Preparation, Characterization and Biological Activity Study of Manganese (II) Phthalocyanine Complex

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Abstract

This study presents the preparation, purification, identification and applications of Manganese (II) phthalocyanine complex. Non-solvent method was used for the preparation of the complex at high temperature (sand bath technique). Phthalic anhydride was used as a precursor for the preparation. Characterization of as-synthesized complex was carried out by different instrumentation methods including ultraviolet-visible, infrared, mass spectroscopy, elemental and thermaogravimetric analyses. Magnetic susceptibility of the studied complex was also determined. Antimicrobial activities of the tested compound were carried out using bacteria and fungi. Human heptacellular Carcinoma (Hep-G2) was used for studying the complex antitumor activity. The results of characterization showed that the complex was prepared and separated in a pure form whereas, the biological activity data depicted that the complex has valuable activity against cell line (Hep-G2) and bacteria.

Keywords - *Manganese (II) Phthalocyanine, Non-solvent method, Biological activity, (Hep-G2) cell.*

I. INTRODUCTION

Phthalocyanines (Pcs), (Fig. 1), are planar, macrocyclic aromatic compounds isoelectronic with phorphyrine molecule consisting of four isoindole units linked together by nitrogen atoms [1-3]. They are tetrabenzo tetraazaporphyrins [4,5,6,7,8]. They are pigment dyes contain л-electron system in the molecular structure which account for their unique spectroscopic and photoelectric properties, and they have received extensive attention due to their peculiar and unconventional chemical and physical properties. They form an important class of macrocyclic compounds which do not occur in nature [5,9]. Phthalocyanines are man's analogues of nature's pigments of life, the porphyrins, such as chlorophyll and hemoglobin [10,11]. Moreover, they were some of the earlier macrocyclic substances synthesized in laboratories.

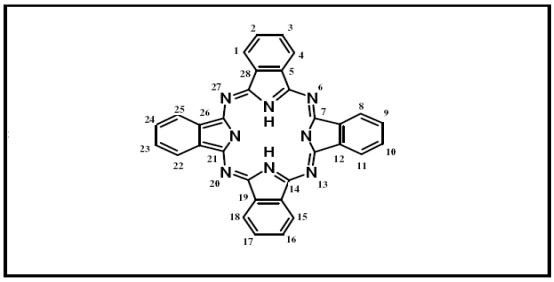

Fig 1: Phthalocyanine structure

They are important nitrogen containing planar 18 л -electron heterocyclic conjugated compounds [12]. Among tetrapyrrole compounds, phthalocyanines which are full-aromatic molecules, due to these л -electron their structure are not only capable of undergoing classical displacement reactions, but they can also be substituted by a large number of functional groups [5,6,13].

The use of phthalocyanine has recently extended to many high technological processes. They can be used in many applications with appropriate substitution in the peripheral position of the macrocycle, such as in optical recording materials, optical limiters, field-effect transistors, Langmuir-Blodgett films [14], thin films (solar cells) [15], gas sensors, [16] and liquid crystals [7]. Furthermore, they are used as catalysts for photo or oxidative degradation of pollutants, and as photosensitizers [17]. They have attracted great attention because of their versatile applications [4,5]. One of their most promising aspects is functioning as photosensitizers for photodynamic therapy of cancer or photodegradation of pollutants [18,19]. The most fundamental property of a photosensitizer is its ability to generate reactive oxygen species (ROS), in particular singlet oxygen, under light irradiation [18]. The photosensitized ROS production of phthalocyanines is strongly affected by the nature of their central metal ions [20]. The phthalocyanines coordinated with closed shell, diamagnetic ions possess high ROS yield [8]. Otherwise, the photosensitizing efficiency of the phthalocyanine is largely influenced by its molecular aggregation [19,21]. Molecular aggregation of phthalocyanines, which is an intrinsic property of these large л-conjugated systems, provides an efficient non-radiative energy

relaxation pathway, thereby shortening the excited state lifetimes and greatly reducing the photosensitizing efficiency [20,22].

