OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE www.BiointerfaceResearch.com ISSN 2069-5837

*Volume 2, Issue 4, 2011, 374-379*

**Received: 19.04.2012 / Accepted: 26.07.2012 / Published on-line: 24.08.2012**

# Synthesis and antimicrobial activity on Manganese tetra (n-carbonylacrylic) aminephthalocyanine

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**ABSTRACT**

Phthalocyanines (Pcs) being analogous to naturally occurring Porphyrins and having the ability of generating singlet oxygen, are under incessant studies for their activity in Photodynamic Therapy (PDT) and antimicrobial activity. Water soluble Pcs are of enormous importance for this category of activity. Pcs show extensive variation in properties with change in the substitution at the peripheral position and also the central metal atom. The synthesis of a novel water soluble Manganese tetra (n-carbonylacrylic) aminephthalocyanines (MnTCPc) and antimicrobial activity against bacteria strains, such as *Escherichia coli* (*ATCC 8739), Salmonella typhi (ATCC 14028), Pseudomonas aeruginosa (ATTC 9027), Bordetella bronchiseptica (ATCC 4617), Staphylococcus epidermidis (ATCC 12228)* and *Bacillus subtilis (ATCC 6633)* have been discussed. The effect of solvent on the antimicrobial activity is noticed and the probable mode for the destruction of the cell wall based on the generation of single oxygen.

**Keywords:** *Aminophthalocyanine, Maelic anhydride, Aquous soluble phthalocyanine, Antibacterial activity, Pathogens.*

## 1. INTRODUCTION

Phthalocyanine (Pc) is the class of tetrapyrol having wide applications not only in industrial field but also in biological field. Their applications are widely studied and there can be series of Pcs having different substituents at the peripheral positions. These are coloured aromatic molecules having the capacity to produce singlet oxygen. The Pcs are studied for their photodynamic activity, for which their solubility in aqueous phase is very important [1-4]. Number of methods are reported to make the Pc molecule water soluble, either by sulfonation at peripheral positions or attacking phosphate moieties [5-8]. The main aim of this work is to prepare new water soluble phthalocyanine adopting simple and safe method, ambient condition, less time and less use of organic solvent, so that the product formed can be used for different functions. Earlier there are few reported methods for the synthesis of Pc using melt and microwave assisted reactions [5, 9-11]. Synthesis of Tetranitro and Tetra amino Pc is reported in the literature [12]. In the present investigation the new water soluble Manganese tetra (n-carbonylacrylic) aminophthalocyanine has been prepared and it was further characterized using FTIR, elemental analysis, UV-visible, TG for its thermal stability and attempted by NMR. Manganese tetra (n-carbonylacrylic) aminephthalocyanines (MnTCPc) was studied for antimicrobial activity against pathogenic bacteria such as *Escherichia coli* (*ATCC 8739), Salmonella typhi (ATCC 14028), Pseudomonas aeruginosa (ATTC 9027), Bordetella bronchiseptica (ATCC 4617), Staphylococcus epidermidis (ATCC 12228)* and *Bacillus subtilis (ATCC 6633).* The comparison is drawn between the different strains of Organism.

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## 2. EXPERIMENTAL SECTION

**2.1. Materials and Methods.** 4-Nitrophthalimide was synthesized from phthalic anhydride as per reported method [13]. Urea, $MnCl_2.4H_2O$, Ammonium molybdate, maleic anhydride and dimethyle formamide (DMF) reagent of analytical grades were used without further purification.

**2.2. Synthesis of Manganese Phthalocyanine.** The synthetic route adopted for the synthesis of Manganese tetra (n-carbonylacrylic) aminophthalocyanine is given below in scheme 1.

