

**SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZONE DERIVED FROM 4-
[N,N-BIS(2-CHLOROETHYL)AMINO]BENZALDEHYDE**

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in Chemistry

By

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**DEPARTMENT OF CHEMISTRY
SCHOOL OF SCIENCE AND HUMANITIES**

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BONAFIDE CERTIFICATE

This is to certify that this project Report is the Bonafide work of **R.SWETHA (40030040)**, **S.RUBASRI (40030028)**, **A.SUBALASKSHMI (40030037)** who carried out the project entitled " **SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZONE DERIVED FROM -4-[N,N-BIS (2-CHLOROETHYLE)AMINO] BENZALDEHYDE**" under our supervision from November 2022 to April 2023.

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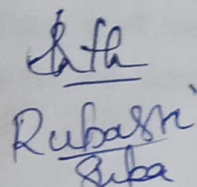
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DECLARATION

We R.SWETHA(40030040), S.RUBASRI(40030028), A.SUBALAKSHMI(4030037) hereby declare that the project Report entitled "SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZONE DERIVED FROM ALDEHYDE-4-[N,N-BIS(2-CHLOROETHYL)AMINO]BENZALDEHYDE" Done by us under the guidance of Dr .J. KARTHIKEYAN Ph.D, Professor, Department of chemistry, Sathyabama Institute of Science and Technology Chennai-600 106 is submitted in partial fulfillment of the requirements for the award of Bachelor of science degree in Chemistry.



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ABSTRACT

Semicarbazones are compound obtained by condensation of semicarbazides with suitable aldehydes and ketones. They are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. The new semicarbazone was made by combining semicarbazide with aldehyde 4-[N,N-Bis(2-chloroethyl)amino] benzaldehyde in 10ml of ethanol. The mixture is stirred until it is fully dissolved and add 1drop of concentrated HCl. The structure of the compounds have been characterized on the basis of their FT-IR, UV-visible, NMR, HRMS Spectroscopic methods

CONTENTS

CHAPTER NUMBER	TITLE	PAGE NUMBER
1)	INTRODUCTION..... 1.1. Reaction of semicarbazone 1.2 Uses of semicarbazone	7-9
2)	LITERATURE SURVEY.....	10-15
3)	AIM AND SCOPE..... 3.1 Aim 3.2 Scope	16
4)	EXPERIMENTAL METHODS..... 4.1 Materials 4.2 Methods	17
5)	RESULT AND DISCUSSIONS..... 5.1 UV –visible spectroscopy 5.2 NMR spectroscopy 5.3 FT-IR spectroscopy 5.4 HRMS	18-23
6)	CONCLUSION..... REFERENCE.....	24 25-

INTRODUCTION

Semicarbazone is a derivative of imines formed by a condensation reaction between an aldehyde or ketone and semicarbazide. Semicarbazide (SEM) is a derivative of urea or hydrazine that possess several important functions in medicinal and health-related issues. SEM motifs constitute the core structures of several drugs and herbicides such as nitrofurazone, tolazamide, larmustine, cafenstrole, and diflufenzopyr. In addition, SEM is applied in food as a marker to detect the illegal usage of the banned antibiotic nitrofurazone. They also reveal a stabilizing effect on the liquid crystalline state of chloroplast membrane lipids, and some are known as surfactants. Another report exhibited that SEMs are also applied as stabilizing agents in the polymer industry. Semicarbazide is a characteristic metabolite of nitrofurazone and often used as a marker to monitor the illegal use of nitrofurazone in foods. Semicarbazide is also released as a byproduct of azodicarbonamide used as a food additive or when packaging is decomposed at a high temperature. Semicarbazide could also be detected in food by treating with hypochlorite and exists naturally in some crustacean products and red seaweed and carrageenan. Toxicological studies have shown that semicarbazide inhibits semicarbazide sensitive amine oxidases, lysyl oxidase, and glutamic acid decarboxylase (GAD). A semicarbazone is a derivative of imines formed by a condensation reaction between a ketone or aldehyde and semicarbazide. They are classified as imine derivatives because they are formed from the reaction of an aldehyde or ketone with the terminal $-NH_2$ group of semicarbazide, which behaves very similarly to primary amines. The semicarbazide which are the raw material of semicarbazone have been known to have biological activity against many of the most common species of bacteria. containing organic compounds with diverse biological activities. They are schiff's bases formed by the condensation product of aldehyde or ketones with different amines. These derivatives are urea and thiourea derivatives depend on the attached aldehyde or ketones moiety. Antibacterial, antifungal,

anticonvulsant, anti-inflammatory, antioxidant, antiviral, and other biological activities are all possible with semicarbazide and thiosemicarbazide derivatives. The structure of synthesized compound were confirmed by an various spectroscopic techniques such as UV, FT-IR, NMR, MSESI-MS methods.

