



## Effects of protonation degree on photodynamic activity of zinc phthalocyanine substituted with 1,2-diethylamino



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### ABSTRACT

This work reports on the effects of protonation degree on the photodynamic activity of zinc phthalocyanine substituted with 1,2-diethylamino (**ZnPc1**). Photophysical properties, singlet oxygen generation ability and photodynamic anticancer activity of derivatives of **ZnPc1** with different protonation degree were studied and compared. Results indicated that the protonation degree can greatly influence the photophysical and photochemical properties of **ZnPc1**, thereby affecting its photodynamic activity. When the mole ratio of **ZnPc1** and hydrochloric acid (HCl) was 1:8, the derivative of **ZnPc1** showed the best photodynamic activity.

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Photodynamic therapy (PDT) is a noninvasive technique for the treatment of various cancers [1,2]. It involves the combined use of three separately nontoxic components, namely, photosensitizers (PSs), molecular oxygen and low energy light. Upon activation by light with specific wavelength, PSs can transfer energy to neighboring oxygen molecules [3]. Then, singlet oxygen ( $^1\text{O}_2$ ) which is considered as the major cytotoxic reactive oxygen species (ROSs) in PDT generates and results in cancer cell death by both apoptosis and necrosis [4,5].

PS is the determining factor in the therapeutic outcome of the PDT [6]. Pcs, especially zinc phthalocyanines (ZnPcs), as efficient phototherapeutic agents, have been receiving intensive attention over the past decades, because of their rather intense long-wavelength absorption band and high  $^1\text{O}_2$  generating efficiency [7,8]. Besides, Pcs substituted with amino groups have been reported as promising PS due to their enhanced  $^1\text{O}_2$  generating efficiency and anticancer activity [9]. However, poor solubility in water limits their application in PDT [10].

We have reported the comparison of two strategies for conferring water solubility to a zinc Pc substituted with 1,2-diethylamino (**ZnPc1**) [11]. The results suggested that preparing hydrochloride derivatives is an easy way to improve the water solubility and PDT activity of amino substituted Pcs. It is well known that quaternization degree can influence the solubility, aggregation tendency and absorption of the PSs, finally affecting their photodynamic activities [12,13]. With that, we think that the protonation degree of amino substituted PSs might also be closely related to their photodynamic activities. In this paper, we extend our

research to the relationship between protonation degree and photodynamic activity of **ZnPc1**. It is expected that the protonation degree would influence the hydrophilicity and aggregation of **ZnPc1** and the pH of the system, therefore resulting in the change of photodynamic activity. Here, the derivatives of **ZnPc1** with different protonation degree were prepared, and their absorption properties and photodynamic activities were studied and compared.

**ZnPc1** was prepared in our laboratory according to the literature (Fig. S1) [11]. The different protonation degree derivatives of **ZnPc1** were prepared according to the following procedures: firstly, a fixed concentration of **ZnPc1** stock solution was prepared; then the corresponding equivalent HCl was added to the above solution for preparing the stock solution of protonated derivatives of **ZnPc1**; the stock solutions were diluted to the final concentrations with water for the following experiments. By adjusting the amount of HCl, the different protonation degree derivatives of **ZnPc1** were acquired.

The pH values of derivatives of **ZnPc1** with different protonation degree in water were measured by a pH meter (Fig. S2). The result shows that when the mole ratio of HCl and **ZnPc1** is less than 8:1, the pH values have only tiny changes. At this stage, it is likely that HCl was mainly used to protonate amino groups. When the mole ratio of HCl and **ZnPc1** is more than 8:1, the pH values change obviously. This may be due to that the amino groups of **ZnPc1** were fully protonated, and the excess HCl would reduce the pH values of the system at this stage.