The present study was set for the preparation of Manganese (II) phthalocyanine complex (MnPc), characterization and the possibility of its use in biological and medical fields.

This work aimed to adjust suitable chemical environment for a preparation methodology of above Manganese phthalocyanine complex. The method involved high temperature conditions provided by a sand path, phthalic anhydride acid was used as starting material.

II. MATERIALS AND METHODS

All the chemicals, reagents and solvents, used were of the analytical grade (AR), and of highest purity, obtained from commercial suppliers.

Spectrophotometric measurements were carried out using automated spectrophotometer UV–Vis (SHIMADZU Lambda 4B) ranged from 200 - 900 nm using 1 cm matched quartz cells. Elemental analysis of the prepared complex was performed using an automatic CHN analyzer. Infrared spectra of the complex was recorded in KBr pellets using (FTIR-460 plus, JASCO, Japan), in 4000 – 400 cm^{-1} region. Magnetic susceptibility measurements were carried out using Gouy magnetic balance consisting of NP-53 type electromagnets with a dc power supply unit and a semimicro electronic balance supplied by AND Electronics, Japan. Pascal's constants were used to calculate the diamagnetic corrections. A mercury tetrathiocyanto cobaltate, $Hg[Co(SCN)_4]$, was used as calibration standard, and double distilled water was used throughout the experiment. Thermogravimetric analysis (TGA) was carried out in a dynamic nitrogen atmosphere (30 cm^3 min^{-1}) with a heating rate of 10 °C min^{-1} using (DTG-60H SIMULTANEOUS DTA-TG APPARATUS –SHIMADZU). Mass spectra measurements were recorded with the aid of a SHIMADZU QP-2010 plus mass spectrometer at 70 eV.

A. Preparation of Manganese (II) Phthalocyanine complex (MnPc)

0.35g (0.00177mol) of manganese(II) chloride tetrahydrate ($MnCl_2.4H_2O$) were placed into a round bottomed flask. The flask was flame-dried with the metal in it until the water vapours were driven off. The flask was cooled to room temperature, (1.047g, 0.0071mol) of Phthalic anhydride, 2.443g (0.0407mol) of urea and 0.15g of ammonium molybdate were added. A dry reflux condenser containing a drying tube at the top was added and the mixture was brought to reflux on a sand bath at 190-220 °C for 2-3 h. Then the mixture was subsequently cooled to room temperature. Water (10 cm^3) was added and after thorough mixing the supernatant was removed by decantation. The residue was boiled for 2 h, first with 150 cm^3 of 1 M HCl and then with 150 cm^3 of 1 M NaOH, filtered, and washed with distilled water until the filtrate was neutral. The solid material was stirred in methanol, suction-filtered, dried in an oven for 6 h at 60 °C. The yield was 0.84 g, (83.6% of theoretical yield).

The structure of the complex and its stability were studied by different physicochemical tools including physical properties (M.P), UV – Vis, IR and mass spectroscopic, elemental and thermogravimetric analyses techniques. The antibacterial and antifungal activities of the tested samples were carried out using a modified Kirby-Bauer disc diffusion method [23], under standard conditions using Mueller – Hinton agar medium, as described by NCCLS [24].

Human hepatocellular Carcinoma (Hep-G2) cancer cell lines was used for in vitro screening experiments; It was obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell was maintained by serial subculturing. Cell culture cytotoxicity assays were carried out as described elsewhere [25, 26].

III. RESULT

A. Preparation and Characterization

The procedure used for the preparation of Manganese (II) Phthalocyanine yielded 83.6% of a compound having a blue colour. The metal complex was thermally stable, and gave a clear solution with concentrated sulfuric acid and was moderately soluble in dimethylsulphoxide (DMSO), dimethylformamide (DMF), chloroform ($CHCl_3$), tetrahydrofuran (THF), toluene and pyridine.

