

SYNTHESIS, GROWTH, SPECTRAL, THERMAL, OPTICAL, BAND GAP STUDIES AND ANTIBACTERIAL ACTIVITY OF PHENYLHYDRAZONE OF 4 –HYDROXY- N –PHENYL ACETAMIDE (PHHPA) CRYSTAL.

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Abstract

Phenylhydrazone of 4-hydroxy-N-phenylacetamide (PHHPA) is a new organic non linear optical material has been successfully grown by slow evaporation solution growth technique at room temperature. The crystal obtained the above technique were subjected to different characterization analysis. Crystalline nature of the sample was confirmed by powder X-ray diffraction method. A functional group present in the crystal was identified by using FT-IR spectral analysis. Optical properties of the crystals examined by UV-Visible studies. The thermal stability of the grown crystal was found by differential thermal analysis and differential scanning calorimetry (DTA/DSC). Proton NMR spectra was recorded to elucidate the molecular structure. Band gap determination of crystal support the application oriented properties of the material. Antibacterial activity of grown crystals were examined by using the Disc Diffusion Assay method.

Keywords: Crystallization, FT-IR, UV-Visible, X-ray diffraction, Thermal analysis, Band gap studies, Antibacterial activity.

INTRODUCTION

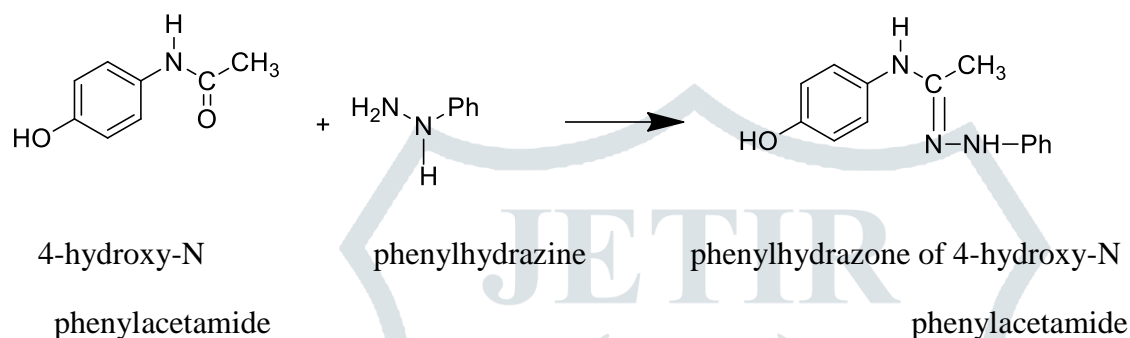
Crystal growth is a wide area of science and technology , which plays a major role in technology of photonics . Rare earth complexes have different properties such as optical, electrical and magnetic. These type of materials utilized in many field for example photoluminescence, electroluminescence and laser etc. The non linear optical (NLO) molecule materials have great impact on information technology and industrial applications.(1-18)

Organic non linear optical crystals with aromatic rings have high non linearity, fast response and tailor made flexibility, low mobility, and higher laser damage threshold when compared to inorganic materials. Thermal, spectral and XRD studies are important tools in the characterization of various materials (1-18). Here crystal of phenylhydrazone of 4-hydroxy N –phenylacetamide has been grown by slow evaporation solution growth technique and the grown crystal were characterized by FT-IR , UV ,H1 NMR , X-ray diffraction (XRD) and TGA-DSC studies. Band gap study and antibacterial activity was additionally determined to ascertain the application oriented properties of PHHPA.

EXPERIMENTAL

The semi organic crystal of phenylhydrazone of 4-hydroxy-N –phenylacetamide (PHHPA) is prepared by adopting general procedure (19). To a hot solution of phenyl hydrazine in methanol, a solution of 4-hydroxy-N –phenylacetamide in methanol was added drop wise during 30 minutes. The mixture was stirred and refluxed for 4 hours. Then it was filtered and the filtrate was concentrated to half the volume. After a slow evaporation of the concentrate at room temperature, crystals were collected by filtration, washed with cold ethanol and then dried. The grown crystals were purified by repeated-recrystallization. These crystals were grown by slow evaporation solution growth technique using methanol as a solvent. The harvested crystals are shown in Figure 1.