Electronic spectra are particularly fruitful to establish the structure and existing form of the Pc [14,15]. The changes in UV–Vis spectrum of derivatives of **ZnPc1** with different protonation degree in water are gathered in Fig. 1. All of the derivatives are typical aggregate Pcs because

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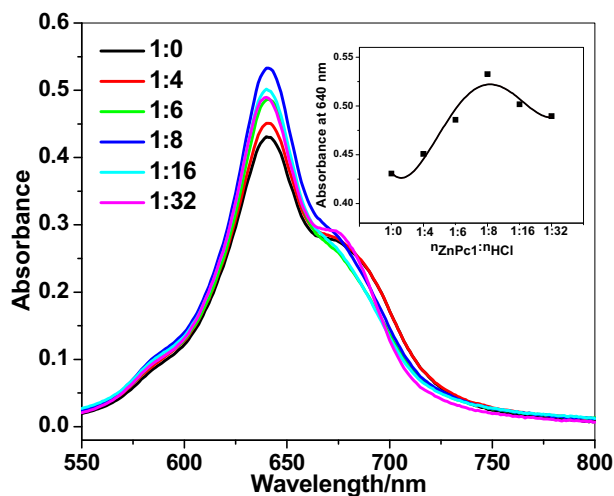


Fig. 1. The UV-Vis absorption spectra derivatives of **ZnPc1** with different protonation degree in water ( $C = 5 \times 10^{-6}$  M). (Inset: change curve of absorption intensity at 640 nm).

of the appearance of the additional Q band at 640 nm [16]. It can be seen that though the absorption spectra shapes of these compounds are similar, the absorption intensity changes obviously. As shown in the illustration, the absorption intensity at 640 nm increases when the protonation degree is increased. This can be attributed to the improvement of water solubility with increasing degree of protonation. When the mole ratio of HCl and **ZnPc1** is more than 8:1, at which mole ratio **ZnPc1** is fully protonated, excess HCl addition would reduce the absorption intensity which can be attributed to the reduction of pH [17].

Fluorescence properties of Pcs are particularly important in PDT [18, 19]. Upon excitation at 610 nm in **ZnPc1**, there was almost no fluorescence. This result may be due to the presence of amino groups, which quench the singlet excited state by intramolecular photoinduced electron transfer (PET) [20]. Though the aggregate Pcs are not known to fluorescence [21], with the additional of HCl, the fluorescence emission intensity of **ZnPc1** increased obviously (Fig. 2). This can be attributed to the protonation of amino groups of **ZnPc1**, resulting in the inhibition of PET process [20] and the increase of fluorescence intensity. Finally, the fluorescence emission spectra of these compounds in water were the competition interactions between protonation and aggregation. Furthermore, when the mole ratio of HCl and **ZnPc1** is more than 8:1, the fluorescence intensity increases as always. This is consistent with the

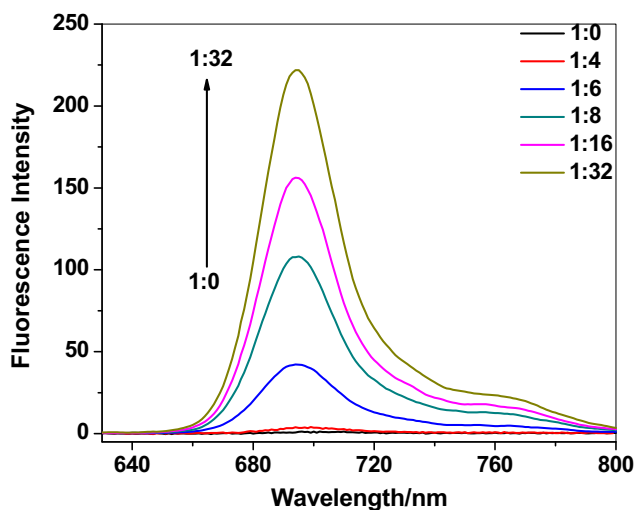


Fig. 2. The fluorescence emission spectra of derivatives of **ZnPc1** with different protonation degree ( $\text{Ex} = 610$  nm).

pH values. In general, the fluorescence intensity increases as pH value decreases [20].