UV-Vis spectral data for $1X10^{-5}$ M solution of the studied complex, recorded in the range 200–900 nm using DMSO as a solvent, showed maximum absorption (λ_{max}) at 674 nm (Fig. 2).

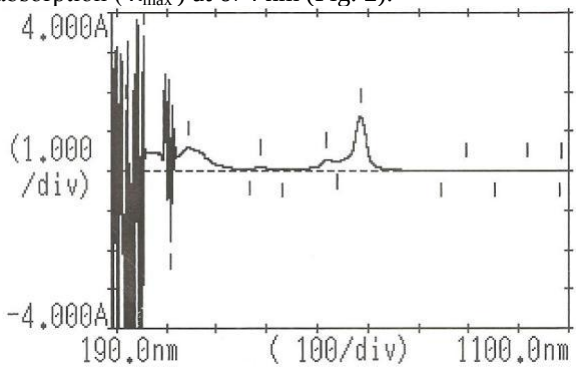

Fig 2: UV-Vis spectrum of $1X10^{-5}$ M standard MnPc complex

IR spectral data (Fig. 3) of the title complex, showed characteristic absorption bands at 3431, 3049, 1611, 1520, 1503, 1450, 1417, 1284, 1163, 1117, 1070, 896 and 723 cm^{-1}.

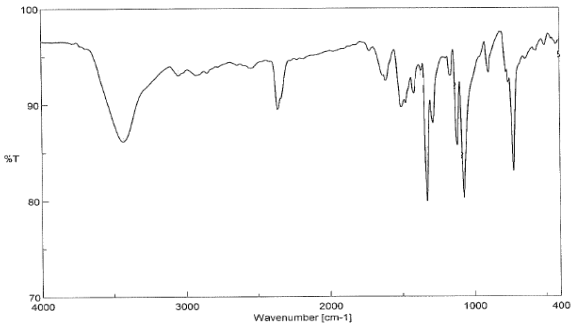

Fig 3: IR spectra of MnPc complex

The MS spectrum of Manganese (II) phthalocyanine complex (Fig. 4) showed a strong peak at m/z= 567. Other important signals were recorded at m/z= 295, 141 and 127.

Results of elemental analysis for C, H, N, and metal content of the compound (Table 1) are in agreement with the molecular formula of the complex.

TABLE I
Elemental analysis and physical properties of Manganese (II) phthalocyanine Complex

General formulae	Colour, (yield %)	M.P (^oC)	Elemental analysis Found and calculated (%)			
			C	H	N	M
MnPc ($C_{32}H_{16}MnN_8$), mole mass =567.48	Blue (83.60)	<300	67.64, 67.73*	2.78, 2.84*	19.67, 19.75*	9.56, 9.68*

* = Calculated

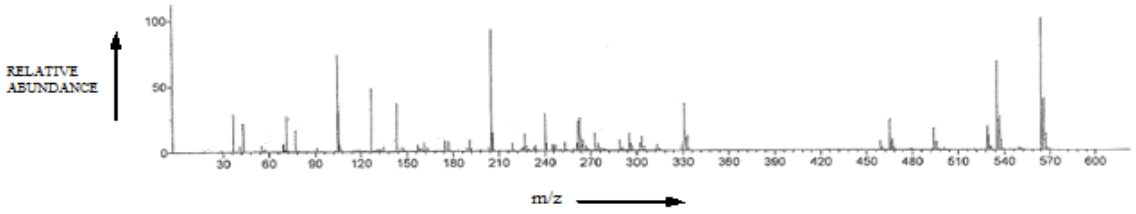

Fig 4: Mass spectrum of MnPc complex

Thermogravimetric curve for Manganese (II) phthalocyanine complex (Fig. 5), showed that the minor and major temperature for thermal decomposition were 243 ^oC and 568 ^oC, respectively, with loss of weight about 76.8 %