REACTION



RESULT AND DISCUSSION

FT-IR SPECTRAL STUDIES

Fourier transform infrared spectra study was carried out in the range of 400-4000 cm^{-1} using PERKIN ELMER FT-IR spectrometer. The absorption range of the grown crystal of PHHPA is appeared in Fig2. The peak at 3201.13cm^{-1} relating to phenyl hydroxide group (20). The presence of peak at 1566.48cm^{-1} is certify to the C=N immine group. The observed peak at 1505.42 and 1444.41cm^{-1} is due to presence of phenyl ring.

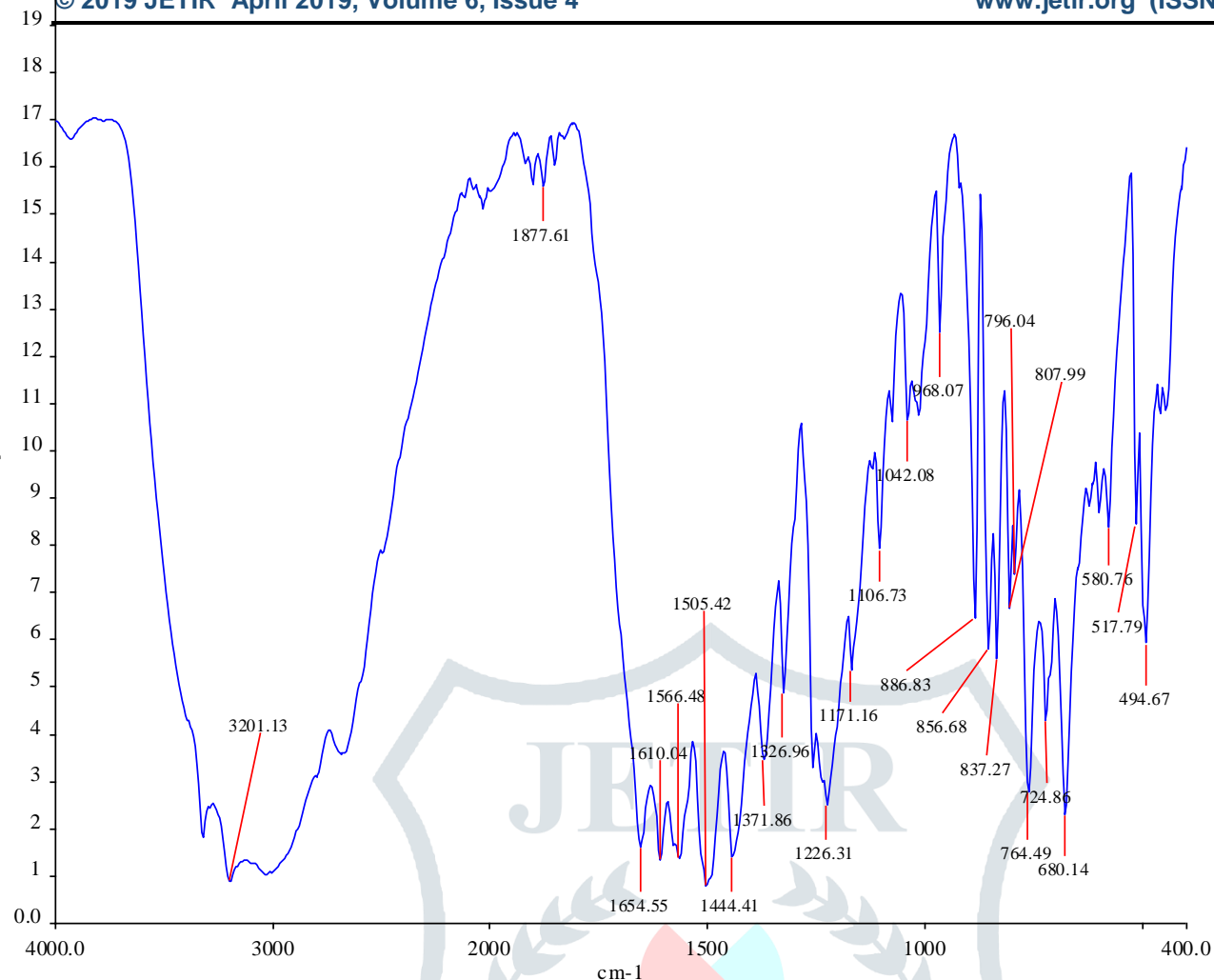


Figure.1 FT-IR Spectrum of phenyl hydrazone of 4-hydroxy- N -phenylacetamide

Table.1. FT-IR Spectral data of phenyl hydrazone of 4-hydroxy- N -phenylacetamide

S.No	Frequency cm^{-1}	Group identification
1	1566.48	C=N Stretching
2	1505.42 1444.41	Phenyl ring
3	3201.13	OH – Stretching
4	1610	NH-Bending
5	1326	Phenyl nitrogen

UV –VISIBLE ANALYSIS

The UV visible spectrum of phenylhydrazone of 4-hydroxy- n-phenylacetamide crystal was recorded in LAMBDA 25 spectra meter in the range of 200-900 nm. The recorded range is appeared is shown in the Figure 3. The characteristics absorption of PHHPA is found at 200-300 nm. Two sharp peak is observed at 222.33 and 268.94 nm (21). There is no absorption between 300-900nm. The UV-Visible spectrum is support the optical nature of the phenylhydrazone of 4-hydroxy- n-phenylacetamide. It further shows that the harvested crystal may have appreciable NLO property.

