ORIGINAL RESEARCH



Synthesis and evaluation of antibacterial activity of water-soluble copper, nickel and zinc tetra (*n*-carbonylacrylic) aminephthalocyanines

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Abstract Water-soluble copper, nickel, and zinc tetra (*n*-carbonylacrylic) aminephthalocyanines were synthesized with an aim to evaluate their role as therapeutic materials. They were characterized by UV–Vis spectrophotometer, infra-red spectra, elemental analysis, nuclear magnetic resonance, and thermogravimetry. Efforts were made to optimize the synthesis of intermediates and final products. Photoluminescence studies were carried out on these compounds. The investigation of antibacterial activity of these aqueous soluble phthalocyanines was carried out on *Staphylococcus citreus*, *Serratia marcescens*, *Proteus vulgaris*, *Bacillus subtilis*, and *Pseudomonas fluorescence*, showed good inhibitory activity.

Keywords

Copper tetra (*n*-carbonylacrylic) aminophthalocyanine · Nickel tetra (*n*-carbonylacrylic) aminophthalocyanine · Zinc tetra (*n*-carbonylacrylic) aminophthalocyanine · Photoluminescence · Antibacterial activity

Introduction

Phthalocyanines (Pcs) are the class of compounds known as tetrapyrolic macromolecules and have comparatively

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S. S. Patil · I. Furtado Department of Microbiology, Goa University, Goa 403206, India more thermal stability than many other metal organic compounds. Pcs have multifunctional applications in oxidation-reduction reactions (Pan et al., 2005; Liu et al., 2007), in photoreduction processes (Premkumar and Ramaraj, 1997), the light harvesting property of Pcs is used for the degradation of organic molecules, dyes etc. in the presence of light (Xia et al., 2008; Borkar and Salker, 2006), and the property of Pcs is also being studied for their use in photodynamic therapy (PDT) and other similar applications (Brasseau and Lier, 1987; Sharman and Lier, 2005). Use of Pcs in PDT is, however, restricted due to their very low solubility in aqueous media. Synthesis of Pcs and that of tetra-nitro and tetra-amino Pc using melt and microwave assisted reactions (Kantra et al., 2008; Chen et al., 2006) and other reactions (Reddy and Keshavayya, 2002), respectively, are well-documented. Methods reported for synthesizing more water-soluble Pcs involve sulfonation at peripheral position (Shaabani et al., 2003) or attack on the phosphate moieties (Boyle and Lier, 1995). This communication details synthesis of novel, watersoluble copper, nickel, and zinc n-carbonylacrylic aminesubstituted Pcs using easily available chemicals, minimum organic solvents, less time, and ambient condition, so that the product formed can be evaluated in aqueous solution for different therapeutic applications such as antimicrobial activities (Nas et al., 2011) and others. Analysis of photoluminescence is also presented and discussed herein.

Materials and methods

4-Nitrophthalimide was synthesized from phthalic anhydride as per the reported method (Young and Onyebuagu, 1990). Urea, NiCl₂·6H₂O, CuCl₂·2H₂O, ZnCl₂, Ammonium molybdate, maleic anhydride, and DMF of pure analytical grade chemicals were used without further purification.

Synthesis of metal phthalocyanine

The route adopted for the synthesis of metal tetra (*n*-carbonylacrylic) aminephthalocyanine is given in Scheme 1.

Synthesis of nickel tetranitro-phthalocyanine (NiTNPc)

Finely ground, 4-nitrophthalimide 9.75 g (0.05 mol), 15 g (0.25 mol) urea, 2.97 g of NiCl₂·6H₂O (0.0125 mol), and 0.15 g of ammonium molybdate were taken in a 250 ml beaker and heated between 180 and 200 °C, for 1 h. The solid product was treated with HCl (1 M) followed by NaOH (1 M). The product was filtered out, washed to neutralization with water, and dried in vacuum. The yield was 76 %, and λ_{max} in DMF gave 635 and 272 nm absorptions, respectively. Copper tetranitro-phthalocyanine (CuTNPc) and zinc tetranitro-phthalocyanine (ZnTNPc) were also prepared following the above procedure.