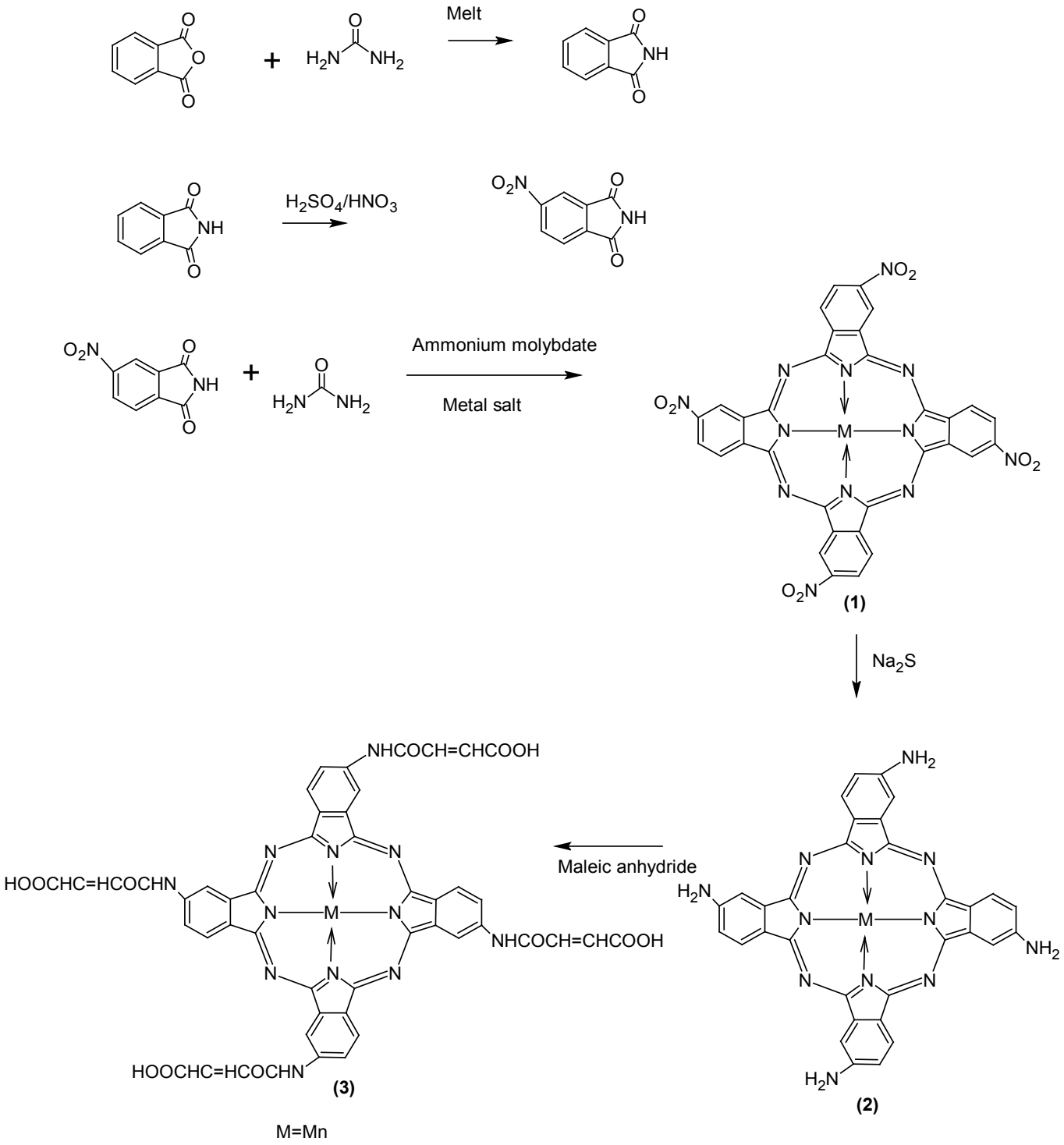
**2.2.1. Manganese Tetranitro Phthalocyanine (MnTNPc, product no. 1).** Nitrophthalimide 9.75 g (0.05 mol), 15 g urea (0.25 mol), 2.47 g of $MnCl_2.4H_2O$ (0.0125 mol) and 0.15 g of ammonium molybdate were finely grounded and placed in a 250 ml beaker with a glass rod. The temperature was maintained between 180 - 200 $^{O}$C for 1 h. The solid product formed was treated with HCl (1.0 M) and NaOH (1.0 M) respectively. After filtration the product was washed to neutralization with water and dried in vacuum.