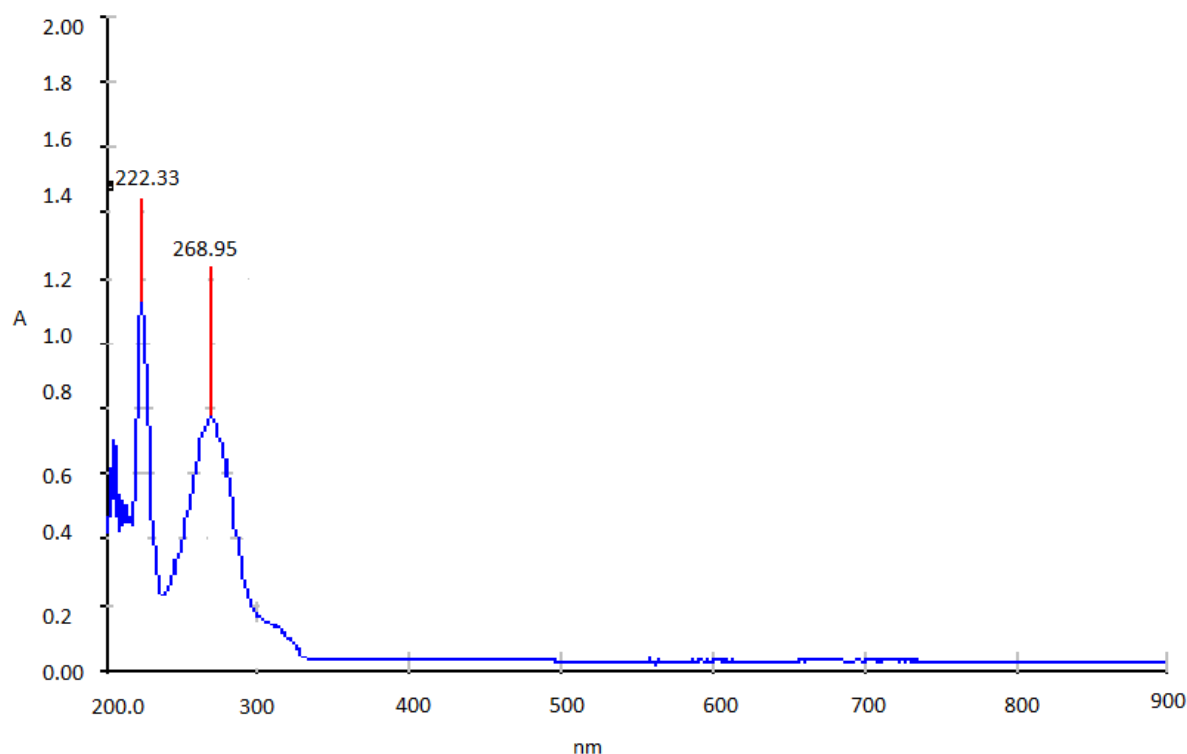


Figure.2.

UV-Visible Spectrum of phenylhydrazone of 4 –hydroxy- N –phenyl acetamide

BAND GAP ENERGY CALCULATION

The Band gap is the major application to determine the electrical conductivity of solid. The UV-Visible spectrum wavelength of grown crystal of Phenylhydrazone of 4-hydroxy- N –phenylacetamide was found to be 222.33 and 268.94 nm. The band gap energy of PHHPA crystal is assessed from the relationship between absorption coefficient and photon energy.

$$(\alpha h\nu) = A(h\nu - E_g)^n$$

Where A is a constant, E_g is the band gap of the absorption coefficient, $h\nu$ is an incident photon energy and n is the directly allowed transition. Figure.4. Show the plot between $(\alpha h\nu)^2$ and eV of PHHPA crystal. The value of band gap energy for prepared crystal is 4.19 eV. It refers to the energy difference between the top of the valence band and bottom of the conducting band in insulator and semi conductor.

