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A Study on Unsubstituted Cu(II) Phthalocyanine and Bovine Serum Albumin Bioconjugation

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Abstract

The ground state electronic and fluorescence spectra of unsubstituted copper (II) phthalocyanine (CuPc) have been studied in the presence of bovine serum albumin (BSA) in water as a solvent. The effect of sodium dodecyl sulfate (SDS) on the solution properties of CuPc: BSA bioconjugate has also been investigated. FT-IR, UV-Vis, and fluorescence analysis have been carried to evaluate the BSA: CuPc bioconjugation. The optimum bioconjugate ratio of BSA: CuPc has been studied via UV-Vis and fluorescence spectral techniques. The collaborative effect of SDS with BSA on the aggregation of CuPc suspension has also been studied in terms of UV-Vis, fluorescence, and FT–IR analysis.

1. Introduction

As a member of aromatic macrocycle compounds based on a delocalized 18- π electron system, phthalocyanines (Pcs) exhibit very attractive chemical and physical properties in many fields [1–4]. Unique properties as high thermal stability, planarity, and symmetry encourage Pcs to use in many application fields as dyes, photodynamic therapy (PDT), semiconductors, Langmuir–Blodgett films, non-linear optics, liquid crystals, catalyst, information storage systems among others [5–11].

Besides their excellent chemical and physical properties, unique electronical and optical properties promote Pcs utilization in many other fields. Phthalocyanines exhibit characteristic Q (600 – 750 nm) ascribed to the $\pi \Rightarrow \pi^*$ transitions from the HOMO to the LUMO of the Pc^{2-} core, and B (300-400 nm) bands from the deeper $\pi \Rightarrow \pi^*$ transitions, respectively in UV-Vis spectrum. Despite all these properties, their poor solubility in water and aggregation restricts the efficiency of Pc applications [12–14].

Usually, spectroscopic techniques are utilized to understand the aggregation phenomenon of the phthalocyanines [15]. The two characteristic peak areas, Q- and B-band (Soret band) areas, dominate the UV-Vis spectra of the phthalocyanines [16]. The main characteristic Q-band peaks in the UV-Vis spectrum can be attributed to the free phthalocyanine molecules or stacked molecules within the phthalocyanine aggregates. Therefore, the degree and the nature of the aggregation in a specific solution can be deduced by examining the relevant peak intensities [17,18]. The overlapping between the π-systems of phthalocyanine molecules leads to Pc aggregates, namely dimeric or oligomeric Pc complexes, resulting in a peak at around 620–630 nm and usually a blue shift in the UV-Vis spectrum [17, 19–21].

Unsubstituted phthalocyanines are known to be poorly soluble in water and in most of the common organic solvents, strongly influencing the bioavailability, singlet oxygen production efficiency, and in vivo distribution [8,

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22–24]. The planar nature of unsubstituted phthalocyanines with extended π electron density stimulates the aggregation property arising from the π⇒π* interactions between Pcs molecules [19, 25–29].

Having both hydrophilic and hydrophobic sites, protein molecules may be introduced to phthalocyanine molecules to diminish aggregation behavior in solution [30–32]. Serum albumins, as bovine serum albumin (BSA) and human serum albumin (HSA), are known to be the most abundant plasma protein in blood [33]. Serum albumins act as carriers for many molecules like bilirubin, fatty acids, and hemin in plasma [34,35]. Having both hydrophilic and hydrophobic sites and being easy to handle makes BSA a good candidate to investigate the effect of the BSA and the water-soluble phthalocyanine molecules interaction in photodynamic therapy (PDT) [36,37]. There have been several studies about the effect of BSA protein on PDT of Pc molecules after binding or encapsulation processes [37–41]. However, there are limited investigations in the literature on the effect of the interaction of unsubstituted phthalocyanines and BSA on the aggregation of phthalocyanines, except for the study of Larroquel, et al., in which unsubstituted zinc (II) phthalocyanine and BSA coordination are studied [42]. We have previously studied the dispersion effect of degraded cellulase enzyme on copper (II) phthalocyanine pigment [43]. Here in this work, best of our knowledge, the non-covalent binding of BSA protein to unsubstituted CuPc has been investigated for the first time in the literature. We also studied the effect of the increase of BSA concentration and the effect of SDS as a low molecular weight dispersant together with BSA on CuPc in terms of UV-Vis and fluorescence spectra of CuPc.

2. Materials and Methods

2.1 Materials

Bovine serum albumin, Cu (II) phthalocyanine, and sodium dodecyl sulfate were supplied from Sigma-Aldrich. In this work, purchased chemicals were used as received and solvents used after the purification process according to the literature [44].