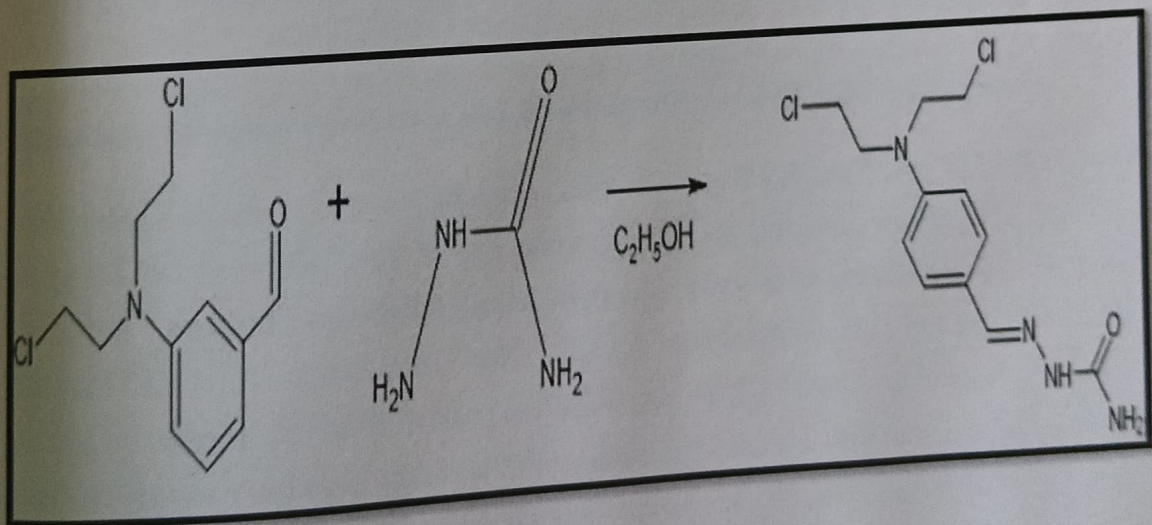
1.1 REACTION OF SEMICARBAZONE

Semicarbazone is a derivative of imines formed by a condensation reaction between a ketone or aldehyde and semicarbazide.

Derivatives of ammonia can also be used in place of ammonia to give the corresponding nitrogen substituted derivatives.

The action of semicarbazide.

When semicarbazide reacts With a ketone(or aldehyde) in ethanol medium to form **semicarbazone**. Only one nitrogen atom of semicarbazide acts as a nucleophile and attack the carbonyl carbon of the aldehyde.



SCHEME 1 SYNTHESIS OF COMPOUND

CHAPTER-2

LITERATURE SURVEY

Green synthesis of semicarbazone: A comparison of two green solvents.

Daniel Brush, Cassidy McGinn, Dr. Eric Helms (2019) An array of semicarbazones were synthesized by the reaction of semicarbazide hydrochloride with substituted benzaldehydes. The reactions were performed in two different novel green solvents, ethyl lactate and dimethyl isosorbide. The yields and purities of each product were compared to determine the optimal solvent:water proportion. The products for each reaction were analyzed using ^1H NMR spectroscopy as well as LC-MS. The results indicate that the reaction is most efficient when the ratio of ethyl lactate:water is 80:20, and the ratio of dimethyl isosorbide to water is 92:8. Generally the semicarbazones synthesized in ethyl lactate displayed a higher yield and degree of purity.

Microwave-assisted Synthesis and Bioevaluation of Some Semicarbazone.

Laila Jafri, Farzana L. Ansari, Maryam Jamil, Saima Kalsoom, Sana Qureishi, Bushra Mirza (2012) In continuation to our efforts in finding potential therapeutic agents, a variety of biologically significant semicarbazones were synthesized by the reaction of different carbonyl compounds with phenyl semicarbazides through microwave irradiation. Initially, 18 semicarbazones were studied for their antimicrobial, antitumor, and antioxidant potential. None of the tested compounds showed any antibacterial activity; however, some compounds showed significant antifungal activity. Interestingly, all compounds showed antitumor activity when tested against tumors grown on potato discs. These compounds were also tested for their effect on OH radical-induced oxidative DNA damage. All the compounds showed DNA protection to varying extent. Based on the promising results of antitumor and antioxidant activities, another set of 24

semicarbazones was synthesized, and all of these semicarbazones were evaluated for their antioxidant potential. The results showed that the semicarbazones derived from 2-nitrobenzaldehyde and acetophenone were the most active 2,2-diphenyl-1-picrylhydrazyl 9 (DPPH) free radical scavengers. The overall results have led to the identification of some interesting compounds which seem to have great potential to be developed into effective anticancer drugs.

Anticancer inhibition relevance of semicarbazones: structure

Semicarbazide: occurrence in food products and state-of-the-art in analytical methods used for its determination

Maria Beatriz de la calle & Elke Anklam et al (2005) provides an overview of the information currently available about the presence of semicarbazide (SEM) in food. Likely source of SEM in food matrices are summarized and discussed. Detailed information is given about the analytical methods used to determine SEM; features and drawbacks associated with them are carefully evaluated. Performance criteria characterising the analytical methods discussed are also given.