**2.2.2. Manganese Tetraamino Phthalocyanine (MnTAPc, product no. 2).** The 7 g of MnTAPc was transferred to 500 ml beaker with about 100 ml water and kept on boiling in water bath for 20 min, similarly 21 g of $Na_2S$ was separately dissolved in 250 ml beaker and kept on boiling in water bath for 20 min . After 20 min both the beakers were removed from the water bath and $Na_2S$ solution was slowly transferred to 500 ml beaker containing MnTNPc solution with proper mixing, this mixture was allowed to stand at room temperature for 15 min. Further, the solid residue obtained was washed with HCl (1.0 N) and NaOH (1.0 M) respectively. After filtration the product was washed to neutralization with water and dried in vacuum.

**2.2.3. Manganese Tetra (n-carbonylacrylic) Aminophthalocyanine (MnTCPc, product no. 3).** The 4 g of MnTAPc was dissolved in 75 ml DMF, to that further 2.5 g of maleic anhydride was added and the reaction was kept at 50 $^{0}$C for 3 h. Later the solution was poured into 1 L water, filtered and dissolved in 0.1 N NaOH. This was precipitated with 0.1 N HCl. The pink precipitate obtained was washed till free from chloride ions, then further washed with Ethanol, diethyl ether and finally dried in vacuum.

**2.3. Instrumentation.** Chemical structure was characterized with FTIR spectrophotometer (Simadzu IR spectrometer) using KBr Pellets. Elemental analysis was carried out with Thermofinnigan. The UV-visible spectra were obtained using Simadzu spectrophotometer in DMF and in 0.1 N NaOH for aqueous soluble Pc. NMR was recorded in DMSO (model Bruker 400 MHz).

**2.4. Bioactivity of Pthalocyanines.** MnTCPc was individually dissolved in alkaline buffer and DMSO to obtain a corresponding solution having a final concentration of 2 mg/ml and 40 µg/ml. Antibacterial activity of Pthalocyanines was tested against bacterial reference strains *Escherichia coli* (*ATCC 8739), Salmonella typhi (ATCC 14028), Pseudomonas aeruginosa (ATTC 9027), Bordetella bronchiseptica (ATCC 4617), Staphylococcus epidermidis (ATCC 12228) and Bacillus subtilis (ATCC 6633).* Cultures were grown in nutrient broth at 37 °C for 24 h and then plated on to separate Mueller hinton agar, for matt growth. Subsequently, wells were bored into the agar, with a sterile 10 mm diameter cork borer, aseptically filled with 100 µl of test sample for agar well diffusion assay. All pathogens were tested against MnTCPc (4µg/ml to 1mg/ml) and plates were incubated at appropriate temperature and monitored for growth inhibition zones. Experiments were performed in triplicate.




**Scheme 1:** Reaction scheme for the preparation of the phthalocyanine

## 3. RESULTS SECTION

**Characterization of MnTCPc.** In this method the conversion of phthalic anhydride is shown in scheme I. further the nitration of phthalimide to 4-nitrophthalimide was performed, the obtained product being used for the synthesis of tetranitro Phthalocyanine. This tetranitro Phthalocyanine was converted to tetra aminophthalocyanine and which was then converted to tetra (n-carbonylacrylic) aminophthalocyanine. The final product exists in 4 isomeric forms.

The yield of MnTNPc (product No. 1) was 86% and $\underline{\lambda}_{max}$ values obtained in DMF at 683, 639 and 279 nm respectively. The yield of MnTAPc (product No. 2) was 80% and $\underline{\lambda}_{max}$ obtained in DMF at

791 and 347 nm respectively. The yield of MnTCPc (product No. 3) was 85% and $\lambda_{max}$ obtained in DMF were 751, 291 and 272 nm respectively, $\lambda_{max}$ in 0.1 N NaOH were at 657, 290 and 255 nm respectively as shown in Fig. 1. The Elemental analysis calculation for $C_{48}H_{28}N_{12}O_{12}Mn$ is as follows: C, 56.53; H, 2.75; N, 16.49 and experimentally found: C, 56.86; H, 2.90; N, 16.90% respectively confirming with good agreement the composition. As Manganese exist in +2 oxidation sate in complex, which is paramagnetic in nature and therefore relevant NMR signals are not observed.