2.2. Equipment

The UV-Vis spectra of the bioconjugates were recorded on A T80+ UV/VIS Spectrometer in a 1 cm pathlength quartz cell between 190-900 nm in water as a solvent. Agilent Cary Eclipse spectrophotometer was used to determine fluorescence spectra of the bioconjugate solutions via 1 cm pathlength quartz cuvettes at room temperature in water. The FT-IR spectra of the samples were studied with Shimadzu FTIR-8201 PC in the spectral range of 4000–400 cm^{-1}. KBr pellets with a 1:1000 mass ratio KBr: the sample was prepared to record the transmission. The sonication of the bioconjugate solutions was performed with QSonica, Q500 Sonicator equipped with a standard needle titanium probe (1/2 inch) submerged approximately 5 mm into the solutions. The sonication process was carried out at 20 kHz. All samples were sonicated three times for 20 seconds at 10-second intervals.

2.3. Methods

Stock BSA solutions were prepared at 2.0 mg/mL using the buffer pH 7.4, as physiological pH (adjusted with 0.2 M Na_2HPO_4). CuPc-BSA bioconjugations were prepared as follows: BSA solutions at 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1.0 mg/L concentrations using the stock BSA solution and 2 mg/L (~$3.5x10^{-6}$ M) CuPc compound was added to each one. The suspensions were stirred for 20 mins with a magnetic stirrer before the homogenization using an ultrasonic apparatus. Ultrasonication was performed in a 20 s duty cycle, with 10 s of rest and 10 s generator acting.

3. Results and Discussion

3.1. FT-IR Spectroscopy

FT-IR spectrum was conducted to investigate the interaction between unsubstituted CuPc and BSA. As depicted in Figure 1, unsubstituted CuPc showed characteristic peaks at 1620 and 1592 cm^{-1} respectively attributed to the vibration bands of aromatic –C=N–, –C=C– and –C–H peaks of the phthalocyanine core ring [45]. In-plane and out plane vibration peaks arising from the bending were appeared between 725–877 cm^{-1} and 1000–1180 cm^{-1} as expected [46,47]. After the bioconjugation process, two new peaks have appeared at 2972 and 3214 cm^{-1} attributed to the -C-H vibration and to the primary amine, respectively. In addition to the bands corresponding to CuPc peaks with slight shifts, the characteristic amide I band and amide II bands of BSA at 1647 cm^{-1} and 1574 cm^{-1}, and the –NH and $–NH_2$ vibration band at around 700 cm^{-1} were also detected in the FT-IR spectrum [47–49]. It can be said from Figure 1 that BSA has been successfully adsorbed on CuPc molecules according to the FT-IR spectrum.

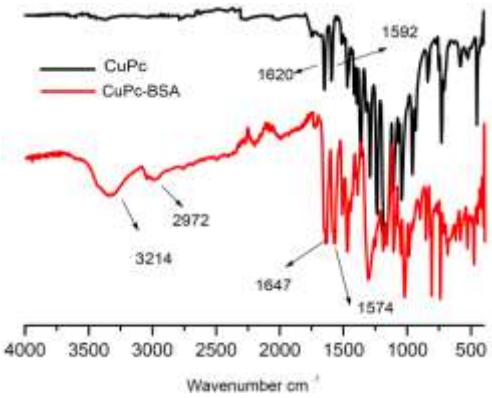

Figure 1. FT-IR spectra of CuPc and CuPc-BSA bioconjugate.

3.2. Ground State Electronic Spectroscopy

UV-Vis spectrum of CuPc is given in Figure 2. The characteristic Q-band peaks attributed to the $\pi \rightarrow \pi^*$ transitions of metallophthalocyanines can be clearly seen in the UV-Vis spectrum of CuPc at 730 and 642 nm and the Soret band at 345 nm in water [14, 51,52]. The narrow peak at 730 nm is attributed to the monomeric CuPc species and the peak at 642 nm is assigned to the aggregated CuPc molecules in water [53]. The bands at around 259 and 232 nm in Figure 2 correspond to the other aromatic sites and bis-triazine groups of the CuPc [54].

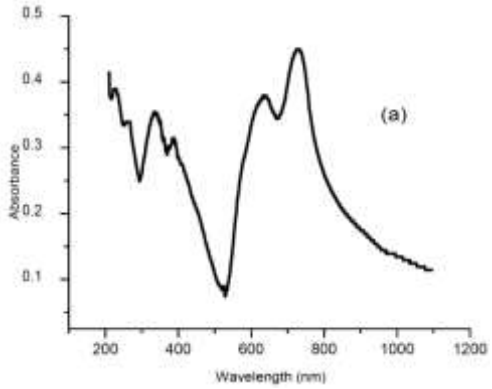

Figure 2. UV-Vis spectrum of CuPc in water (C:~3.5x10^{-6} M)

The UV-Vis spectra of CuPc after bioconjugation with BSA together with that of CuPcs' is depicted in Figure 3. The decrease in the Q- and B-bands peak intensities in the UV-Vis spectrum may be due to not only to the dilution effect as expected but also to the non-covalent interaction of CuPc and BSA molecules. Furthermore, the peak at 232 nm has been lost in Figure 3 owing to the bioconjugation [43].