Some properties of semicarbazide-sensitive amine oxidases

BA Callingham, MA Barrand (1987) semicarbazide-sensitive amine oxidases (SSAOs) comprise a substantial but diffuse group of enzymes separable from classical monoamine oxidase in several respects. Difference in cofactor requirements, molecular weight and subcellular distribution are crucial for such separation. Differential sensitivity to enzyme inhibitors, characterized by resistance to inhibition by acetylenic MAO inhibitors coupled with sensitivity to semicarbazide and some related compounds are characteristic of these enzymes. SSAO enzyme have been found in plasma of man, ox, pig and horse, for example as well as in the solid tissues of many species. Extensive so far failed to produce any conclusive evidence to indicate what the precise function of many of these enzymes may be. Indeed in most cases there is no clear idea as to the

nature of the preferred physiological substrate, although many amines with pharmacological activity have been shown to be substrates. The actions of these amines may be potentiated following inhibition of SSAO, but as yet little is known whether or not these actions can be important *in vivo*. An attempt is made in this review to bring together some of the evidence to see if there are indications for future endeavours.

Corrosion inhibition relevance of semicarbazides: electronic structure, reactivity and coordination chemistry

Chandrabhan verma, Mumtaz A Quraishi, Kyong Yop Rhee (2022) Semicarbazide ($\text{OC}(\text{NH}_2)(\text{N}_2\text{H}_3)$) and thiosemicarbazide ($\text{SC}(\text{NH}_2)(\text{N}_2\text{H}_3)$) are well-known for their coordination complex formation ability. They contain nonbonding electrons in the form of heteroatoms (N, O and S) and π -electrons in the form of $>\text{C}=\text{O}$ and $>\text{C}=\text{S}$ through they strongly coordinate with the metal atoms and ions. Because of their association with this property, the Semicarbazide (SC), thiosemicarbazide (TSC) and their derivatives are widely used for different applications. They serve as building blocks for synthesis of various industrially and biologically useful chemicals. The SC, TSC and they derivatives are also serve as strong aqueous phase corrosion inhibitors. In the present reports, the coordination ability and corrosion protection tendency of Semicarbazide (SC), thiosemicarbazide (TSC) and their derivatives is surveyed and described. These compounds are widely used as inhibitors for different metals and alloys. Through their electron rich sites they adsorb on the metal surface and build corrosion protective film. Their adsorption mostly followed the Langmuir adsorption isotherm. Through their adsorption they increase the value of charge transfer resistance and decrease the value of corrosion current density. Computational studies adopted in the literature indicate that SC, TSC and their derivatives adsorb flatly and spontaneously using charge transfer mechanism.

Semicarbazone Analogs as Anticonvulsant Agents:

Mohamed Jawed Aham (2013). Semicarbazones are synthesized by the condensation of semicarbazide and aldehyde/ketone. The literature survey revealed that semicarbazones had been emerged as compounds with diverse biological activities including anticonvulsant, antitubercular, anticancer, and antimicrobial activities. The anticonvulsant activity of semicarbazones is mainly attributed due to the presence of an aryl binding site with aryl/alkyl hydrophobic group, a hydrogen bonding domain and an electron donor group and they are suggested to act by inhibiting sodium ion (Na^+) channel. Dimmock et al., reported an extensive series of semicarbazones and reported 4-(4-fluorophenoxy) benzaldehyde semicarbazone (C0102862, V102862) as lead molecule. In MES (oral) screening C0102862 showed protective index ($\text{PI} > 315$) more than carbamazepine ($\text{PI} 101$), phenytoin ($\text{PI} > 21.6$) and valproate ($\text{PI} > 2.17$). This review briefly describes the information available about semicarbazone analogs and their anticonvulsant activity.

Synthesis and Antimicrobial Evaluation of Novel Derivatives of Semicarbazide and 1,2,4-triazol

Anna Pachuta-Stec, Anna Biernasiuk, Anna Malm, Monika Pitucha(2017) Reaction of phenoxyacetic acid hydrazide with isocyanate was used to the synthesis of new semicarbazide derivatives. Cyclization of these compounds in a 2% aqueous solution of sodium hydroxide led to formation of 1,2,4-triazole-3-one. The chemical structure of synthesized compounds was confirmed by elemental analysis and spectroscopic methods (^1H and ^{13}C NMR). On the basis of the NMR, spectra were found that cyclic compounds 1,2,4-triazole exist in the -one form. Moreover, all derivatives were examined for their *in vitro* activity against some species of bacteria. New compounds presented mild or moderate antimicrobial activity only against reference Gram-positive bacteria. Two derivatives (one semicarbazide and one triazole) showed bactericidal or bacteriostatic activity.

Semicarbazide is a minor thermal decomposition product of azodicarbonamide used in the gaskets of certain food jars.