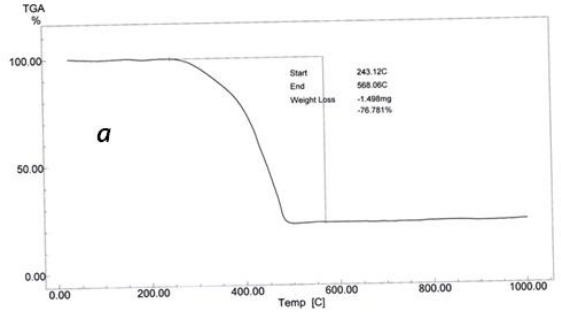

Fig 5: Thermal analysis (TGA) of MnPc complex

The magnetic susceptibility (X g) and magnetic moments (μeff) values of Manganese (II) phthalocyanine complex, in the solid state, average of the three independent determinations, were (0.185 x10-6 cgs units), and 4.35, respectively.

B. Biological activity

1. Antimicrobial activity

The complex showed considerable activity against bacteria (Table 2). The inhibition zones were 12, 20, 12 and 18 for Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus, respectively. However on the fungal species the compound showed negligible activity.

2. Antitumor activity

The studied compound showed considerable activity against the cell line used (Hep-G2), The inhibitory activity was concentration dependent with IC_{50} of 23.1. (Fig. 6).

TABLE 2
Anti bacterial and antifungal activities of the tested compound

Microorganism	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus cereus	Aspergillus flavus	Candida albicans
Gram stain reaction	Negative	Negative	Positive	Positive	Fungus	Fungus
Inhibition zone diameter (mm/mg sample)	12	20	12	18	0	0

TABLE 3
Inhibitory activity of MnPc complex against Hepatocellular carcinoma cells (IC_{50}=23.1 µg)

Sample conc. (µg)	50	25	12.5	6.25	3.125	1.56	0
Viability %	33.58	47.60	70.38	86.52	95.82	100	100

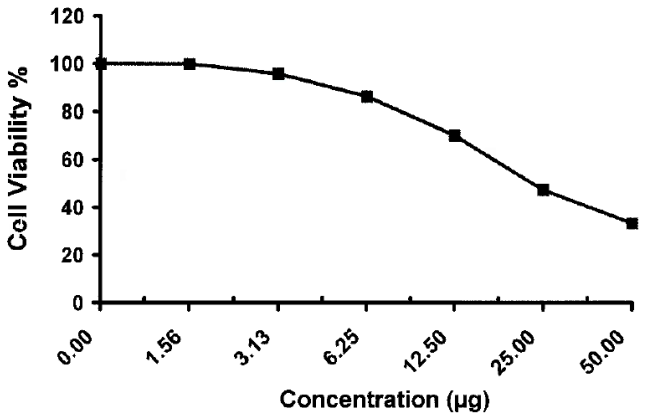

Fig 6: Inhibitory activity of MnPc complex

IV. DISCUSSION

The data revealed that the adopted methodology resulted in a Manganese (II) phthalocyanine complex with a high yield (83.6%). The antimicrobial and antitumor activities were studied.

The complex showed a major Q-band absorption around= 674 nm and a shoulder like absorption around 595–620 nm, (Fig. 2). These absorption bands are consistent with the electronic configuration of porphyrin system [27]. The deep bluish colour displayed by the complex is due to the absorption peaks in the Q band region [28].

The IR spectrum of the Manganese (II) phthalocyanine complex (Fig. 3) showed several bands. The band at 3431 cm^{-1} assigned as an OH vibration of adsorbed water. The band at 3049 cm^{-1} is due to CH asymmetric and symmetric stretching vibrations in the ring. The band at 1611cm^{-1} is assigned to the C–C stretching vibration in pyrrole and that at 1417 cm^{-1} is assigned to C–C stretching in isoindole. The band at 1450 cm^{-1} are assigned to the C–H in plane bending vibration and the two bands at 1520 and 1503 cm^{-1} is assigned to the C–H bending in aryl. The two bands at 1117 and 1070 cm^{-1} are assigned to C–H bending in plane deformations. The band at 723 cm^{-1} is assigned to C–H bending out of plane deformations. The two bands at 1284, 1163 cm^{-1} are assigned to the C–N in isoindole in plane and in pyrrole stretching vibration, respectively.

