipid bilayer of the cell membrane, facilitating the localized generation of reactive oxygen species and thereby increasing cell damage. This mechanism of action is particularly relevant for ZnPc, which not only generates a significant amount of reactive oxygen species but also exhibits a remarkable affinity for cell membranes, thereby amplifying its hemolytic effect.

Conversely, the findings on the photohemolytic potential of the synthesised phthalocyanines exhibit a discernible correlation with the central metal. For example, zinc phthalocyanine (ZnPc) displays the highest capacity to induce hemolysis, as evidenced by the consistent value of the straight line equation (0.0596), while copper (CuPc) and nickel (NiPc) phthalocyanines exhibit lower values (0.0136).

In this regard, and in comparison with the results of research on AlPCS-induced photohemolysis [17], they also indicate a significant efficiency in inducing hemolysis, although not linear as a function of the concentration of the photosensitizing compound. The efficiency of AlPCS photohemolysis is not affected by the presence of D_2O or glycerol, indicating that neither singlet oxygen nor hydroxyl radicals are involved in the process of membrane damage. This discrepancy in the mechanism of action may be attributed to the magnetic properties of the phthalocyanine molecules. For instance, zinc phthalocyanine (ZnPc), a diamagnetic molecule, demonstrates high efficiency in generating reactive oxygen species. In contrast, aluminum phthalocyanine (AlPCS) may induce hemolysis through disparate mechanisms.

It is important to note that ZnPc exhibits a greater photohemolytic capacity than other porphyrins, including NiPc, CoPc, FePc, CuPc, and MnPc. This is attributed to its strong interaction with the human erythrocyte membrane. The low photohemolytic capacity of the latter compounds may be attributed to their limited ability to interact with the human blood cell membrane. Moreover, the photohemolytic activity of ZnPc is predominantly mediated by the Type I phototoxic mechanism, which involves the generation of free radicals rather than singlet oxygen. The photohemolytic activity of PcZn was found to be significantly reduced when samples were irradiated in the presence of free radical inhibitors SOD and GSH, in comparison to NaN_3. This suggests that the damaging effect on human erythrocyte membranes is less dependent on singlet oxygen.

Ultimately, it is demonstrated that diamagnetic molecules, such as ZnPc, possess excited state lifetimes that are conducive to the generation of both type I and type II phototoxic mechanisms. This is a crucial prerequisite for the advancement of efficacious photosensitive molecules for biomedical applications. Indeed, numerous studies have proposed porphyrin-derived compounds linked to metals such as zinc as

optimal candidates for enhancing photodynamic therapy [20, 25].

5. Conclusion

Zinc phthalocyanine (ZnPc) demonstrated a markedly enhanced photohemolytic impact on human red blood cells relative to other phthalocyanines, including nickel phthalocyanine (NiPc), cobalt phthalocyanine (CoPc), iron phthalocyanine (FePc), copper phthalocyanine (CuPc), and manganese phthalocyanine (MnPc). This was attributed to the superior ability of ZnPc to interact with the erythrocyte membrane, thereby triggering cell damage and free radical generation (type I mechanism), which ultimately leads to hemolysis. Moreover, experiments employing free radical scavengers indicate that singlet oxygen plays a comparatively minor role in ZnPc-induced hemolysis, with free radicals exerting a more pronounced effect. These findings underline the significance of membrane interaction in phthalocyanine phototoxicity, indicating that PcZn may be a valuable subject for further investigation in photodynamic therapy, potentially due to its effective type I phototoxic mechanism.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

[1] Zhang Y, Lovell JF. Recent applications of phthalocyanines and naphthalocyanines for imaging and therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2017;9(1):10.1002/wnan.1420. [DOI: 10.1002/wnan.1420] [PMID] [PMCID]

[2] Li X, Peng XH, Zheng BD, Tang J, Zhao Y, Zheng BY, Ke MR, Huang JD. New application of phthalocyanine molecules: from photodynamic therapy to photothermal therapy by means of structural regulation rather than formation of aggregates. Chem Sci. 2018;9(8):2098-04. [DOI: 10.1039/c7sc05115h] [PMID] [PMCID]

[3] Kahn G, Fleischaker B. I. Red blood cell hemolysis by photosensitizing compounds. Journal of Investigative Dermatology. 1971;56(2):85-90. [DOI: 10.1111/1523-1747.ep12260639]

[4] Chidawanyika W, Ogunsipe A, Nyokong T. Syntheses and photophysics of new phthalocyanine derivatives of zinc, cadmium and mercury .New J Chem. 2007;31(3):377-84. [DOI:10.1039/B615663K]

[5] Kluson P, Drobek M, Strasak T, Krysa J, Karaskova M, Rakusan J. Sulphonated phthalocyanines as effective oxidation photocatalysts for visible and UV light regions. J Mol Catal A Chem. 2007;272(1-2):213-9. [DOI: 10.1016/j.molcata.2007.03.024]

[6] Fernandez L, Esteves VI, Cunha A, Schneider RJ, Tome JP. Photodegradation of organic pollutants in water by immobilized porphyrins and phthalocyanines. J Porphyrins Phthalocyanines. 2016;20(01n04):150-66. [DOI: 10.1142/S108842461630007X]

[7] Liu Y, Qin R, Zaat SAJ, Breukink E, Heger M. Antibacterial photodynamic therapy: overview of a promising approach to fight antibiotic-resistant bacterial infections. J Clin Transl Res. 2015;1(3):140-67. [PMID] [PMCID]