Richard H Stadler, Pascal Mottier, Phillipe Guy, Eric Gremaud, Natalia Varga, Sam Ialjie, Evidence is presented for the first time showing that semicarbazide (SEM) is a minor thermal decomposition product of the blowing agent azodicarbonamide (ADC). A novel direct analytical method based on liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESIMS/MS) has been developed to determine SEM in foamed polyvinyl chloride (PVC) seals of metal lids, as well as in commercially available ADC. The direct LC-MS/MS method for gaskets entails extraction of the gaskets in hot water, addition of ($^{15}\text{N}_2^{13}\text{C}$)-SEM as internal standard, and injection of an aliquot directly into the LC-MS system, achieving good sensitivity ($\text{S/N} = 348$ for 2 ng injected on-column) and monitoring three characteristic mass transitions ($m/z\ 76 \rightarrow 31$; $76 \rightarrow 44$; $76 \rightarrow 59$). Semicarbazide can be detected in thermally treated ADC, reaching up to $0.93\ \text{mmol mol}^{-1}$ at $220\ ^\circ\text{C}$, as determined by the direct LC-MS/MS method. This new method is also compared to the classical derivatization method using 2-nitrobenzaldehyde (2-NBA) that is routinely employed to determine SEM as an indicator of the usage of the antimicrobial drug nitrofurazone, the use of which is not authorized in the European Union (EU). Both methods revealed proportional results, with approx. 3-fold higher levels recorded by the direct SEM approach, probably due to differences in the extraction procedures used. A limited survey of plastic seals from used press twist and twist-off metal lids on food jars (non-foamed and foamed) revealed levels of SEM ranging from 2 to $8689\ \mu\text{g kg}^{-1}$ (average = $1593\ \mu\text{g kg}^{-1}$, $n = 57$ determinations).

Manganese(III) oxidations of benzoylhydrazine, aroxyacetylhydrazine, semicarbazide, thiosemicarbazide and their derivatives in pyrophosphate media.

B. S. Sherigara, Ivan Pinto & K. Ishwar Bhat (1994) Manganese(III) ion was stabilised in weakly acidic solution by means of pyrophosphate. Conditions for the quantitative oxidation of hydrazine, benzoylhydrazines, aroxyacetylhydrazines, semicarbazide, semicarbazones, thiosemicarbazide and thiosemicarbazones were investigated by titrimetric and potentiometric methods. The stoichiometries of these oxidations were pH-dependent. At pH 5 benzoyl hydrazines were oxidised by donating two electron each, the nitro derivative being the sole exception to show a three-electron change. Aroxyacetylhydrazines, semicarbazide and semicarbazones underwent three-electron oxidations. The free radical nature of these reactions was illustrated. Thiosemicarbazides reacted faster than semicarbazides. Metal complexes of thiosemicarbazide also yielded stoichiometric results, indicative of the number of TSC ligands in the complex. The oxidation products have been identified. At pH $\approx$ 1 these reactions conformed to different stoichiometries.

Synthesis and antimicrobial study of copolymer resins derived from p-hydroxybenzoic acid, semicarbazide and formaldehyde.

Kamlakar Nandekar and Wasudeo Gurnule(1913) The Copolymer p-HBSF-I resin was synthesized from p-Hydroxybenzoic acid, semicarbazide and formaldehyde with hydrochloric acid as a catalyst in 1:1:2 molar ratio by polycondensation technique. The copolymer characterization was done with IR and ^1H NMR Spectra. The antibacterial and antifungal screening of copolymer p-HBSF-I was done by using a well-known agar diffusion method. The bacterial strains used were Staphylococcus Aureus, Bacillus Subtillus, Escherchia Coli, Salmonella Typhi and the fungal strains used were Aspergillus Niger, Candida Albicans. The present study includes the antimicrobial investigation of copolymer resins in order to know the effectivity of semicarbazide group.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim of the project to synthesize and characterization of semicarbazone derived from 4-[N,N-Bis(2-chloroethyl)amino]benzaldehyde

3.2 SCOPE

- The detailed spectral assignments for the synthesized compounds were made.
- Characterization of the compounds using various spectroscopic methods such as UV-visible , FT-IR , ^1H NMR and HRMS .
- Vibration property of the molecule was studied by FT-IR Spectroscopy.

CHAPTER-4

MATERIALS & METHODS

4.1 MATERIALS

The starting material 4-[N,N-Bis(2-chloroethyl)amino]benzaldehyde, semicarbazide, ethanol was used in the synthesis process and concentrated hydrochloric acid.

4.2 EXPERIMENTAL METHODS

The Title of the compound was synthesized by taking 0.246g of 4-[N,N-Bis(2-chloroethyl)amino] benzaldehyde into ethanol and 0.111g of semicarbazide into 10 ml of ethanol. Both the mixture is stirred for 1 hour until it is completely dissolved in it. Now transfer both the solution into a 250 ml RB Flask and add 1 drop of concentrated HCl.

The mixture was kept stirring for 3 hours using magnetic stirrer.

