Preparation, characterization and biological activity of chromium phthalocyanine chloride complex

Aziza. A. K. and El Mugdad A. A

Department of Chemistry, College of Science, Sudan University of Science and Technology (SUST)

ABSTRACT: This work presents the preparation, purification, identification and applications of chromium (III) phthalocyanine chloride complex. Non-solvent method was used for the preparation 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: *Chromium phthalocyanine*, *non-solvent method*, *biological activity*, *(Hep-G2) cell*.

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].

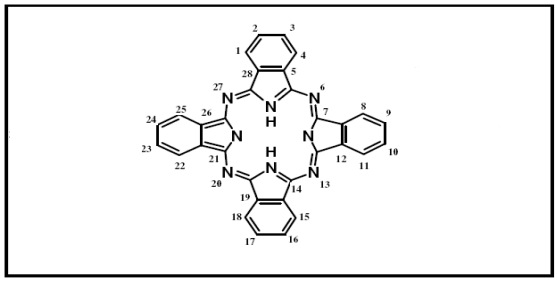

Fig. 1. Phthalocyanine structure

They are tetrabenzo tetraazaporphyrins [4, 5,6, 7, 8] They are pigment dyes that 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.

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 [18, 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].

There are literally thousands of chromium(III) complexes that with a few exceptions, are all hexacoordinate [23]. The principal characteristic of these complexes in aqueous solutions is their relative kinetic inertness. Ligand displacement reactions of Cr (III) complexes are only about 10 times faster than that of Co (III), with half-times in the range of several hours. It is largely because of this kinetic inertness that so many complex species can be isolated as solid as they persist for relatively long period of time in solution, even under conditions of marked thermodynamic instability [23].

The present study was set for the preparation of chromium phthalocyanine chloride complex (CrPc.Cl), characterization and the possibility of its use in biological and medical fields.

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

MATERIALS and METHODS

All chemicals, reagents and solvents, used were of the analytical grade (AR), and of highest purity, obtained from commercial suppliers. This study was carried out at Cairo University, Cairo, Egypt.

Spectrophotometric measurements were carried out using automated spectrophotometer UV–Vis (SHIMADZU Lambda 4B) ranged from 200 to 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 were 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 and DrTGA) 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.

Preparation of Chromium (III) Phthalocyanine chloride complex (CrPc.Cl)

Chromium(III) chloride hydrate salt (0.487g, 0.0031mol), 1.82g (0.0123mol) of phthalic anhydride, 4.243g (0.071 mol) of urea and 0.15g of ammonium molybdate were placed into a test-tube and heated in a sand-bath at around 190-220°C for 2-3 h and subsequently cooled to room temperature. Water (10 cm^3) was added and after thorough mixing the supernatant was removed by decantation. The residue 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 0.963 g, (87.7% 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[24], under standard conditions using Mueller–Hinton agar medium, as described by NCCLS [25].

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 sub-culturing. Cell culture cytotoxicity assays were carried out as described elsewhere [26, 27].

RESULTS

1. Preparation and Characterization:

The procedure used for the preparation of chromium phthalocyanine chloride yielded 87.7% of a compound having a blue colour with a green tinge. The metal complex was thermally stable, and gave a clear solution with concentrated sulfuric acid and was fairly soluble in dimethylsulphoxide (DMSO), dimethylformamide (DMF), chloroform ($CHCl_3$), tetrahydrofuran (THF), toluene and pyridine but insoluble in water and various organic solvents including ethyl alcohol, diethyl ether, carbon tetrachloride and benzene

UV-Vis spectral data for $1X10^{-5}$ M solution of the studied complex, recorded in the range 200–900 using DMSO as a solvent, showed maximum absorption (λ_{max}) at 685 nm with molar absorptivity ($\varepsilon = 2.7X10^3$ L mol^{-1} cm^{-1}). (Fig. 2).

