CHAPTER-III

Experimental Methods

The details of experimental techniques including the synthesis of compounds and spectroscopic measurements are outlined in this chapter.

III 1 List of chemicals & reagents

Benzaldehyde (S D Fine)

N- bromosuccinamide (Merck)

Copper(II)acetate (S D Fine)

Copper(II)sulphate (S D Fine)

3, 4-dimethoxy benzaldehyde (S D Fine)

N,N-dimethylethylenediamine (Merck)

Iron(III)chloride (S D Fine)

Manganese(II)chloride (S D Fine)

Nickel(II)chloride (Merck)

Propionic acid (SRL)

Pyridine (S D Fine)

Pyrrole (SRL)

Quinoline (S D. Fine)

Sodium cyanide (S D Fine)

were commercially obtained and purified wherever necessary according to known procedures. All solvents were either laboratory reagent or analytical reagent quality. Appropriate purification procedures were employed whenever necessary.
III.2 Synthesis of Precursors

III.2.1 Copper(I) cyanide \([\text{I}]\)

Copper(I) cyanide was synthesized by mixing three different solutions under stirring conditions, viz., copper sulphate (10 g, 40 mmol) in 30 mL of water at 60° C, potassium dithionate (3 g, 15.8 mmol) in 10 mL of water at 50° C and sodium cyanide (2.8 g, 60 mmol) in 10 mL of water at 50° C. The copper sulphate solution was then made slightly acidic by adding few drops of dilute sulphuric acid (pH = 5.5). The cuprous cyanide precipitate was filtered, washed with hot water and then with rectified spirit and dried at 110° C for 24 h. Cuprous cyanide was stored in dessicator. Yield 2.85 g (80%).

III. 3. Synthesis of Porphyrins and Metalloporphyrins

III.3.1 Meso-5,10,15,20-tetraphenylporphyrin \([\text{I}]\)

Porphyrin \(\text{I}\) was synthesized by following Adler's method\(^2\) using pyrrole and benzaldehyde. Freshly distilled pyrrole (3.35 g, 3.5 mL, 50 mmol) and freshly distilled benzaldehyde (5.3 g, 5 mL, 50 mmol) were refluxed in propionic acid for 30 min. The mixture was cooled to 30° C and it was evaporated to dryness under vacuum. The solid residue was dissolved in benzene and the organic layer was twice washed with water. The crude porphyrin was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2 g, 9 mmol) and the mixture was refluxed for 1 h to oxidize chlorin impurity. The resultant solution was concentrated to dryness under vacuum.
The solid residue was dissolved in small volume of benzene and purified using a silica gel column with 1:1 mixture of benzene and hexane as eluant. The eluant was concentrated to dryness under vacuum. The R_f value on silica gel coated plate was found to be 0.8 in 9:1 hexane and chloroform. The purity of the sample was checked preliminarily by TLC followed by absorption spectrum for the other batches of preparation. Yield 1.38 g (18%); UV-Vis (CH_2Cl_2) λ, nm(log ε): 417 (5.64), 514 (4.17), 548 (3.80), 592 (3.65), 648(3.62). ^1H NMR (400 MHz, CDCl_3) δ 8.75 (s, 8H, βH), 8.3 (d, 8H, ArH_2,6), 7.8 (m, 12H, ArH_3,4,5), -2.6 (s, 2H, -NH); IR (KBr): 3052, 3023, 964 cm\(^{-1}\).

![UV-Vis spectrum of meso-5,10,15,20-tetraphenylporphyrin](image)

Fig. III.1 UV-Vis spectrum of meso-5,10,15,20-tetraphenylporphyrin
Complex III was obtained by refluxing I (0.12 g, 0.2 mmol) with freshly recrystallized N-bromosuccinamide (0.0994 g, 0.5 mmol) in 30 mL of chloroform for 1 h. A few drops of pyridine were added at cool condition for neutralizing the mixture. The solvent was removed, washed with methanol and dried in air. The product was then purified using preparative thin layer chromatography using silica gel. Benzene and cyclohexane in the ratio of 35:65 was used as eluant. Yield 0.063 g (37%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm($\log \varepsilon$): 429.0 (5.45), 524 (4.26), 558 (3.55), 604 (3.62), 665 (3.82). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.72 (s, 1H, $\beta$H), 8.30-8.10 (m, 4H, $\beta$H), 8.42 (d, 8H, ArH$_{3,5}$), 8.15 (m, 4H, ArH$_4$), 8.05-7.9 (m, 8H, ArH$_{2,6}$), -2.74 (s, 2H, N-H), IR (KBr): 3054, 3022 and 697 cm$^{-1}$; Anal. Calcd for C$_{44}$H$_{27}$N$_4$Br$_3$: C, 62.1; H, 3.2; N, 6.6%; found: C, 61.8; H, 2.9; N, 6.0%.