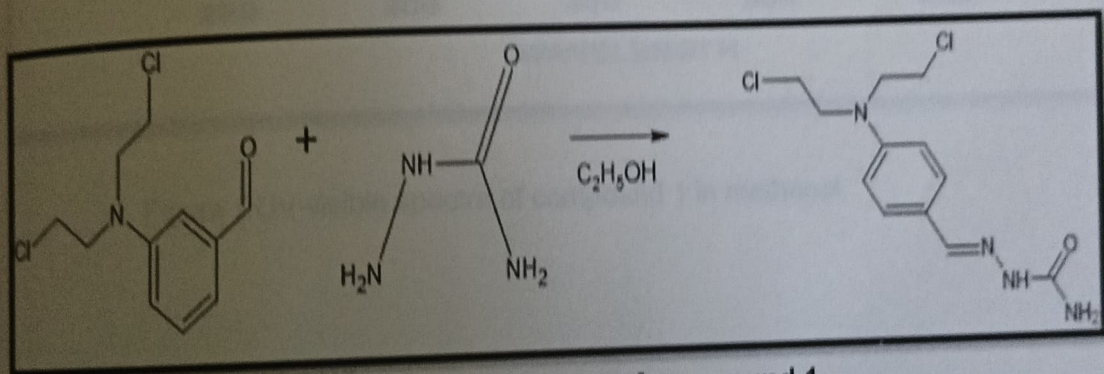
Dried the test tube until the water gets evaporated. Transfer the mixture into a clean test tube. Then keep the test tube in the stand without any disturbance for 2-3 weeks for crystallization.

CHAPTER-5

RESULTS AND DISCUSSIONS

5.1 Synthesis

The synthesis of semicarbazone was summarized in scheme 1 shown below. The compound 1 was synthesized by reacting Aldehyde -4-[N,N-Bis(2-chloroethyl)amino]benzaldehyde with semicarbazide in ethanol as solvent through condensation reaction under basic condition in good yield. The synthesized compounds were found to be soluble in chloroform, ethanol. The FT-IR, NMR, UV and HR-ESI-MS data of these compounds are in good agreement with the proposed chemical structure and molecular formula.



Scheme 1 synthesis of compound 1

5.2 UV-visible spectrum

The uv-visible spectrum of the synthesized compound was measured in the range of about 200-800nm by Using methanol as solvents. The absorbance is the rough neighbourhood of 270-300nm is common for molecules containing a C=O groups (such

as ketone and aldehyde). The given compound contains carbonyl group, it is expected to show two absorption band pertaining to the $n-\pi^*$ and $\pi-\pi^*$ transition. The compound 1 showed strong absorption band at 340nm which is due to the $n-\pi^*$ transition.

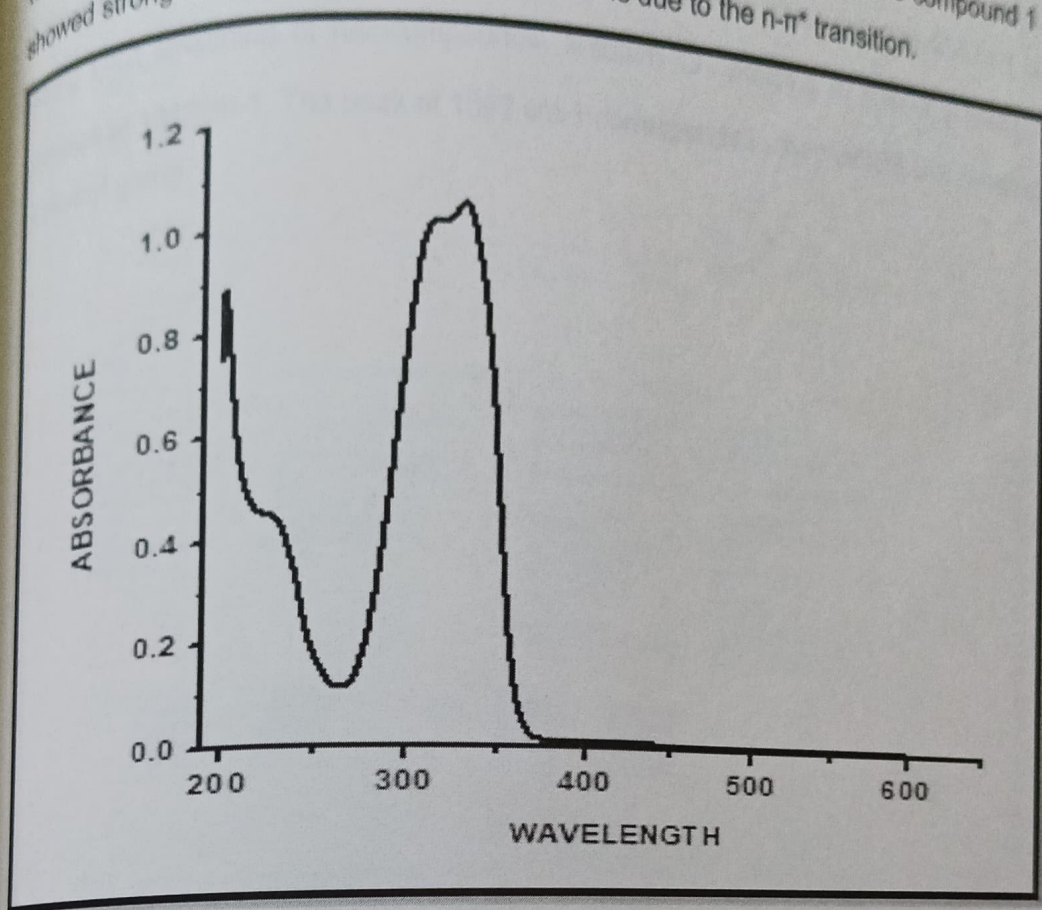
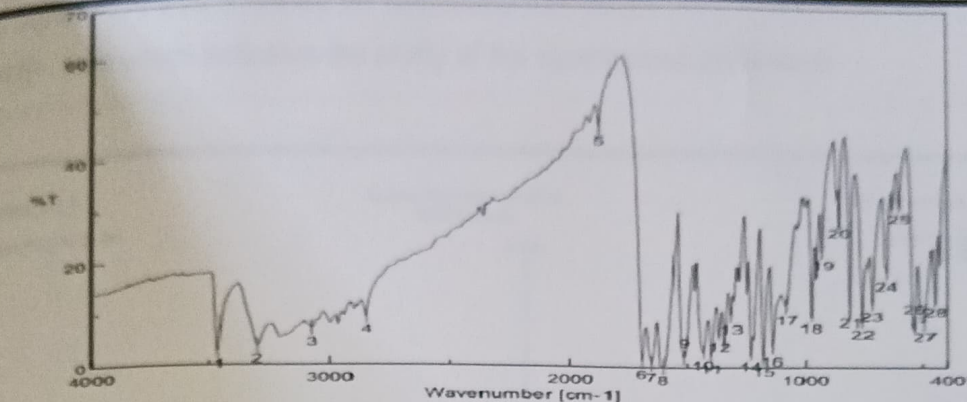