Based on mass spectrum data of Manganese (II) complex (Fig. 4), this complex has a strong molecular ion peak at m/z= 567. The peaks at m/z= 295, 141 and 127 are due to the formation fragment ions $[C_{16}H_6N_3Mn]$, $[C_8H_3N_3]^+$ and $[C_8H_3N_2]^+$ respectively . The signal appears at m/z = 128 is due

to the fragment $[C_8H_4N_2]$. The peak appear at m/z = 27 is due to the formation of fragment ions $[HCN]^+$.

The results of elemental analysis for carbon, hydrogen, nitrogen and metal (Table 1), are in good agreement with the calculated values and are consistent with the proposed structure of the complex.

The data (Fig. 5) revealed that the complex was stable up to 243 °C and showed a loss in weight at a temperature range of 240–600 °C. The loss in weight could be attributed to pyrolysis by a minor decomposition reaction at about 243 ^oC and a major decomposition reaction at 568 °C.

The magnetic susceptibility measurements gave an indication for the coordination environment of the Mn(II) center in the complex.

The antimicrobial data showed that the complex has a broad spectrum of antibacterial activity as it displayed substantial inhibitory effects on the bacterial species tested (Table 2). However the complex showed negligible antifungal activity. The remarkable activity of this compound may arise from the isoindole ring, which may play an important role in the antimicrobial activity. The mode of action may involve the formation of a hydrogen bond through the tertiary nitrogen of the isoindole ring with the active centers of the cell constituents, resulting in interference with the normal cell metabolic process. These results may be of significant value to biological applications.

The data (Fig. 6) indicate that the compound displayed a considerable antitumor activity against the studied cell line at 100 μdm^3 with IC_{50}=23.1µg, this finding suggests that the compound is of value in the medical fields for tumor treatment and could be of immense of value to biological applications.

V. CONCLUSION

Manganese (II) phthalocyanine complex was prepared at high temperature using a sand bath technique. The spectroscopic data are in agreement with the electronic and chemical structure of this complex. The biological importance as indicated by the antitumor activity and inhibition of bacterial growth suggest possible applications in the medical field.

REFERENCES

[1] Moser, F. H and Thomas, A. L. (1963). Phthalocyanines Compounds, Rehinhold Publishing Corporation, New York.

[2] Leznoff, C. C., Lever, A. B. P. (1989). Phthalocyanines, Properties and Applications,1, VCH Publications, Inc; New York.

[3] Gregory, P. (2000). Industrial applications of phthalocyanines, Journal of Porphyrins and Phthalocyanines 4: 432- 437.

[4] Puddephatt. RJ. Advances in organometallic polymers, ring-opening polymerization and the

[5] self-assembly of supramolecular polymers. Journal of Inorganic and Organometalic Polymer. Material.;15, 371–88, (2005).

[6] AS.Abd-El-Aziz, Carraher Jr CE, Pittman Jr CU, M. Zeldin "Macromolecules containing metal and metal-like elements". Wiley-Interscience, John Wiley & Sons, Inc ; New York, (2003–2007).

[7] Gregory, P. (2000). Industrial applications of phthalocyanines, Journal of Porphyrins and Phthalocyanines 4: 432- 437.

[8] Leznoff, C. C., Lever, A. B. P. (1986– 1993). The Phthalocyanines: Properties and Applications, 1–4, John Wiley and Sons Inc; New York

[9] Myers, J. F., Canham, G. W. R. and Lever, A. B. P. (1975). Inorganic Chemistry 14: 461-468.