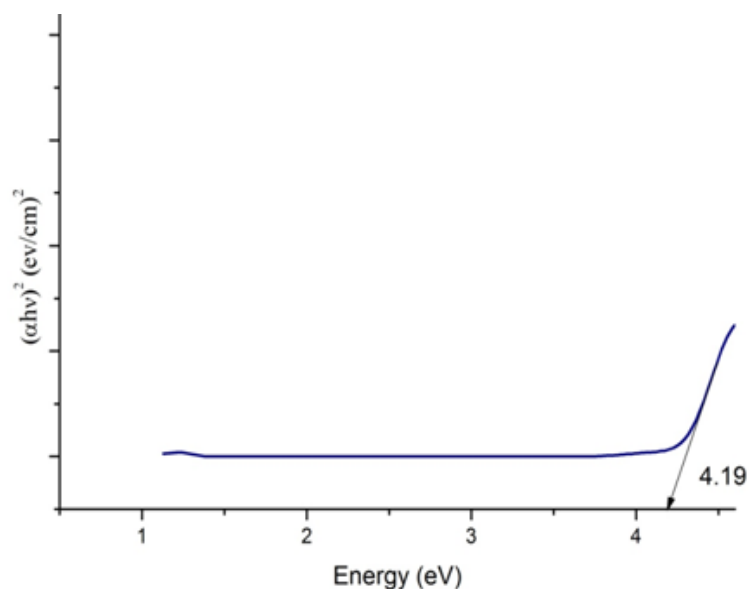


Figure: 3. Band gap energy of phenylhydrazone of 4-hydroxy N-phenylacetamide

NMR SPECTRAL ANALYSIS

The nuclear magnetic resonance spectral analysis is useful in the determination of the molecular substance based on the chemical environment of the crystal. ¹H NMR spectrum is determined by using BRUKER AV300. The ¹H NMR spectra of PHHPA is appeared in figure.5. A peak is observed at 1.979 ppm is affirm the methyl group. The signal at 2.507 ppm confirms the C-NH proton. The doublet peak at 6.661-6.690 ppm affirm the phenyl hydroxide (22-26). The multiplet peak at 6.923-7.358 ppm is due to the presence of aromatic group. The peak at 10.197 ppm is affirm the presence of aromatic N-H proton.