Figure 1 UV-visible spectra of compound 1 in methanol

5.3 FT-IR spectroscopy

The measurement of infrared radiations interaction with matter through absorption, emission or reflection is known as infrared spectroscopy (IR-spectroscopy or vibrational spectroscopy). Its used to research and classify chemical compounds or functional groups that occurs in solid, liquid or gaseous forms. The compound gives the characteristic IR peak that proved the presence of C=O and N=C. The FT-IR spectrum of the synthesized product showed a strong C=O stretching vibration at 1690.3cm^{-1} . The

peak 1517.7cm^{-1} corresponds to strong N-O stretching which is nitro compounds. And the peak 1430cm^{-1} indicates the presence O-H bending of carboxylic acid. The peak 1231cm^{-1} corresponds to medium C-N stretching of amine. The peak $850\text{-}550\text{cm}^{-1}$ is strong C-Cl stretching of halocompounds. Medium OH bending of phenol stretching vibration at 1313cm^{-1} . The peak at 1362 cm^{-1} corresponds to the medium C-H bending of methyl group.



[Comments]
Sample name
Comment
User
Division
Company

Sathyabama Institute of Science and Technology

[Detailed Information]

Creation date 29-03-2023 13:12
Data array type Linear data array
Horizontal axis Wavenumber [cm-1]
Vertical axis %T
Start 349.053 cm-1
End 7800.65 cm-1
Data interval 0.964233 cm-1
Data points 7729

[Measurement Information]

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Serial Number B049361790
Measurement Date 29-03-2023 13:12
Light Source Standard
Detector TGS
Accumulation 16
Resolution 4 cm-1
Zero Filling On
Apodization Cosine
Gain Auto (4)
Aperture Auto (7.1 mm)
Scanning Speed Auto (2 mm/sec)
Filter Auto (10000 Hz)

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3464.49	2.76974	2	3301.54	3.72986
3	3075.9	7.08955	4	2851.24	9.48696
5	1872.54	46.5083	6	1690.3	1.26447