Fig. III.2 UV-Vis spectrum of 2,3,12-tribromo-5,10,15,20-tetraphenylporphyrin
Synthesis of this complex involved a procedure analogous to that of III, $H_2TPP(\text{Br}_3$ except that four equivalents of N-bromosuccinamide (0.187 g, 0.9 mmol) were used instead of 0.5 mmol. Yield 0.056 g, (30%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm (log $\varepsilon$): 436.0 (5.32), 533 (4.22), 575(3.53), 618(3.53), 689 (3.98). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.50 (m, 4H, $\beta$H), 8.40(d, 8H, ArH$_{3.5}$), 8.17 (m, 4H, ArH$_4$), 8.1-7.9(m, 8H, ArH$_{2,6}$), -2.74 (s, 2H, N-H); IR (KBr): 3373, 3053 and 690 cm$^{-1}$; Anal. Calcd for C$_{44}$H$_{26}$N$_4$Br$_4$: C, 56.8; H, 2.8; N, 6.0%; found: C, 56.3; H, 2.6; N, 5.5%.

Fig. III.3 UV-Vis spectrum of 2,3,12,13-tetrabromo-5,10,15,20-tetraphenylporphyrin
III.3.4 [2,3,12-tribromo-5,10,15,20-tetraphenylporphyrinato]copper(II) (V)

This complex was obtained by refluxing III (0.5 g, 0.7 mmol) in 50 mL of dimethylformamide with saturated solution of copper(II)acetate in methanol for 1 h. The completion of the reaction was checked by thin layer chromatography and absorption spectrum. The reaction mixture was concentrated and purified through silica gel column (100 – 200 mesh) using hexane and chloroform in the ratio of 7:3 as eluant. Yield 0.061 g (95%).

III.3.5 [2,3,12,13-tetrabromo-5,10,15,20-tetraphenylporphyrinato]copper(II) (VI)

This composite was set and purified by following the procedure as given for V, except that IV (0.5 g, 0.7 mmol) was used in place of III. Yield 0.066 g (95%).
III.3.6 [2,3,12-tricyano-5,10,15,20-tetraphenylporphyrinato]copper(II) $VII$

& [2,3,12,17-tetracyano-5,10,15,20-tetraphenylporphyrinato]copper(II)$^3$ $VIII$

About 0.08 g (0.9 mmol) of copper(I) cyanide and 0.080 g (0.094 mmol) of corresponding $VII$ were taken in 15 mL of quinoline and refluxed for about 1h. The reaction mixture was diluted with 100 mL of chloroform after 1 h of reaction. Then the solution was washed with 1:5 dilute hydrochloric acid for about 4 to 5 times. The organic layer was then evaporated to dryness. Cu(II)TPP(CN)$_3$ (5%) was also obtained as minor product along with Cu(II)TPP(CN)$_4$ (40%). These mixtures were then separated by column chromatography using chloroform and hexane in the ratio of 2:8 as the eluant. Cu(II)TPP(CN)$_3$ comes out of the column first followed by the Cu(II)TPP(CN)$_4$. A similar procedure was followed for synthesizing Cu(II)TPP(CN)$_3$ using compound V. IR (KBr): 2219 cm$^{-1}$ for tricyano copper(II)porphyrin and tetracyano copper(II)porphyrin. UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm($\log \varepsilon$): 436.0 (5.51), 638(4.12)

![UV-Vis spectrum](image)

Fig. III.4. UV-Vis spectrum of [2,3,12,17-tetracyano-5,10,15,20-tetraphenylporphyrinato]copper(II)
III.3.7 2,3,12-tricyano-5,10,15,20-tetraphenylporphyrin [I\lambda]

About 0.05 g (0.7 mmol) of \textit{VII} was dissolved in 5 mL of concentrated sulphuric acid, stirred for 3 min at room temperature, poured in concentrated aqueous ammonia and then extracted with chloroform. The organic layer was then evaporated to dryness and then chromatographed on silica gel column. Yield 0.37 g (80\%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm (log e): 440.0 (5.37), 542 (4.23), 584(4.01), 640(3.89), 701 (4.36). 