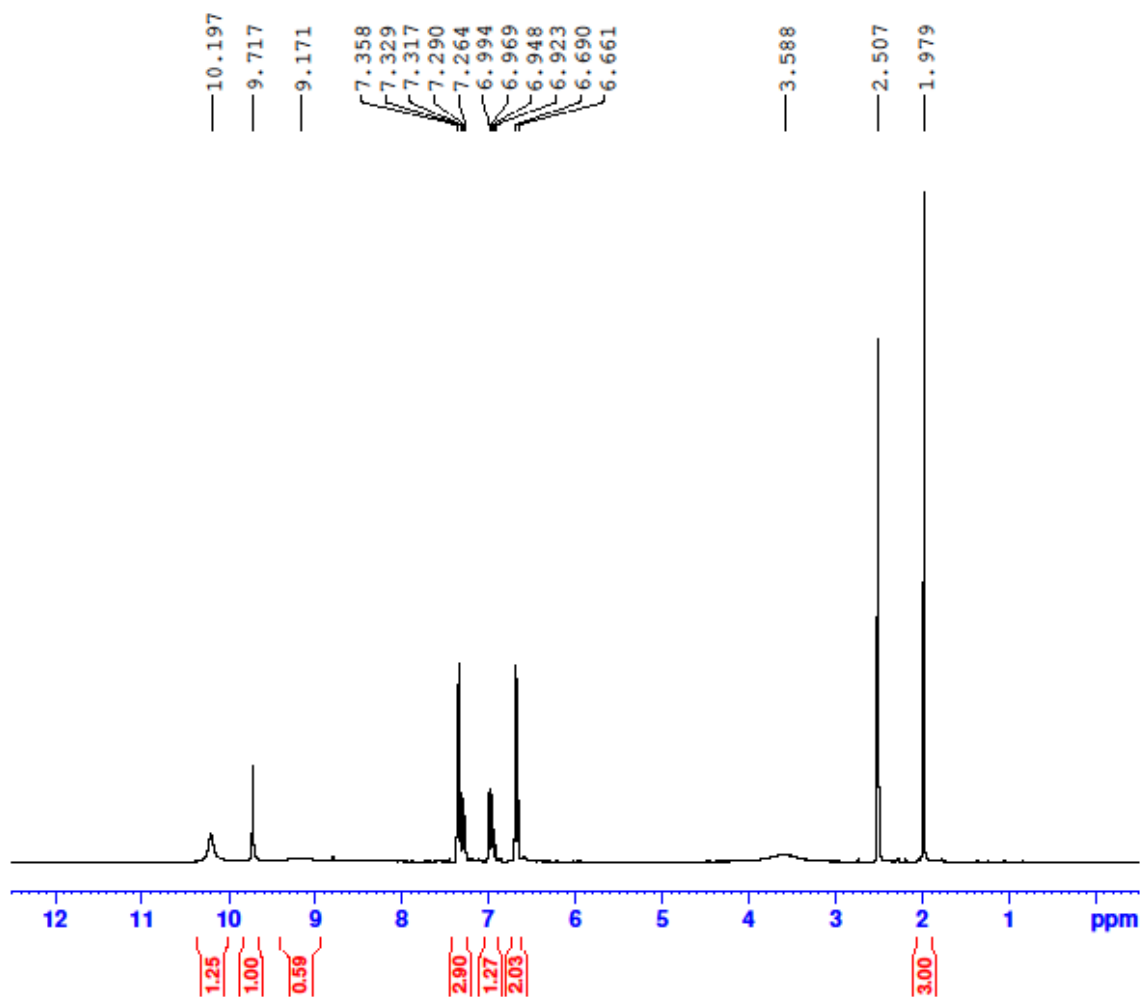


Figure.4. ^1H NMR Spectrum of phenylhydrazone of 4 –hydroxy- N –phenylacetamide

Table .2. ^1H NMR Spectral data of phenylhydrazone of 4 –hydroxy- N –phenyl acetamide