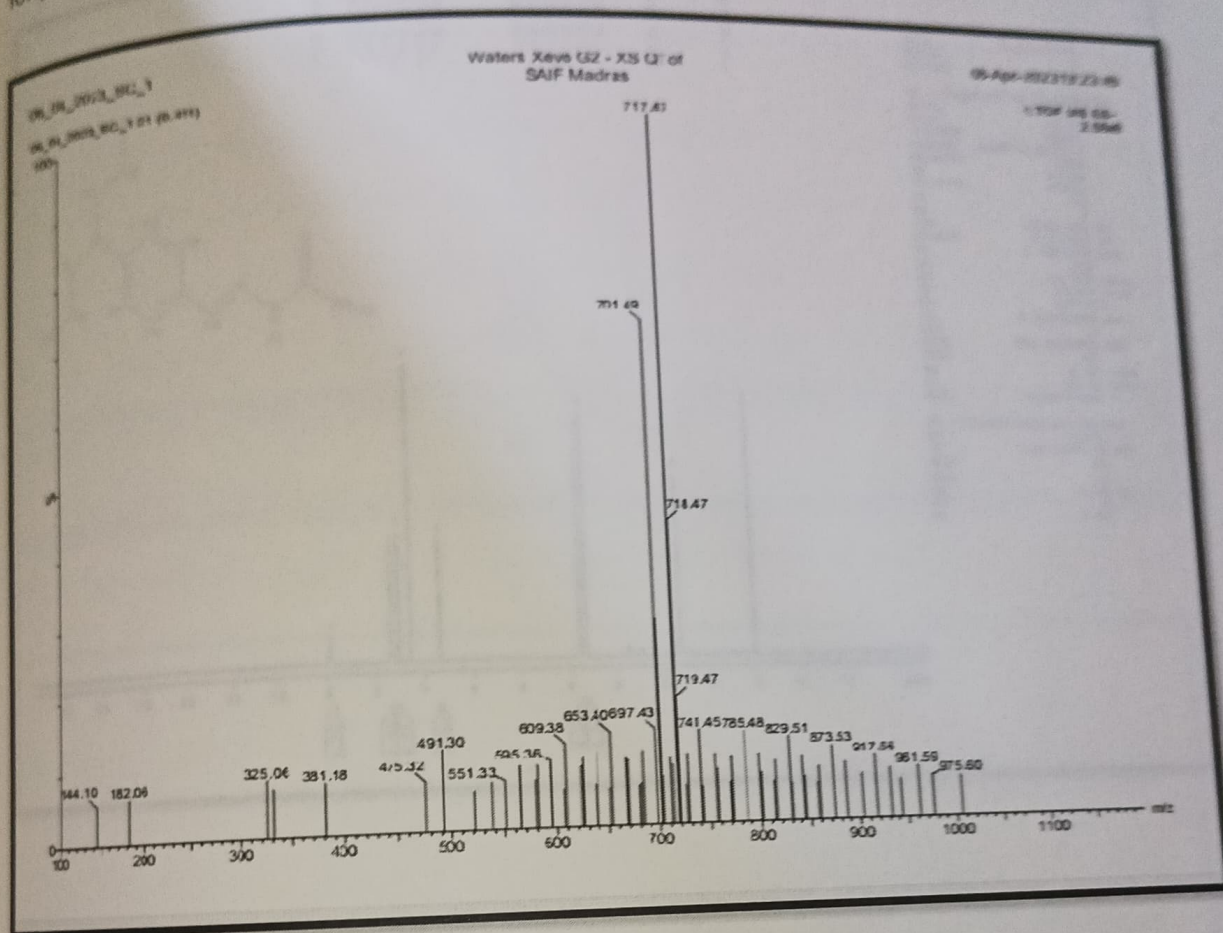
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
7	1649.8	0.687317	8	1597.73	0.199748
9	1517.7	2.05109	10	1430.92	3.02841
11	1399.1	2.43226	12	1362.46	6.68512
13	1313.29	10.1423	14	1231.33	2.81295
15	1177.33	1.89345	16	1138.76	3.8798
17	1079.94	11.8901	18	975.804	10.3006
19	931.45	22.5072	20	854.311	28.6847
21	808.992	11.4505	22	754.995	9.20733
23	718.354	12.5936	24	655.679	18.5637
25	600.717	31.83	26	538.042	8.70821
27	493.688	8.73676	28	451.261	13.5096

5.3 Mass spectroscopy

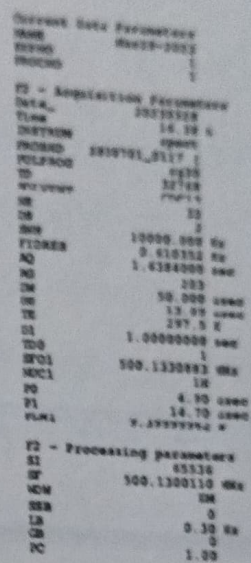
The semicarbazone was used to record the High resolution ESI MS spectrum in positive mode. The mass peak observed as $[M+H]^+$ at m/z 325.06 for the compound.