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.50 (s, 1H, $\beta$H), 9.20-9.05 (m, 4H, $\beta$H), 8.30(d, 8H, ArH$_{3,5}$), 8.15 (m, 4H, ArH$_{4}$), 8.05-7.95(m, 8H, ArH$_{2,6}$), $\sim$2.68 (s, 2H, N-H); IR (KBr): 2219 cm$^{-1}$; Anal. Calcd for C$_{47}$H$_{27}$N$_{7}$: C, 81.9; H, 3.9; N, 14.2%; found: C, 80.9; H, 3.3; N, 14.1%; El-MS, m/z 690, [M]$^+$

![UV-Vis spectrum of 2,3,12-tricyano-5,10,15,20-tetraphenylporphyrin](image)

Fig. III.5 UV-Vis spectrum of 2,3,12-tricyano-5,10,15,20-tetraphenylporphyrin
This compound was obtained by following the procedure as mentioned for IX except that for VIII (0.05 g, 0.7 mmol) instead of VII Yield 0.37 g (80%) UV-Vis (CH₂Cl₂) λ, nm (log ε) 442 0 (5.51), 553 (4.01), 599 (4.24), 665 (3.89), 728 (4.56)

¹H NMR (400 MHz, CDCl₃) δ 8.97 (s, 4H, βH), 8.13 (d, 8H, ArH₃,3), 7.98 (d, 4H, ArH₄), 7.85 (m, 8H, ArH₂,6), -2.69 (s, 2H, N-H), IR (KBr) 2219 cm⁻¹, Anal Calcd for C₄₈H₂₆N₈ C, 80.7, H, 3.6, N, 15.7%, found C, 80.3, H, 3.2, N, 14.9%, El-MS, m/z 715, [M]⁺

Fig. III 6 UV-Vis spectrum of [2,3,12,17-tetracyano-5,10,15,20-tetraphenylporphyrin]
This complex XI was obtained by refluxing (50 mg, 0.07 mmol) IX in 50 mL of dimethylformamide with excess of iron(III)chloride for about one hour. The completion of the reaction was checked by thin layer chromatography and absorption spectrum. The reaction mixture was concentrated and purified through silica gel column using chloroform. The eluate was then shaken with excess of 2M hydrochloric acid for 48 h. The organic layer is evaporated and re-crystallized from a mixture of dichloromethane and petroleum ether. Yield 0.04 g (73%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm (log $\varepsilon$): 308(4.07), 442(4.35), 509 (4.18), 611(3.51).

Fig. III.7. UV-Vis spectrum of [2,3,12-tricyano-5,10,15,20-tetraphenylporphyrinato]iron(III)chloride
This compound was obtained by following the procedure similar to that of $XI$ except that compound $X$ was used in place of $IX$. Yield 0.04 g (71%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm (log $\varepsilon$): 380 (5.02), 446 (4.79), 629 (4.74).

Fig. III.8 UV-Vis spectrum of [2,3,12,13-tetracyano-5,10,15,20-tetraphenylporphyrinato]iron(III)chloride
This complex was synthesized similar to that of \textit{XI} except that manganese(II) acetate was used in place of iron(III) chloride. The completion of the reaction was checked by thin layer chromatography and absorption spectrum. The reaction mixture was concentrated and purified through silica gel column using chloroform. The eluate was then shaken with excess of 2M hydrochloric acid for 48 h. The organic layer is evaporated and re-crystallized from a mixture of dichloromethane and petroleum ether. Yield 0.035 g (64\%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm ($\log e$) 438 (4.62), 506 (5.31), 594 (4.09), 638 (4.22), 688 (4.67).
III.3.12 [2,3,12,13-tetracyano-5,10,15,20-tetraphenylporphyrinato] manganese(III) chloride [XIV]

This compound was obtained by following the procedure similar to that of XIII except that $X$ was used in place of $IX$. Yield 0.04 g (71%). UV-Vis (CH$_2$Cl$_2$) $\lambda$, nm ($\log \varepsilon$): 442.0 (4.82), 512 (5.31), 598(4.19), 643(4.42), 714 (4.67)

![UV-Vis spectrum](image)