Synthesis of nickel tetraamino-phthalocyanine (NiTAPc)

The above prepared NiTNPc (7 g) was transferred into a 500 ml beaker with approximately 100 ml water and kept boiling on water bath, for 20 min; similarly, 21 g of Na₂S was dissolved in a separate 250 ml beaker and kept boiling on water bath, for 20 min. After 20 min, both the beakers were removed from the water bath, and Na₂S solution was slowly transferred to 500 ml beaker containing NiTNPc solution while still warm. The resulting mixture was allowed to stand at ambient temperature for 15 min. Further, the solid residue thus obtained was washed with HCl (1 M) followed by NaOH (1 M). The filtered out product was washed to neutralization with water and dried in vacuum. The yield was 80 %, and λ_{max} in DMF gave 713 and 310 nm absorptions, respectively. Copper tetraaminophthalocyanine (CuTNPc) and zinc tetraamino-phthalocyanine (ZnTNPc) were prepared as above.

Synthesis of nickel tetra (*n*-carbonylacrylic) aminephthalocyanine (NiTCPc)

One mole of 4 g of nickel tetraamino phthalocyanine (NiTAPc) was dissolved in 75 ml DMF, to which 2.5 g (4 mol) of maleic anhydride was added, and the reaction mixture was kept at 50 °C for 3 h. Later, the entire solution was poured into 1 l distilled water, filtered, and the residue dissolved in 0.1 M NaOH and precipitated with addition of 0.1 M HCl. The precipitate, thus obtained, was firstly washed with water till free from chloride ions, then with

ethanol followed by diethyl ether, and finally dried in vacuum.

Water-soluble copper and zinc tetra (*n*-carbonylacrylic) Pcs (CuTCPc and ZnTCPc) were prepared using the above procedure. Salts of CuCl₂·2H₂O and ZnCl₂ were used for the preparation of CuTCPc and ZnTCPc for the central metal atoms.

Characterization of synthesized Pcs

Elucidation of chemical structure was characterized with FTIR spectrophotometer employing Shimadzu IR prestige 21 spectrometer using KBr Pellets; the UV-Vis spectra for each Pc was obtained using Shimadzu spectrophotometer in DMF and in 0.1 N NaOH for aqueous soluble Pc. Elemental analysis of each of the synthesized Pcs was carried out employing Prostar Varian C, H, N analyzer. NMR was recorded in DMSO using Bruker 400 MHz spectrometer. The thermal stability of individual Pcs was checked using TG (model NETZSCH STA 409PC). Photoluminescence studies were done by loading sample on powder holder, and measurements were done using Shimadzu RF-530IPC spectrofluorophotometer with a xenon flash lamp, and a UV-35 filter was used at the emission slit to avoid harmonic effects from the grating. The Gouy method was used to measure magnetic susceptibility at ambient temperature with a field of 8,500 G using mercury tetrathiocyanatocobaltate as a standard.

Effect of phthalocyanines on bacterial cultures

Phthalocyanine conjugates of copper, nickel, and zinc (CuTCPc, NiTCPc, and ZnTCPc) were individually dissolved in alkaline buffer (pH 8) to obtain a corresponding solution, having a final concentration of 100 µg/ml and 2 mg/ml, respectively. Antibacterial activity by each of these Pcs was tested against five emerging opportunistic bacterial pathogens obtained from the Department of Microbiology, Goa Medical College, Goa. Twenty-four hour old seed cultures of Staphylococcus citreus/Serratia marcescens/Bacillus subtilis/Proteus vulgaris and Pseudomonas fluorescence pre-grown in nutrient broth, were plated on to separate nutrient agar, for matt growth. Subsequently, wells were bored into the agar with a sterile 10-mm diameter cork borer, aseptically for agar well diffusion assay (Cooper, 1955). Different concentrations of individual phthalocyanines in the range of 0.2 µg to a maximum of 1 mg were added into separate wells. All plates were incubated at appropriate temperature and monitored for growth, zones of inhibition of growth, and/ exhibition around the well, if any, periodically.







Results and discussion

FTIR peaks around $1,525-1,532 \text{ cm}^{-1}$ correspond to the presence of nitro group, those around $3,300-3,200 \text{ cm}^{-1}$ indicate the presence of primary amino group, and the one

in between 1,712 and 1,713 cm^{-1} corresponds to the presence of carbonyl group. The characteristic FTIR peaks of different functional groups are observed with change in substitution on the ring in case of (1) NiTNPc, (2) NiTAPc, and (3) NiTCPc (Fig. 1).



Fig. 1 IR spectra of (1) NiTNPc, (2) NiTAPc, and (3) NiTCPc

UV–Vis measurements were done to observe the shift in Q and B band with change in substitution on the ring and change in solvent in case of water-soluble Pc. The appearance of Q band in the visible region is responsible for the color of the compound. Figure 2a shows representative UV–Vis spectra of (1) CuTNPc, (2) CuTAPc, (3) CuTCPc in DMF, and (4) CuTCPc in 0.1 N NaOH. Figure 2b shows representative UV–Vis spectra of (1) NiTNPc, (2) NiTAPc, (3) NiTCPc in DMF, and (4) NiT-CPc in 0.1 N NaOH. Figure 2c shows representative UV–Vis spectra of (1) ZnTNPc, (2) ZnTAPc, (3) ZnTCPc in DMF, and (4) ZnTCPc in 0.1 N NaOH, respectively.