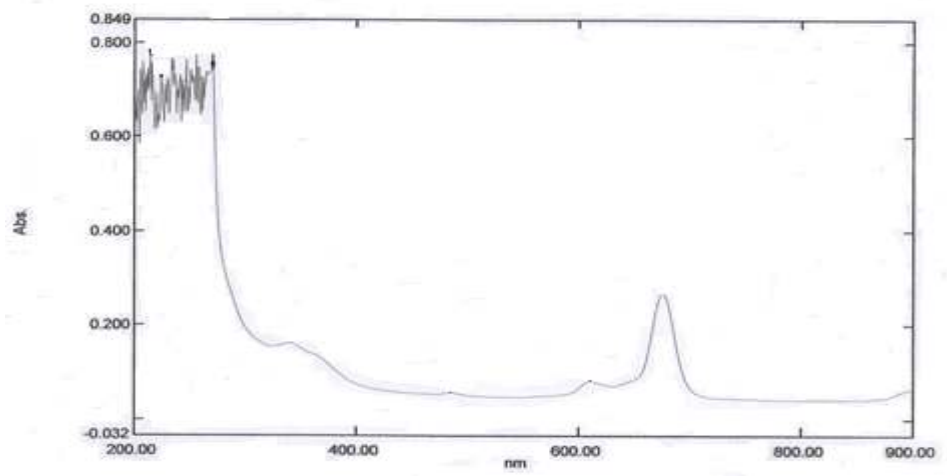

Fig. 2. UV-Vis spectrum of 1X10-5 M standard CrPc.Cl complex

IR spectral data (Fig. 3) of the title complex, showed characteristic absorption bands at 3431, 3050, 1611, 1520, 1470, 1450, 1332, 1289, 1160, 1114, 1072, 910 and 724 cm^{-1}. The MS spectrum of Chromium phthalocyanine complex (Fig. 4) showed a strong peak at m/z= 564. Other important signals appeared at m/z= 292, 141 and 127, 240 and 142. Results of elemental analysis for C, H, N, and metal content of the compound (Table1) are in agreement with the molecular formula of the complex.

Thermogravimetric curve for chromium phthalocyanine complex (Fig. 5), showed that the minor and major temperature for thermal decomposition was 360^{o} C and 605^{o} C, respectively.

The magnetic susceptibility (X_m) and magnetic moments (μ_{eff}) values of chromium phthalocyanine complex, in the solid state, average of three independent determinations, were 0.209 and 4.57, respectively.

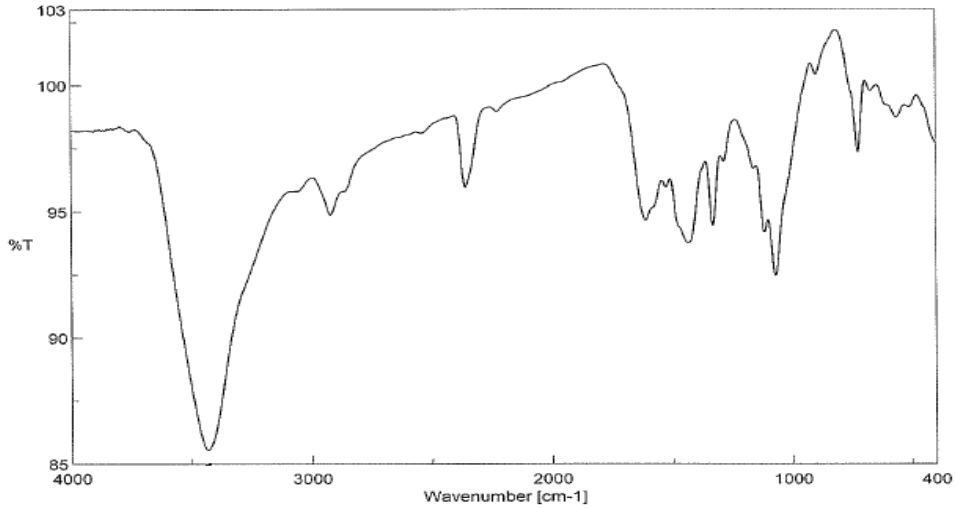

Fig. 3. IR spectrum of solid CrPc.Cl complex

Table 1: Analytical and physical data of chromium phthalocyanine complex

general formulae	Colour, (yield %)	m.p (^{o}C)	Elemental analysis Found (calcd %)			
			C	H	N	M
CrPc.Cl ($C_{32}H_{16}CrN_{8}.Cl$), mole mass =599	blue with green tinge (87.70)	<300	64.02, 64.17	2.42, 2.69	18.53, 18.71	8.31, 8.68