FTIR technique was used for the characterization of functional group in molecule for product No. 1, the absorbance peaks at 1339, 1256 $cm^{-1}$ are stretching of aromatic C-N, 1530 $cm^{-1}$ is N-O stretching, 1140, 1072, 1011, 908, 849, 758 and 731 $cm^{-1}$ are for Pc skeletal. For product No. 2, the peaks at 3341 and 3215 $cm^{-1}$ are N-H stretching of primary amine, 1605 $cm^{-1}$ is C- N stretching, 1134, 1072, 827 and 744 $cm^{-1}$ are for Pc skeleton. For product No. 3, the absorbance peak at 1715 $cm^{-1}$ is C=O stretching.

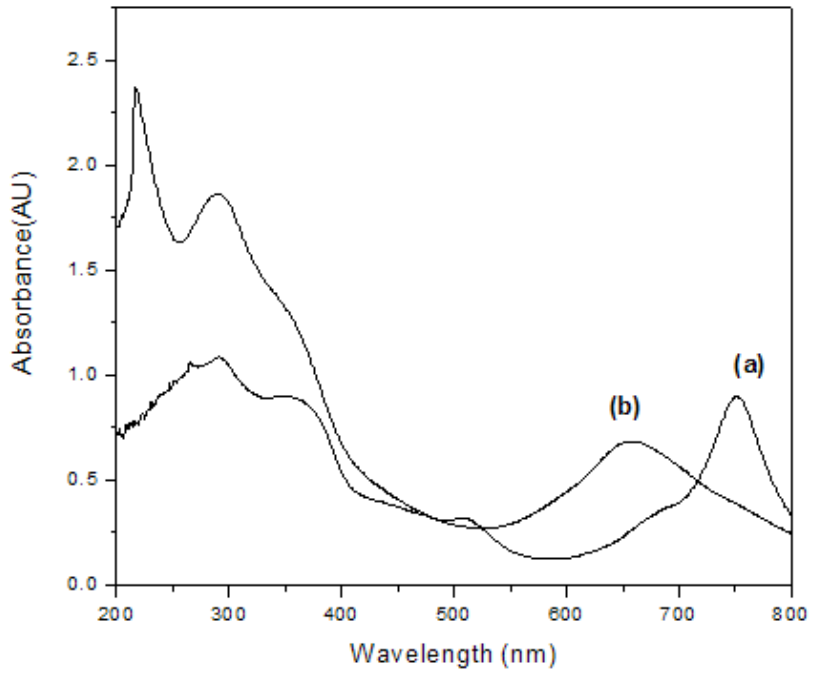



**Figure 1:** UV-Visible spectra of MnTCPc (a) DMF, (b) 0.1 N NaOH

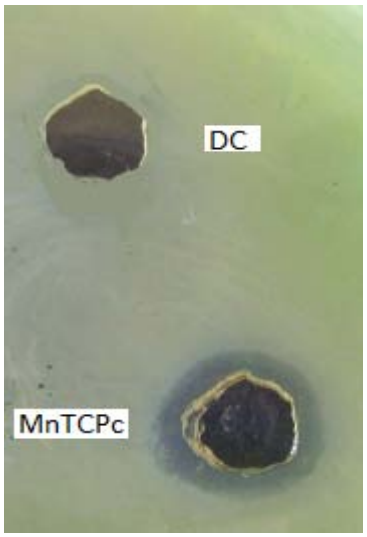



**Figure 2:** Representative test for Zone of inhibition growth on *Pseudomonas aeruginosa (ATTC 9027)* by MnTCPc (DC = diluents control)

**Antimicrobial Activity.** MnTCPc was inactive when dissolved in alkaline buffer even at very high concentration such as 1 mg/ml but showed very good activity when dissolved in DMSO at a very low concentration such as 4 µg/ml. Fig. 2 shows the representative test in DMSO and growth inhibition zones for *Psedomonas aeruginosa (ATTC 9027)* by MnTCPc. The Variation in activity for different strains of microorganisms is shown in Fig. 3.