As the absorption spectra and fluorescence emission spectra of **ZnPc1** are closely related to its protonation degree, we believe that its singlet oxygen generation would also be influenced by the protonation. The  $^1\text{O}_2$  generation ability of the compounds was monitored using the molecular probe disodium ADPA [22]. The net loss of ADPA absorption at 378 nm was measured and recorded over time after irradiation by 665 nm LED light. As shown in Fig. 3A ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:8$  as an example), the compounds can induce photo-oxidation of ADPA and the absorption intensity of ADPA continued to decrease as the irradiation time increases. As shown in Fig. 3B, the  $^1\text{O}_2$  generation ability of the compounds varies by the protonation degree. The order of the  $^1\text{O}_2$  generation rates is ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:8, k = 11.01 \times 10^{-3}$ ) > ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:6, k = 10.56 \times 10^{-3}$ ) > ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:4, k = 10.18 \times 10^{-3}$ ) > ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:16, k = 9.73 \times 10^{-3}$ ) > ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:32, k = 9.05 \times 10^{-3}$ ) > ( $n_{\text{ZnPc1}}:n_{\text{HCl}} = 1:0, k = 5.8 \times 10^{-3}$ ). The best  $^1\text{O}_2$  generation rate was obtained when the mole ratio of HCl and **ZnPc1** is 8:1, which is in accordance with the absorption spectra results. It is believed that the amino groups of **ZnPc1** are fully protonated, which enhance the absorption intensity and inhibit PET process, resulting in the high  $^1\text{O}_2$  generation rate. When the mole ratio of HCl and **ZnPc1** is more than 8:1, since the amino groups

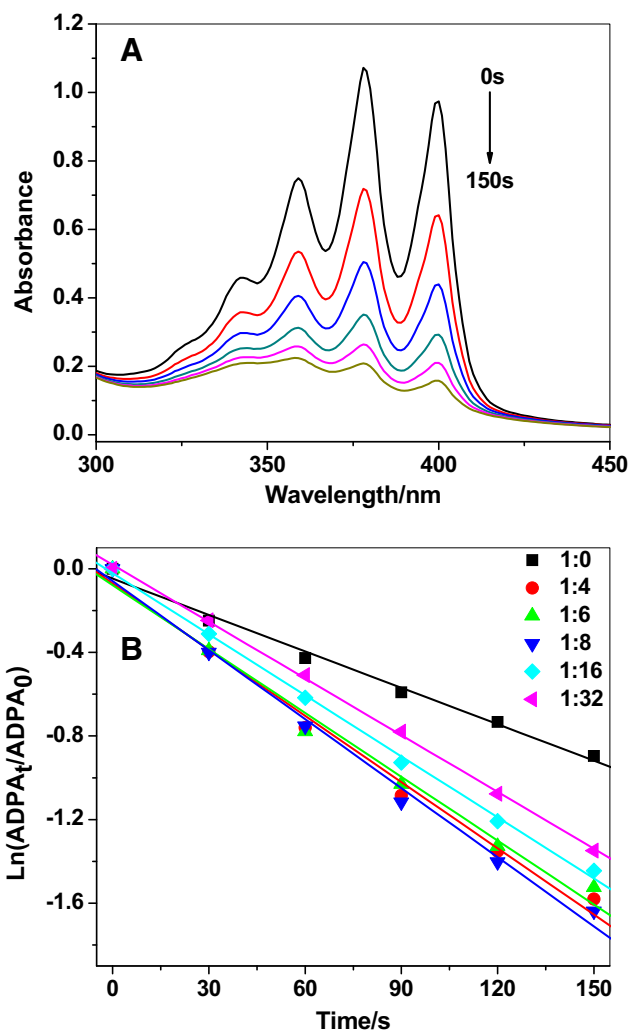


Fig. 3. (A) UV-Vis spectrum for the determination of the  $^1\text{O}_2$  production rate of drugs use ADPA as quencher in water ( $C = 5 \times 10^{-6}$  M, mole ratio of **ZnPc1**:HCl = 1:8 as an example); (B) first order plots of ADPA absorbance versus time.

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