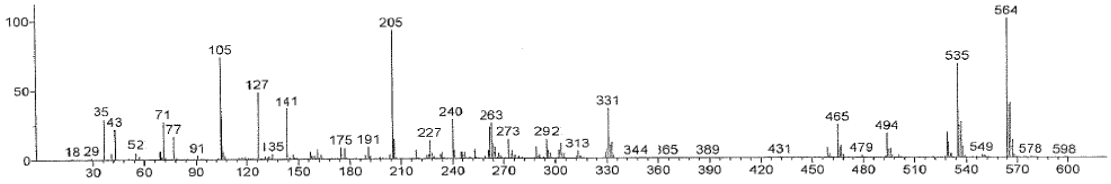

Fig. 4. Mass spectrum of CrPc.Cl complex

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2. Biological activity

2.1. Antimicrobial activity

The complex showed considerable activity against bacteria (Table 2). The inhibition zones were 12, 9, 10 and 11 for *Escherichia Coli*, *Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus,* respectively. However on the fungal species the compound showed negligible activity.

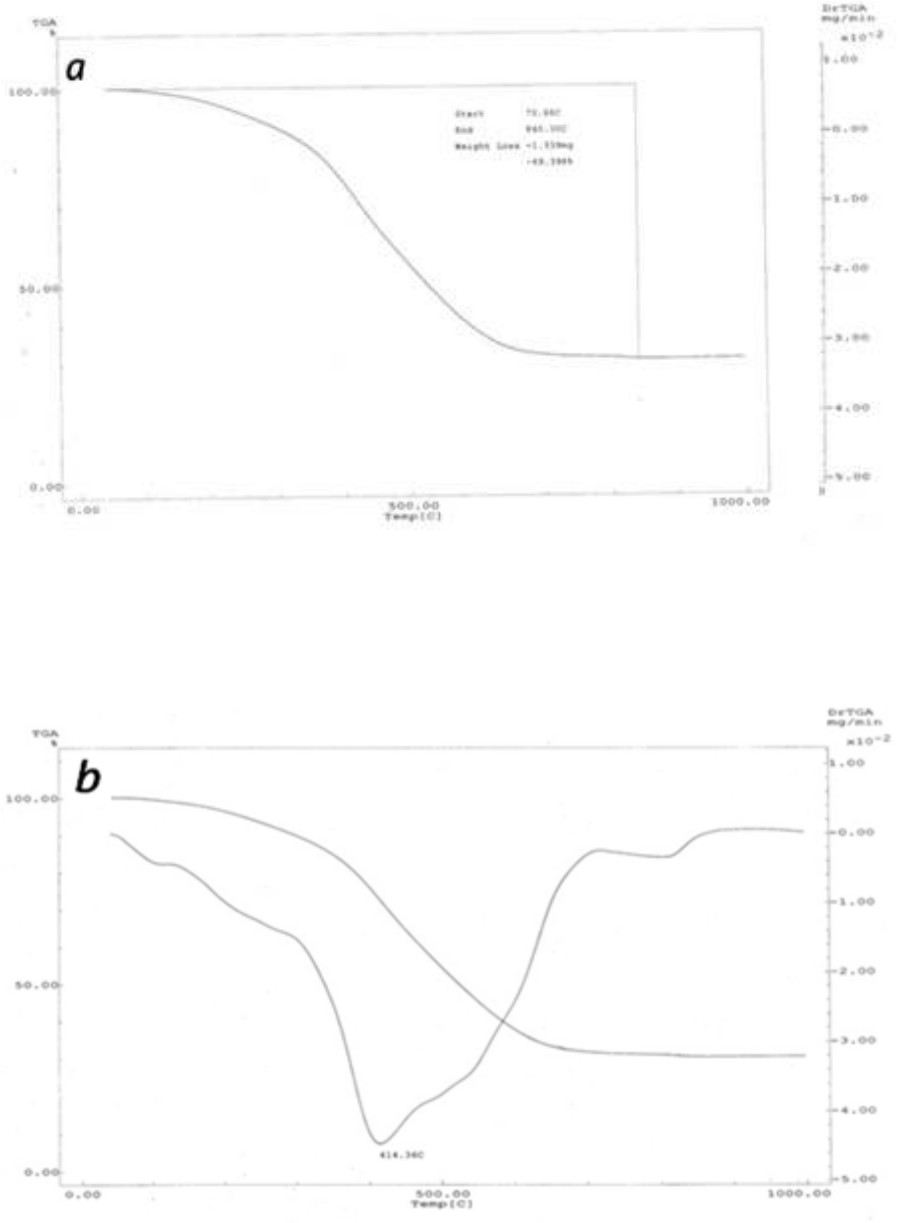

Fig. 5. Thermal analysis of CrPc.Cl(a) TG/DTG, (b) DTA

Table 2: The anti bacterial and antifungal activities of the tested compounds

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	9	10	11	0	0

2.2. 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 37.3. (Fig. 6).

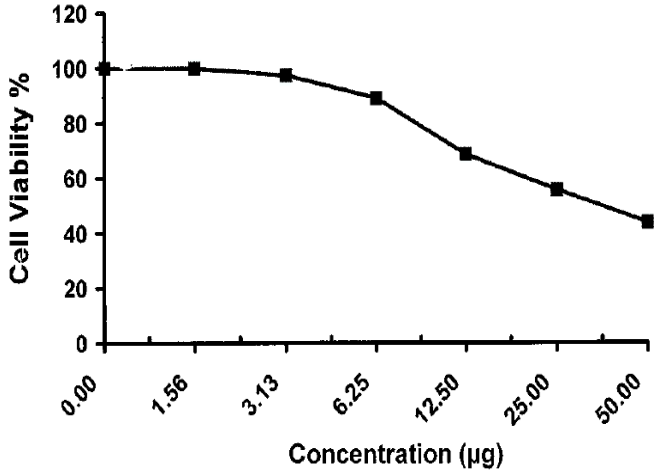

Fig. 6. Inhibitory activity of CrPc.Cl complex

DISCUSSION

The data revealed that the adopted methodology resulted in a chromium phthalocyanine complex with a high yield (87.70%). The antimicrobial and antitumor activities were studied.

The studied complex showed a major Q-band absorption around 685 nm and a shoulder like absorption around 595–620 nm, (Fig. 2). These absorption bands are consistent with the electronic configuration of porphyrin system. The deep bluish colour with a green tinge displayed by the complex is due to the absorption peaks in the Q band region [28].

The IR spectrum of the chromium phthalocyanine complex (Fig. 3) showed several bands. The band at 3431 cm^{-1} assigned as an OH vibration of adsorbed water. The band at 3050 cm^{-1} is due to CH asymmetric and symmetric stretching