S.No	Signal at ppm	Group identification
1	1.979	Methyl
2	2.507	C-NH proton
3	6.661-6.690	phenyl hydroxide
4	6.923-7.358	aromatic group
5	10.197	aromatic N-H proton

X-RAY DIFFRACTION STUDIES

Phenylhydrazone of 4-hydroxy- N –phenylacetamide was carried out using BRUKER D8 advanced powder diffractometer with $\text{Cu K}\alpha$ radiation (1.5418 Å). The grown crystal was scanned at a rate of $1^\circ/\text{min}$ in the range of temperature $20-80^\circ\text{C}$. The X-ray diffractometer shown in figure.6. The sharp peak is observed in the powder XRD pattern and it confirms the crystalline nature of the harvested crystal (27-28). PHHPA shows the application oriented properties of harvested crystals.

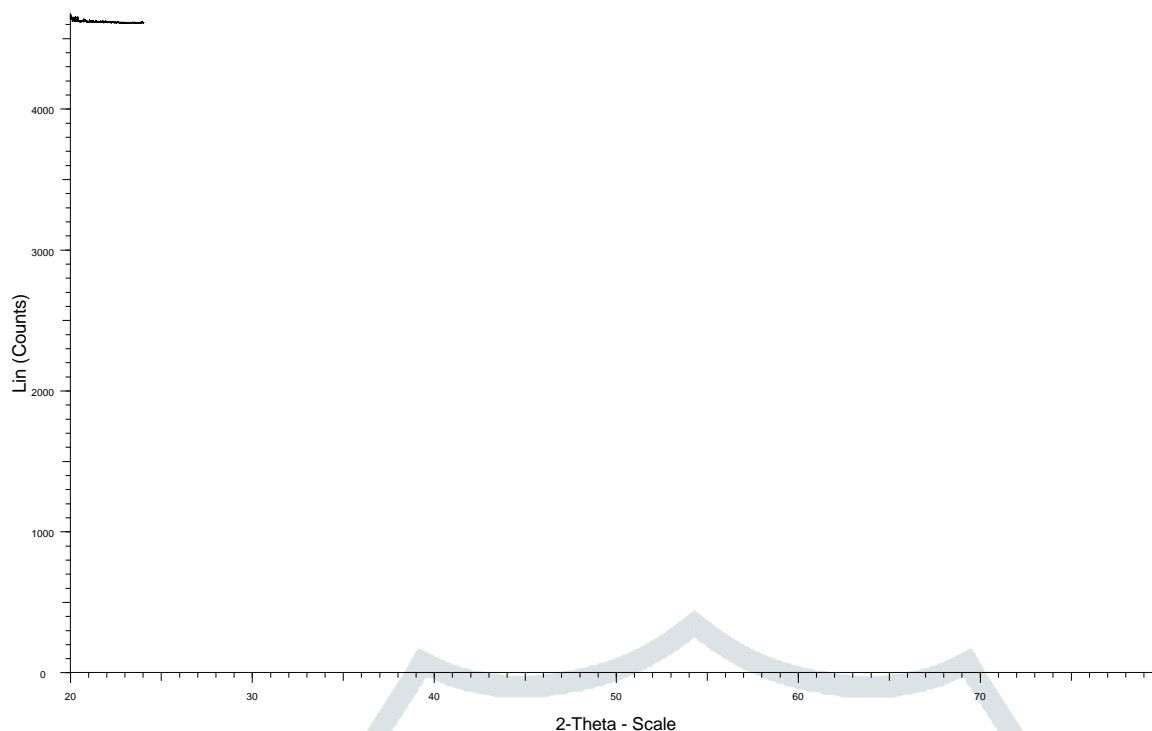


Figure.5. X-ray diffraction pattern of phenylhydrazone of 4 –hydroxy- N –phenyl acetamide

THERMAL ANALYSIS

Phenylhydrazone of 4-Hydroxy-N –Phenylacetamide (PHHPA) was analysed in thermally using SDTQ 600V 209 BUILD 20 instrument between the temperature 0°C to 500°C at a heating rate of 10°C/min under nitrogen atmosphere. The thermogram values shown in figure 7. It provides information about thermal properties of the material. The PHHPA sample weighing 4.6810 mg is taken for measurement. The crystal start with melting at 140°C. The endothermic peaks at 160°C, 250°C, and 320°C. The thermogram shows the thermal stability and crystalline nature of the grown crystal (29). The sharpness of the endothermic peak confirms the sample shows the good degree of crystallinity and purity of the crystal.