[10] Moser, F. H and Thomas, A. L. (1983). The Phthalocyanines , 1–2, CRC Press, FL.

[11] Dent C. E., Linstead R. P. and Lowe A. R. (1934). The structure of the phthalocyanines, Journal of Chemical Society 1934: 1033-1039.

[12] Myers, J. F., Canham, G. W. R. and Lever, A. B. P. (1975). Inorganic Chemistry 14: 461-468.

[13] Kobayashi, N. and Opin, C. (1999). Phthalocyanines, Journal of Solid.

[14] Leznoff, C. C. and Lever, A.B.P. (1989). Phthalocyanines Properties and Applications, VCH,Weinheim.

[15] Gerdes, R. W., orhle, D., Spiller, W., Schneider, G., Schnurpfeil, G. and Schulz- Ekloff, G. (1997). Photo-oxidation of phenol and monochlorophenols in oxygen saturated aqueous solutions by different photosen-sitizers, Journal of Photochemistry and Photobiology A Chemistry 111: 65-74.

[16] Snow, A. W., Shirk, J. S. and Pong, R. G. S. (2000). Oligo oxyethylene liquid phthalocyanines, Journal of Porphyrins and Phthalocyanines 4: 518-524.

[17] Liu, S. G., Liu, Y. Q, Xu, Y., Zhu, D. B., Yu, A. C. and Zhao, X. S. (1998). Synthesis, Langmuir−Blodgett Film, and Second-Order Nonlinear Optical Property of a Novel Asymmetrically Substituted Metal-Free Phthalocyanine, Langmuir 14: 690-695.

[18] Hirth, A.G. amd Trp,nacj. N.(1999). Efficient near-IR sensitization of nanocrystalline TiO2 films by zinc and aluminum Phthalocyanines. Journal of Porphyrins and Phthalaoyanines 3:230-37.

[19] Mac Donald, I.J. and Dougherty, T.J. (2001). Basic Principles of Photo dynamictherrpy Journal of Prophyrins and Phthalocyanines.5:105-129.

[20] W.X. Chen, W.Y. Lu, Y.Y. Yao, M.H. Xu, (2007). Journal of Enviromental. Science. Technology 41: 6240.

[21] Ali, H., Van Lier, J. E. (1999). Metal complexes as photo- and radiosensitizers. Chemical, Reviews 99:2379-2450.

[22] Nyokong, T. (2007). Effects of substituents on the photochemical and photophysical properties of non-transition metal phthalocyanines. Coordination Chemistry Reviews 251: 1707-1722.

[23] Ng, D. K. P. (2003). Dendritic phthalocyanines: synthesis, photophysical properties, and aggregation behavior. Comptes Rendus Chimie 6: 903-910.

[24] Achar, B. N., Fohlen, G. M. and Parker, J. A. (1987). Organic semiconductors-metal phthalocyanine sheet polymers. Journal of Polymer Science Part a 25:443-450.

[25] A.W. Bauer, W.M. Kirby, C. Sherris, M. Turack, , (1966). Antibiotic susceptibility testing by a standardized single disk method, American Journal of Clinical Pathology 45: 493-496

[26] National Committee for Clinical Laboratory Standards. (1993) Performance VOL.41,(1997) antimicrobial susceptibility of Flavobacteria.

[27] T.Mosmann, (1983).Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunology Methods 65: 55-63.

[28] P.Vijayan, C. Raghu, S.A. Dhanaraj, B. Suresh, (2004).Antiviral activity of medicinal plants of Nilgiris. Indian Journal of Medical. Research; 120, 24-29.

[29] A.M.Schaffer, M. Gouterman, E.R. Davidson, (1973) Theoretica Chimica Acta; 30 9.

[30] A.B.P.Lever, (1965).Advances in Inorganic Chemistry and Radiochemistry; 7, 27.