vibrations in the ring. The band at 1611cm^{-1} assigned to the C–C stretching vibration in pyrrole and that at 1332 cm^{-1} assigned to C–C stretching in isoindole. The two bands at 1470 and 1450 cm^{-1} assigned to the C–H in plane bending vibration and that at 1520 cm^{-1} assigned to the C–H bending in aryl. The two bands at 1114 and 1072 cm^{-1} assigned to C–H bending in plane deformations. Also, the two bands at 910 and 724 cm^{-1} assigned to C–H bending out of plane deformations. The bands at 1289 and 1160 cm^{-1} assigned to the C–N in isoindole in plane band in pyrrole stretching vibration, respectively. Based on mass spectral data of chromium complex (Fig. 4). This complex has a strong molecular ion peak at m/z= 564. The peaks at m/z= 292, 141 and 127, may be due to the formation of fragment ions $[C_{16}N_3H_6Cr]^+$, $[C_8N_3H_3]^+$, $[C_8N_2H_3\ ]^+$. The signals at m/z = 240 and 142, these fragment ions may be due to the formation of $[C_{16}N_3H_6]^+$ and $[C_8N_3H_4]^+$, respectively.

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 is stable up to 350 °C and showed a loss in weight at temperature range of 350–920 °C. The loss in weight could be attributed to pyrolysis by a minor decomposition reaction at about 360°C and a major decomposition reaction at 605°C. The magnetic susceptibility measurements could give indication for the coordination environment of the Cr(III) 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 be arising 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 considerable antitumor activity against the studied cell line at 100 μdm^3 with IC_{50}=37.3 μg, 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**.**

CONCLUSIONS

- Chromium (III) phthalocyanine Chloride 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 fields.

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