Fig. III.10. UV-Vis spectrum of [2,3,12,13-tetracyano-5,10,15,20-tetraphenylporphyrinato]manganese(III) chloride
To 50 mL of dry dimethylformamide I (0.5 g, 0.8 mmol) was added followed by addition of saturated anhydrous nickel(II)chloride solution under nitrogen atmosphere. The mixture was refluxed for 6 h under nitrogen atmosphere. The completion of the reaction was checked by thin layer chromatography and electronic absorption spectrum. The reaction mixture was concentrated and purified through silica gel column using chloroform as eluant. Yield 0.35 g (65%). UV-Vis (CH$_2$Cl$_2$) 
\lambda, \text{nm (log } \varepsilon) : 426.0 (5.27), 625 (4.27).
Fig III 12  Mass spectrum of \( V/H \)
Fig III.13 Mass spectrum of VIII
Fig. III 14  NMR spectrum of IX
Fig III.16. Proposed Molecular structure of various $\beta$-substituted porphyrins.
III.4 Instrumental Methods

III.4.1 NMR

$^1\text{H}$ NMR spectra were recorded on Bruker 300MHz spectrometer in CDCl$_3$ solution, using TMS as the internal standard, chemical shifts are reported in $\delta$ units downfield from TMS. Abbreviation used s: singlet, d: doublet, t: triplet, m: multiplet. The NMR spectra were obtained by Dr. H. Surya Prakash Rao, from the University of Nijmegen, Netherlands and also by Dr. C.V. Asokan, University of Leuven, Belgium.

III.4.2 Mass spectrum

Electron impact (EI) mass spectra were recorded at room temperature on a JEOL D-300 mass spectrometer. The mass spectra were obtained by Dr. C.V. Asokan, University of Leuven, Belgium.

III.4.3 Absorption spectrum

Electronic absorption spectra were obtained with OCEAN OPTICS UV-VISIBLE - SD-1000, CCD based spectrophotometer using 1 cm quartz cell in dichloromethane as solvent.

III.4.4 IR

Infrared spectra of the ligands and complexes were recorded on ABB Bomem MB-104 FTIR spectrometer using KBr disks, available in our University.

III.4.5 Elemental analysis

Elemental (C, H and N) analysis was performed with Heraus Rapid analyzer. The data were obtained from Central Drug Research Institute, Lucknow, India.

III.4.6 EPR measurements

X-band EPR spectra were recorded with JEOL JES-TES 100 ESR spectrometer in our laboratory, having 100 kHz field modulation. The variable range
of magnetic field is from 0-650mT. A $\text{TE}_{011}$ cylindrical cavity resonator (model ESUCX2) was used for all the measurements.

EPR spectra at liquid nitrogen temperature (77 K) were obtained using a cold finger Dewar (Model ES-UCD3X). A portion of this dewar, in which the sample has to be placed, employs a structure that prevents bumping of liquid nitrogen. The inner diameter of the sample insertion portion is 5.1mm and the capacity of dewar bottle is 120ml. Dry nitrogen gas was passed through the cavity throughout the measurements.

EPR spectra at 108K were recorded using a Digital Variable Temperature Unit. (Model ES-DVT3). Desired temperatures can be easily obtained by sending the hot air/gas or cold gas which is controlled manually (off-line mode) or by the computer (on-line mode) to the sample area. In the temperature range from $-170 \degree C$ to $+100 \degree C$, the temperature is automatically controlled by evaporating liquid nitrogen in the metal dewar and heating nitrogen gas with heaters. Furthermore, in the temperature range higher than $50 \degree C$ the temperature is automatically controlled by the heater, which heats the air from the air-compressor.

III.5. Estimation of the cavity constant $K_Q$

The cavity constant tells us about how much of the microwave radiation is absorbed by the system. We know that

$$B_1^2 \propto P$$

i.e. $B_1^2 = K_Q P$ where, the cavity constant $K_Q$ is given by

$$K_Q = \frac{1}{P_{1/2}^2 \gamma^2 T_1 T_2}$$  \hspace{1cm} (III.1)

where $P_{1/2}$ is the power for which the signal intensity is one-half the unsaturated intensity and it can be experimentally measured as described in the section (III.5).
The signal intensity can be fitted into the equation (III.1) to find the unknown parameter $K_Q$.

The value of $P_{1/2}$ calculated for tanol (1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine) was about 5.06mW. A solution of tanol in secondary butyl benzene was introduced inside a cold finger dewar inserted inside the cavity. The value of $T_1$ (0.43μsec) for tanol at 30 °C has been taken from the literature. True linewidth is obtained from the Fig. by extrapolating the linear-fit curve to zero power. $T_2$ was calculated using the equation III.2. $T_2$ value calculated using tanol was 0.1597μs.