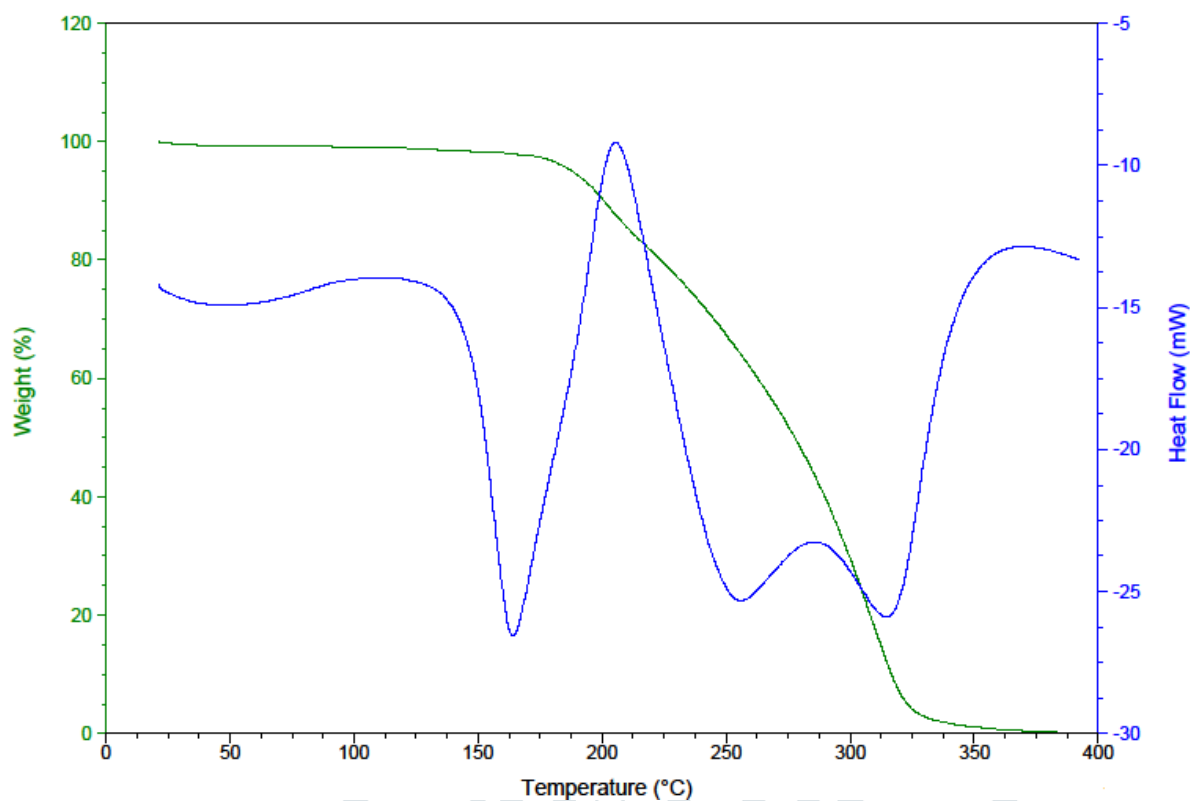


Figure.6. Thermogram of phenylhydrazone of 4 –hydroxy- N –phenylacetamide

ANTIBACTERIAL ACTIVITY

In vitro antimicrobial evaluation of Phenylhydrazone of 4-Hydroxy-N –Phenyl acetamide compound were carried out against 8 bacterial strains, which includes 4 Gram-positiv bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus mutans*) and 4 Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Schigella flexneri*). The selected nonlinear optical compounds were screened for antimicrobial activity using the Disc Diffusion Assay (31). The Petri plates (9 cm dia.) were pre-seeded with 10 ml of Muller Hinton Agar and stock culture was streaked thoroughly to ensure uniform distribution of the micro-organisms. Sterile paper discs (5 mm diameter) containing 100 μ g/ml of compounds screened for antibacterial activity.