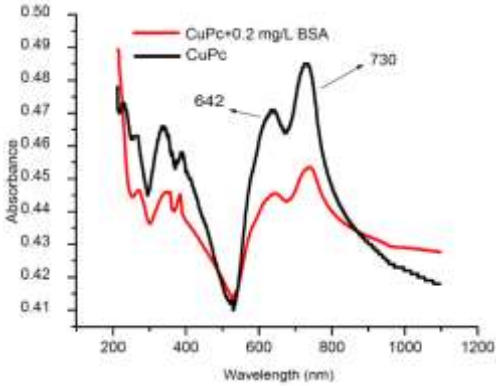

Figure 3. UV-Vis spectra of CuPc and CuPc–0.2 mg/L BSA bioconjugate

In Figure 4 and 5, the UV-Vis spectra of CuPc and various CuPc-BSA bioconjugate concentrations are given in different wavelength ranges. BSA protein exhibits two characteristic bands in the UV-Vis spectrum at around 220 nm and 280 nm due to the α-helix structure and amino acid residues, respectively [55]. According to Figure 4, there is a decrease in the Q-band intensities with the increase of BSA concentration in the bioconjugate until CuPc:0.6 mg/L BSA ratio. After the mentioned concentration ratio, an increase has been observed with the increase of BSA amount. This may be the result of the non-covalent binding of BSA molecules to unsubstituted CuPc molecules. The anionic, cationic, hydrophobic, and hydrophilic nature of proteins arises from the carboxyl, amino and methyl functional groups of the protein molecule. Hence the interactions of proteins with ligands are induced by hydrogen bonding, hydrophobic interactions, electrostatic interactions, and van der Waals forces, meanly non-covalent interactions [56,57]. The supramolecular approach can be used to explain the non-covalent interaction between phthalocyanines and protein molecules such as BSA, HSA and lipoproteins [35, 58,59]. As it is well-known that phthalocyanines are hydrophobic molecules [60,61], the interaction between CuPc and BSA may be attributed to the non-covalent binding [42,62]. At higher concentrations as 0,8 mg/L and 1,0 mg/L BSA, the Q-band absorbance was higher as can be seen in Figure 4. As the Q-band absorbance arises from the $\pi \Rightarrow \pi^*$ transitions of phthalocyanine molecules [12], this behaviour may be attributed to the lack of protein molecules that can form BSA-CuPc bioconjugate after this concentration ratio and the increase of CuPc molecules.

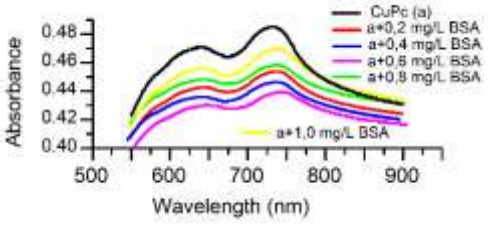

Figure 4. UV-Vis spectra of CuPc and CuPc–BSA bioconjugate at various concentrations between 500-900 nm

Moreover, similar behavior has also been detected between 220-300 nm in Figure 5. As depicted in Figure 5, the bands at around 269 and 233 nm disappeared with the addition of BSA protein as a result of bioconjugation. According to both Figure 4 and Figure 5, with 0.6 mg/L BSA addition, the optimal bioconjugate composition is achieved in our working conditions.

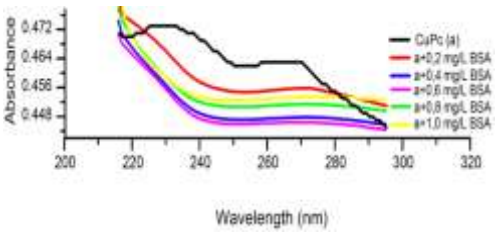

Figure 5. UV-Vis spectra of CuPc and CuPc–BSA bioconjugate at various concentrations between 200-320 nm

3.3. Fluorescence Spectroscopy

Fluorescence spectroscopy is an effective technique to evaluate the structural alterations of proteins due to the susceptible structure of aromatic amino acid, i.e. tyrosine, tryptophan, and phenylalanine, residues to the polarity of microenvironments during the excitation [63,64]. The fluorescence spectrum of CuPc and CuPc-BSA bioconjugates is given in Figure 6. The fluorescence intensity of CuPc and CuPc-BSA bioconjugates increased with the increase of BSA concentration in the bioconjugate due to the increase of aromatic amino acid species. It can be said from Figure 6 that, at CuPc-0.2 mg/L BSA bioconjugate concentration, the surface of unsubstituted CuPc has been covered by BSA molecules. However, there is no red or blue shift was observed at the maxima of the emission bands. It is known that the shifts of emission bands emerge from the changes in the hydrophobicity of the micro-environment around tryptophan residues [64]. As can be seen in Figure 6 emission maxima of the spectra showed a regular increase with the increase of BSA ratio without any shifts, suggesting that there is no change in the local dielectric environment of BSA [65].