![Plot of linewidth (δ) for tanol in toluene at low power (0.1mW-1mW)](Fig. III.17)
Linewidth at zero power is obtained by extrapolating the linear-fit curve

The calculated cavity constant ($K_Q$) using tanol is $3.7246 \times 10^{-7}$ T$^2$/W

**III.6. CW-saturation measurements**

Stock solutions of Cu(II)TPP were prepared in toluene/dichloromethane and the concentration of Cu(II)TPP was kept constant at 1mM throughout. The other reagent was added in varying concentrations in the range of 0.1 to 100mM. The EPR
spectra were recorded at liquid nitrogen temperature using a cold finger dewar or variable temperature unit, for each sample in the range of 0.1 mW to 15 mW. Then the intensity of the outermost copper line in the $g_\parallel$ region was measured for each microwave power, since this line is relatively free from overlap effects. The intensity was obtained by finding the area under the curve, which looks like an absorption curve. The area was obtained by counting the number of small squares in the recorded spectrum. The intensities obtained in this manner are used in the saturation profile plots. The intensity-power plots are fitted using equation (1.27) for a single $P_{1/2}$ value using the ORIGIN package. The package ORIGIN uses either SIMPLEX method or non-linear least-squares method for optimization. Both the methods were found to give identical results.

A plot of $P_{1/2}$ for Cu(II) verses concentration of the other reagent was obtained. The values of $T_2$ are obtained from the EPR spectra recorded in the low power range (0.1 mW-1 mW). The linewidth under non-saturation conditions, for each spectrum was estimated by matching with computer simulated EPR powder spectrum. The linewidth at zero power ($\delta$) was obtained by extrapolation of the linear fit curve. $T_2$ was calculated from the linewidth, using the relation,

$$T_2 (sec) = \frac{2}{\delta (Hz)}$$  \hspace{1cm} (III.2)

Since, $P_{1/2} = \frac{1}{K_Q \gamma^2 T_1 T_2}$ where $K_Q$ is the cavity constant

$$T_1 = \frac{1}{K_Q \gamma^2 T_1 T_2}$$  \hspace{1cm} (III.3)

For a two component system, the integrated spectral intensity, $S(B_1)$ is additive.

$$S(B_1) = f S_A (B_1) + (1-f) S_B (B_1)$$  \hspace{1cm} (III.4)
where \( B_1 \) is the amplitude of the microwave field, \( S_A \) and \( S_B \) are the integral intensities of the two components \( A \) and \( B \), which are normalized in the absence of saturation and \( f \) is the fraction of the integrated spectral intensity contributed by the component \( A \) in the absence of saturation.

We have not made any assumption regarding homogeneous or inhomogeneous broadening. Instead we have tried to use a floating variable to determine the degree of homogeneity. The individual line shape is given by

\[
S_i(B_1) = \frac{A_i B_i}{(1 + \gamma^2 B_1^2 T_i T_a)^b}
\]

\( i = A \) or \( B \)

\( b = 1/2 \) for inhomogeneous broadening and 1 for homogenous broadening and \( B_1 \propto (P)^{1/2} \) where \( P \) is the microwave power.

For a general system with homogenous and inhomogeneous broadening effect the intensity for the two component systems is given by

\[
S(B_1) = A_0 \sqrt{P} \left( \frac{f}{1 + P/P_{1/2}^A} \right)^b + \left( \frac{1-f}{1 + P/P_{1/2}^B} \right)^b
\]

(III.6)

here \( b \) is treated as adjustable parameter. In the ideal case, \( b=1/2 \) for inhomogeneous broadening and 1 for homogenous broadening. The experimental data consists of the values of \( P \) and the corresponding values of \( S \). \( P_{1/2}^A \) and \( P_{1/2}^B \) values are given as input parameters. A non-linear curve fitting procedure available in the graphics package ORIGIN was used to obtain the unknown parameters \( f \) and \( b \).

III.7 Computer simulation

The EPR spectra of frozen solution were simulated using the computer programme 'POWDER' developed in our laboratory over the past few years. The algorithm involves perturbation calculation of energy levels to second order. Numerical integration over angular variables is done using a Gaussian ten-point
quadrature method. The frequency sweep approach recommended by Pilbrow\textsuperscript{9} is used for calculating the spectrum at each field point. The input parameters are the microwave frequency, linewidth (MHz), g-values and hyperfine coupling constant (MHz). The experimental spectrum is taken on a transparency and matched with the simulated spectrum for obtaining the best fitting for the Hamiltonian parameters.

III 8 References

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