The yield of the NiTCPc was 85 %, and λ_{max} obtained in DMF were 692 and 311 nm, respectively, whereas the λ_{max} in 0.1 M NaOH were 632 and 293 nm, respectively. Anal: Calc. for C₄₈H₂₈N₁₂O₁₂Ni. 2H₂O: C 54.41, H 3.04, N 15.86 % and found C 53.63, H 2.99, N 16.91 %. The magnetic susceptibility study indicated that the compound NiTCPc was diamagnetic. The NMR signals due to aromatic protons were observed from 6.63 to 7.23 ppm, signals due to amido proton were seen at 6.03 ppm and that due to aliphatic protons at 2.09 ppm.

The yield of copper tetra (*n*-carbonylacrylic) aminephthalocyanine (CuTCPc) was 87 %, and λ_{max} in DMF were 696 and 344 nm absorptions, respectively, whereas λ_{max} in 0.1 M NaOH were 612 and 297 nm, respectively. Anal: calc. for C₄₈H₂₈N₁₂O₁₂Cu·1H₂O: C 55.10, H 2.89, N 16.06 % and found C 55.95, H 3.11, N 15.64 %. As copper exists in +2 oxidation state in the compound, it is paramagnetic in nature due to which NMR signals were not observed.



Fig. 2 a UV–Vis spectra of (1) CuTNPc, (2) CuTAPc, (3) CuTCPc in DMF, and (4) CuTCPc in 0.1 N NaOH. **b** UV–Vis spectra of (1) NiTNPc, (2) NiTAPc, (3) NiTCPc in DMF, and (4) NiTCPc in 0.1 N NaOH. **c** UV–Vis spectra of (1) ZnTNPc, (2) ZnTAPc, (3) ZnTCPc in DMF, and (4) ZnTCPc in 0.1 N NaOH

The yield of zinc tetra (*n*-carbonylacrylic) aminephthalocyanine (ZnTCPc) was 88 %, and λ_{max} in DMF were 690 and 353 nm, respectively, whereas λ_{max} in 0.1 M NaOH were 661 and 341 nm, respectively. Anal: Calc. For C₄₈H₂₈N₁₂O₁₂Zn·3H₂O: C 53.17, H 3.16, N 15.50 % and found C 52.95, H 3.01, N 16.40 %. The magnetic susceptibility study indicated that this compound was diamagnetic. NMR signals obtained in the case of ZnTCPc were almost similar to those obtained in the case of NiTCPc.

The impact of substitution at peripheral position on the stability of the compound studied using TG revealed that these compounds have good thermal stability up to 400 °C as seen for *n*-carbonylacrylic amine-substituted Pc in Fig. 3. These compounds can be conveniently used at



Fig. 3 TG curves of (1) CuTCPc, (2) ZnTCPc, and (3) NiTCPc



Fig. 4 Emission at 468 nm (1) CuTCPc, (2) ZnTCPc, and (3) NiTCPc

higher temperature till 300 °C without undergoing any decomposition or compositional change.

Photoluminescence data recorded in Fig. 4 show emission maximum at 468 nm for *n*-carbonyl acrylic aminesubstituted Pc, on excitation at 220 nm, Fig. 5 indicates the excitation spectra. Figure 6 shows up-conversion at 450–480 nm with maximum at 468 nm, for the same compounds, after excitation at lower energy wavelength at 625 nm. Interestingly, excitation of the compounds either with higher energy at 220 nm and or lower energy at 625 nm resulted in emissions in the range 450–470 nm. ZnTCPc showed maximum intensity as compared to that of CuTCPc and NiTCPc. The up-conversion was observed in



Fig. 5 Excitation at 220 nm (1) CuTCPc, (2) ZnTCPc, and (3) NiTCPc



Fig. 6 Up-conversion at 468 nm (1) CuTCPc, (2) ZnTCPc, and (3) NiTCPc

all the samples by keeping the slit width same. Sample was excited at different wavelengths in the range 580–650 nm, of which 625 nm showed the up-conversion. The up-conversion pattern was similar to that of excitation at 220 nm indicating that in both the cases, emission centers were the same. The up-conversion process may be explained by anti



Fig. 7 Zones of inhibition of growth by CuTCPc, NiTCPc, and ZnTCPc: **a** *Bacillus subtilis*; **b** *Serratia marcecans*; **c** *Staphylococcus citreus*

stokes two photon theory ,i.e., when the samples were irradiated at 625 nm, the first photon pumps the electron from the conduction band to the intermediate level, and the second photon ionizes the electrons to the conduction band. When the electrons are recaptured or transfer energy in the valence band, intrinsic emission occurs, while the recombination or transfer of energy in the intermediate level gives the emission at lower wavelength.