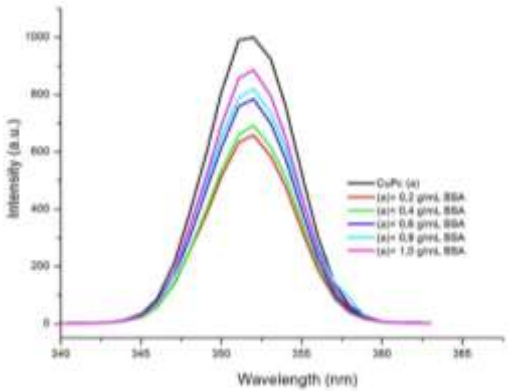

Figure 6. Fluorescence emission spectra of CuPc and CuPc-BSA bioconjugate solutions (excitation wavelength 272 nm)

3.4. SDS Effect on the Bioconjugation of CuPc and BSA Protein

The presence of an ionic detergent can affect the binding and denaturation capacity of proteins [66]. As a surfactant SDS strongly interacts with BSA and induces the proteins partial unfolding [67]. This interaction can be identified by the changes in UV-Vis and fluorescence spectra [68]. First of all, in order to understand the synergistic effect of SDS on the non-covalent binding of BSA to the CuPc molecule, UV-Vis spectra of a series of BSA: SDS mass ratios between 10:0 and 0:10 studied and the favorable mass ratio of BSA to SDS was found to be 8:2. Figure 7 shows the UV-Vis spectra of CuPc, CuPc-BSA, and CuPc-BSA-SDS bioconjugation system. As can be seen from Figure 7, the peak at 272 nm, attributed to the aromatic residues, blue-shifted 2 nm indicating the binding of SDS and BSA molecules.

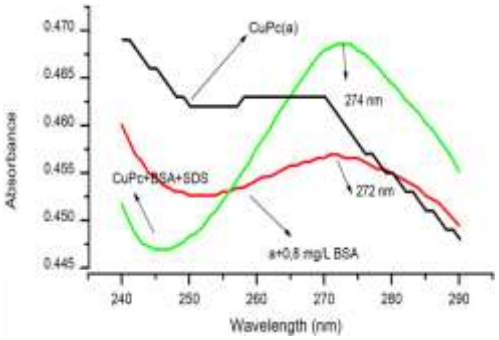

Figure 7. UV-Vis spectra of CuPc, CuPc-BSA and CuPc-BSA-SDS bioconjugation system

The fluorescence spectra of CuPc, CuPc-BSA and CuPc-BSA-SDS bioconjugation system is given in Figure 8. There was an increase in the fluorescence intensity with SDS addition as contrary to the expectation. This behavior may be the result of the increased interaction of CuPC with BSA in presence of SDS [69].

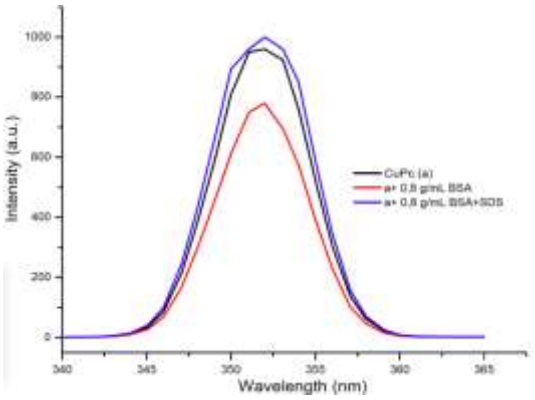

Figure 8. Fluorescence emission spectra of CuPc –CuPc–BSA and CuPc–BSA–SDS bioconjugatio n system (excitation wavelength 272 nm).

4. Conclusions

We have studied the bioconjugation of unsubstituted CuPc and BSA protein in this work. Bioconjugation was investigated via spectroscopic methods. Non-covalent interaction of CuPc and BSA was observed according to the FT-IR spectrum. The nature of the non-covalent interaction was evaluated using UV-Vis and fluorescence spectroscopy and the optimum BSA concentration for a stable bioconjugation is found to be 0.6 g/mL. SDS effect on bioconjugation of CuPc and BSA as a low molecular weight dispersant is also studied and 8:2 BSA: SDS mass ratio is found to be the most effective ratio. According to UV-Vis and fluorescence spectra SDS increased the non-covalent interaction of the CuPc-BSA bioconjugation system.

Declaration of Ethical Standards

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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