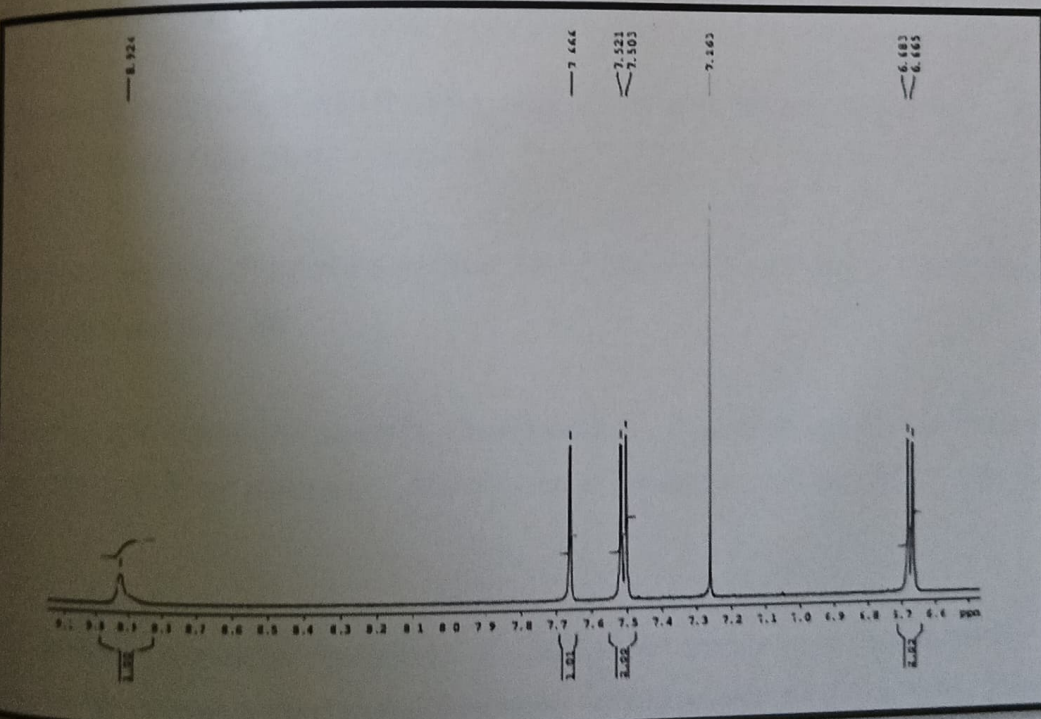
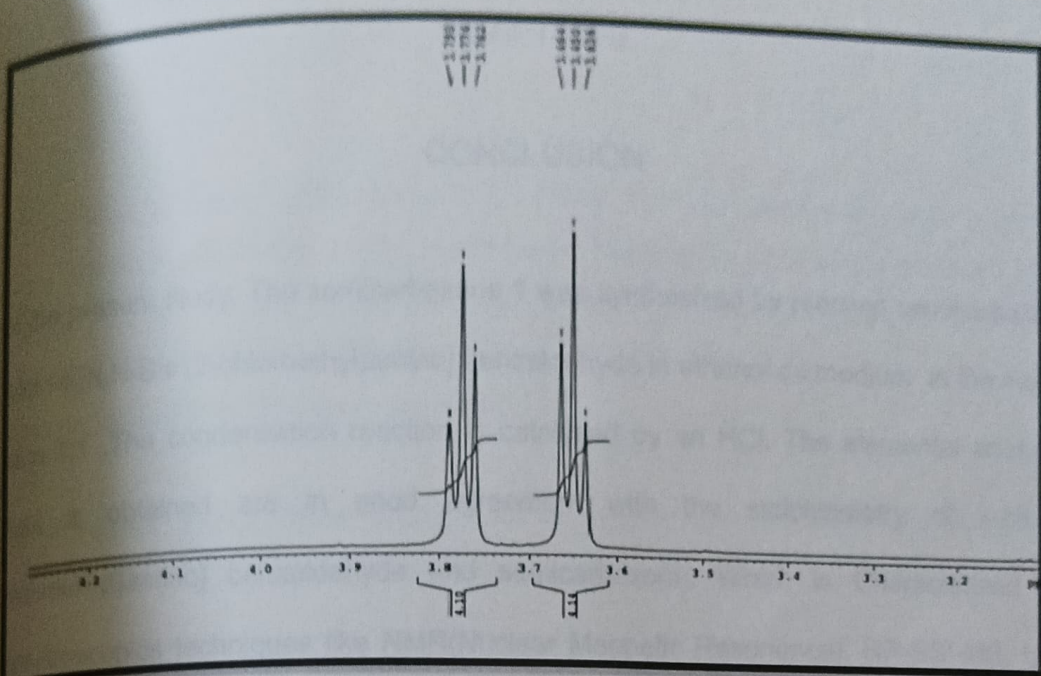
A good agreement was obtained for calculated m/z values and experiments m/z values for $[M+H]^+$ ions which indicates the purity of the synthesized compound.



5.4 ^1H NMR spectral studies

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of sample as well as its molecular structure. The ^1H NMR spectroscopy is used more often than ^{13}C NMR, partly because proton spectra are much easier to obtain than carbon spectra. The NMR spectrum were recorded for the compound 1 with chloroform as solvent. The doublet at 7.50 is due to methylene group 3.63 is due to C-N.





CHAPTER-6

CONCLUSION

In the present study, The semicarbazone 1 was synthesized by reacting semicarbazide with 4-[N,N-Bis (2-chloroethyl)amino] benzaldehyde in ethanol as medium in the molar ratio 1:1. The condensation reaction is catalyzed by an HCl. The elemental analysis data is obtained are in good agreement with the stoichiometry of 4-bis(2-chloroethyl)amino] benzaldehyde and semicarbazone. Which is Characterized by spectroscopics techniques like NMR(Nuclear Magnetic Resonance), HR-ESI-MS, UV-visible spectrum, FT-IR spectrum. The vibration property of the molecule was studied by FT-IR spectroscopy.

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