Images depicted in Fig. 7 showed absence of growth of S. citreus, S. marcecans, and B. subtilis around wells containing the phthalocyanine conjugates of copper, nickel, and zinc. In Fig. 8, different higher concentrations of ZnTCPc showed activity against P. vulgaris, thus suggesting that the conjugates affected growth adversely. As observed in Fig. 9a, b, inhibition of growth with copper and nickel conjugates occurred at minimum of 10 and 20 µg in case of S. citreus, S. marcescens, P. vulgaris, P. fluorescence; S. citreus, S. marcescens, B. subtilis, and P. vulgaris, respectively. The adverse effect of Cu and Ni conjugates, in terms of size of growth inhibition zone was in the order of: P. fluorescence = S. citreus >S. marcescens = P. vulgaris and S. marcescens > B. subtilis > S. citreus, respectively. Further, as evident in Fig. 9c, Zn conjugates at 200, 400, and 600 µg affected growth of S. citreus > S. marcescens, P. vulgaris, and B. subtilis, respectively. It is noteworthy that the growth of P. fluorescence was inhibited only by Cu conjugate. The Cu and Ni conjugates are potent at a tenfold lower concentration than that of Zn conjugate. The observation is, therefore, an evidence of antimicrobiocity/antibiocity of metal phthalocyanines against S. citreus, S. marcescens, P. vulgaris, B. subtilis, and P. fluorescence, which is dose-



Fig. 8 Zones of inhibition of growth on *Proteus vulgaris* by ZnTCPc at (*i*) 200; (*ii*) 400; (*iii*) 600; (*iv*) 800; and (*v*) 1,000 μ g of concentration (*DC* diluent control)



Fig. 9 Inhibition of growth of: *Staphylococcus citreus*, *filled square*; *Serratia marcescens*, *filled circle*; *Proteus vulgaris*, *filled triangle*; *Bacillus subtilis, open square*; and *Pseudomonas fluorescence, open circle*. By Phthalocyanine conjugates of: **a** CuTCPc, **b** NiTCPc, and **c** ZnTCPc

dependent, bacteria- and metal-specific. Such inhibition of growth, seen with antibiotics, is termed as antimicrobial effect and is taken as the criteria for evaluating potency of antibiotics (Cooper and Woodman, 1946). The results are of significance as the bacterial pathogens under study were obtained as emerging pathogenic status from the State

Hospital and also because: *S. citreus* is recommended to be pyogenic and involved in nosocomial infections (Chang and Chen, 2003); *S. marcecans* (Sunenshine *et al.*, 2007), *P. vulgaris* are involved in emerging UTI infections (Trevejo *et al.*, 2005); and *B. subtilis* is often encountered as a laboratory contaminant or in casual handling of food and food poisoning thereof (Parisi, 1985). The inhibition of growth of *P. fluorescence* by Cu conjugates is of special significance as the organism is known to be resistant to many frontline antibiotics (Pitt *et al.*, 2003).

These compounds, therefore, have an excellent property to provide or make available oxygen species for the oxidation, and there is every chance that such reactions may be more effective in the presence of light. Further, these properties of light harvesting and photoluminescence property including up-conversion may be playing a vital role in the antibacterial activity of Pcs. It is also note worthy that the phthalocyanine compounds in solution have potency to generate reactive oxygen species in the presence of dissolved oxygen and light. Thus, the activity may be increased by taking oxygen-enriched solution. The PDT may also work based on a similar phenomenon wherein oxygen species like singlet oxygen may be involved in killing bacteria present around.

Conclusion

A novel aqueous soluble metal phthalocyanines of copper, nickel, and zinc were synthesized, starting from easily available phthalic anhydride. The conditions required for synthesis of intermediate products were optimized. This method developed for the synthesis of water-soluble Pcs is important and can be used further as therapeutic materials. The up-conversion study indicates that the molecule may show some application in laser technology and also for PDT. The preliminary results clearly indicate that the phthalocyanine conjugates are potent '*bacteriocides*' and that their potency can be affixed in terms of minimum inhibitory concentration (MIC) against the bacterial cultures used in the study.

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