Simultaneously, paper discs dipped with pure respective organic solvents were used as positive controls. The Petri plates were then pre-incubated for 3 h at 5°C to permit maximum diffusion of the compounds into the media. Cefalexin and Gentamycine (10 μ g/ml) was used as negative control against gram positive and gram negative bacteria respectively (32) were used as reference standards. After the incubated period the zone of inhibition (diameter) was measured with a scale and the data were tabulated.

Table.3. Antibacterial activity data of Phenylhydrazone of 4-hydroxy- N -phenylacetamide compound (10µg) tested against selected human pathogenic bacteria.

Microorganism tested		Zone of inhibition(mm) diameter	
		Nonlinear optical compound	
		PC*	COM1
Gram positive	<i>Bacillus subtilis</i>	10	8
	<i>Micrococcus luteus</i>	12	13
	<i>Staphylococcus aureus</i>	11	-
	<i>Streptococcus mutans</i>	-	-
Gram negative	<i>Escherichia coli</i>	8	-
	<i>Klebsiella pneumonia</i>	9	-
	<i>Proteus vulgaris</i>	13	-
	<i>Schigella flexnari</i>	11	-

PC*= Positive Control Cefalexin and Gentamycine (10µg)

COM1 = Phenylhydrazone of 4-hydroxy- N -phenyl acetamide

Determination of Minimal Inhibitory Concentration (MIC) and minimal bactericidal concentration (MBC)

A modified agar microdilution method of Lorian, (1996) was used to determine the MIC of Phenylhydrazone of 4-hydroxy- N -phenylacetamide . The test tubes contained serially diluted from 1500µg/ml to 1.46µg/ml were inoculated with the selected bacterial strain suspension and the test tubes were incubated at 35°C for 18 h. Observations were performed in triplicates and results were expressed as the lowest concentration of compounds that produced a complete suppression of colony growth.

Table.4. Minimum Inhibitory Concentrations (MIC) data of Phenyl hydrazone of 4-hydroxy- N -phenylacetamide tested against selected human pathogenic bacteria.

Microorganism tested		Nonlinear optical compounds	
		PC*	COM1
Gram positive	<i>Bacillus subtilis</i>	1.46	2.23
	<i>Micrococcus luteus</i>	5.89	5.89
	<i>Staphylococcus aureus</i>	5.89	11.72
	<i>Streptococcus mutans</i>	2.23	1.45
Gram negative	<i>Escherichia coli</i>	5.87	11.72
	<i>Klebsiella pneumonia</i>	23.4	46.87
	<i>Proteus vulgaris</i>	11.7	23.44
	<i>Schigella flexneri</i>	11.7	5.89

PC*= Positive Control Cefalexin and Gentamycine (10µg)

COM3 = Phenyl hydrazone of 4-hydroxy- N -phenyl acetamide

CONCLUSION:

The semi organic crystal of Phenylhydrazone of 4-Hydroxy-N –Phenylacetamide (PHHPA) was prepared from 4-Hydroxy-N –Phenylacetamide and Phenyl hydrazine in methanol solution by adopting the procedure. The prepared crystal was grown by slow evaporation solution growth technique (SESGT). The grown crystal was characterized by spectral and thermal analysis. The FT-IR and UV-visible spectral analysis to confirm the purity and transparency of grown crystal. PHHPA crystal was also characterized using H^1 NMR spectral studies. The sample confirms the purity and the nature of crystals were studied by X-ray diffraction studies. The DSC/TGA study also confirms the crystalline nature and thermal stability of the TSCPHA. Band gap determination and antibacterial activity studies were confirms the application oriented properties of the material.

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