MnTCPc was not able to show any antimicrobial activity in aqueous solution which could be due to the short life time of singlet oxygen generated in the media in comparison to that of DMSO. These generated singlet oxygen in DMSO further produce more reactive species. These reactive species are responsible for the destruction of lipids and proteins present on the cell wall. Oxidative stress in bacteria is very well known. Lipids are reported as major target among DNA, RNA and proteins. Free radicals can cause lipid peroxidation which decreases membrane fluidity. In addition to that it converts polyunsaturated fatty acids to aldehydes that can damage molecules such as proteins and act as "second toxic messengers" [14]. Phthalocyanines, which are characterized with far red wavelength absorption (> 670 nm), long triplet life time ( approx. 1 ms), and high quantum yields of

singlet oxygen generation (> 0.2), have been studied as drugs in microbial photodynamic inactivation [15].

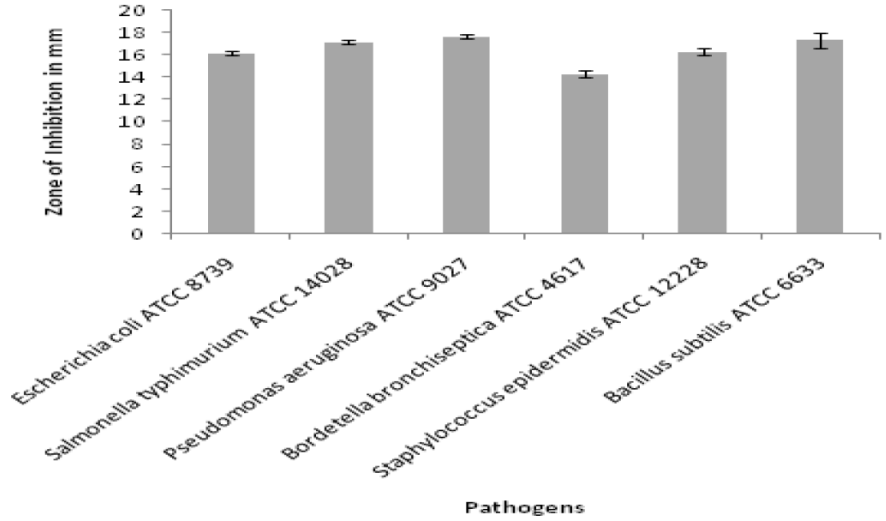



**Figure 3:** Antimicrobial activity of MnTCPc in DMSO on different microbial strains.

As seen in Fig. 3 MnTCPc is effective on gram positive as well as on gram negative microbial human/mammalian pathogens, therefore the activity is a broad spectrum in nature. Further, among the gram negative organisms *Pseudomonas aeruginosa* is affected maximum followed by *Salmonella typhimurium* and *Bacillus subtilis*. *Bordetella bronchiseptica* showed little higher resistance but less than gram positive *Staphylococus epidermidis*. It may be noted that *Pseudomonas aeruginosa* is type IV pathogen having injectisome, while *Bordetella bronchiseptica* is a lower mammalian pathogen and hence the study is of much significance.

## 4. CONCLUSIONS

The intermediate and final products obtained in this study were Tetranitro Manganese Phthalocyanine, Tetraamino Manganese Phthalocyanine and Manganese tetra (n-carbonylacrylic) aminophthalocyanine. MnTCPc was not able to show any appreciable antimicrobial activity in alkaline solution which could be due to the short life time of singlet oxygen generated in aqueous medium but showed good activity in DMSO due to high quantum yield generation of singlet oxygen with sufficient life time for antibacterial activity to proceed.

## 5. ACKNOWLEDGMENT

Authors acknowledge UGC, New Delhi, for the financial support.

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