[8] Agirtas S, Ion RM, Bekaroglu O. Spectral study of the supramolecular assemblies porphyrins–phthalocyanines. Materials Science and Engineering: C. 1999;7(2):105-10. [DOI: 10.1016/S0928-4931(99)00125-3]

[9] Ion RM, Yilmaz I, Bekaroglu O. Supramolecular assemblies of pyridyl porphyrin and diazadithia phthalocyanine. J Serb Chem Soc. 1999;64(7-8):453-62. [DOI: 10.2298/JSC9908453I]

[10] Yeber MC, García G. Photocatalytic degradation of

Kraft Lignin using the S2O82−/Fe0/UV process: optimization with multivariate analysis. Desalination and Water Treatment. 2015;56(7):1793-801. [DOI: 10.1080/19443994.2014.952668]

[11] Chernonosov AA, Koval VV, Knorre DG, Chernenko AA, Derkacheva VM, Lukyanets EA, Fedorova OS. Conjugates of phthalocyanines with oligonucleotides as reagents for sensitized or catalytic DNA modification. Bioinorg Chem Appl. 2006;2006:63703. [DOI: 10.1155/BCA/2006/63703] [PMID] [PMCID]

[12] Ishii K. Functional singlet oxygen generators based on phthalocyanines. Coordination Chemistry Reviews. 2012;256(15-16):1556-68. [DOI:10.1016/j.ccr.2012.03.022]

[13] Ağırtaş MS, Cabir B, Yıldıko Ü, Özdemir S, Gonca S. Synthesis, antioxidant, DNA cleavage and antimicrobial properties of phthalocyanine complexes bearing the poly-hydroxyl groups. Chem Pap. 2021;75:1749-60. [DOI:10.1007/s11696-020-01432-7]

[14] Zavodnik IB, Zavodnik LB, Bryszewska MJ. The mechanism of Zn-phthalocyanine photosensitized lysis of human erythrocytes. J Photochem Photobiol B. 2002;67(1):1-10. [DOI: 10.1016/s1011-1344(02)00246-4] [PMID]

[15] De Guidi G, Chillemi R, Costanzo LL, Giuffrida S, Condorelli G. Molecular mechanism of drug photosensitization. 4. Photohemolysis sensitized by carprofen. J Photochem Photobiol B. 1993;17(3):239-46. [DOI: 10.1016/1011-1344(93)80021-z] [PMID]

[16] León M, Vargas F. Preliminary research on metalized phthalocyanines synthesis. Evaluation as potential photosensitizer in photodynamic therapy. Rev Colomb Cienc Quím Farm 2022;51(1):348-68. [DOI: 10.15446/rcciquifa.v51n1.102717]

[17] Ben-Hur E, Rosenthal I. Photohemolysis of human erythrocytes induced by aluminum phthalocyanine tetrasulfonate. Cancer Lett. 1986;30(3):321-7. [DOI: 10.1016/0304-3835(86)90057-1] [PMID]

[18] Costanzo LL, De Guidi G, Condorelli G, Cambria A, Fama M. Molecular mechanism of drug photosensitization--II. Photohemolysis sensitized by ketoprofen. Photochem Photobiol. 1989;50(3):359-65. [DOI: 10.1111/j.1751-1097.1989.tb04170.x] [PMID]

[19] Staicu A, Pascu A, Nuta A, Sorescu A, Raditoiu V, Pascu ML. Studies about phthalocyanine photosensitizers to be used in photodynamic therapy. Romanian Reports in Physics. 2013;65(3):1032-51. [Link]

[20] Pereira GF, Tasso TT. From cuvette to cells: How the central metal ion modulates the properties of phthalocyanines and porphyrazines as photosensitizers. Inorganica Chim.Acta.2021; 519:120271. [DOI: 10.1016/j.ica.2021.120271]

[21] Shi-Kang W, Hou-Chen Z, Guo-Zhu C, Da-Nian X, Hui-Jun X. A study on the ability of some phthalocyanine compounds for photogenerating singlet oxygen. Acta Chim Sin. 1985;3(1): 21-5. [DOI:10.1002/cjoc.19850030105]

[22] Neagu M, Constantin C, Tampa M, Matei C, Lupu A, Manole E, Ion RM, Fenga C, Tsatsakis AM. Toxicological and efficacy assessment of post-transition metal (Indium) phthalocyanine for photodynamic therapy in neuroblastoma. Oncotarget. 2016;7(43):69718-732. [DOI: 10.18632/oncotarget.11942] [PMID] [PMCID]

[23] Dahl TA, Midden WR, Hartman PE. Pure singlet oxygen cytotoxicity for bacteria. Photochem Photobiol. 1987;46(3):345-52. [DOI: 10.1111/j.1751-1097.1987.tb04779.x] [PMID]

[24] Mackintosh HJ, Budd PM, McKeown NB. Catalysis by microporous phthalocyanine and porphyrin network polymers. J Mater Chem. 2008;18(5):573-8. [DOI: 10.1039/B715660J]

[25] Vargas F, León M, Álvarez Á, Dasilva S, Maldonado A. Photochemical and Photobiological Activity of Zn, Cu, Ni, Co, Fe and Mn Unsubstituted Phthalocyanines. Chem Proc. 2023;14(1):27. [DOI: 10.3390/ecsoc-27-16171]