



SYNTHESIS AND CHARACTERIZATION OF TEMPLATE MEDIATED MACROCYCLIC COMPLEXES BY USING THIOUREA AND ADIPIC ACID (TA)

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Abstract

This study focuses on the synthesis and comprehensive characterization of macrocyclic complexes derived from the reaction between thiourea and adipic acid, referred to as TA complexes. Employing various analytical techniques such as FT-IR spectroscopy, NMR spectroscopy, XRD analysis, and SEM coupled with EDS, the study meticulously elucidates the structural attributes, confirming the successful formation of the TA macrocyclic complex. The investigation reveals significant insights into the molecular structure, crystallinity, and surface morphology of the synthesized compounds. Furthermore, the research extends to evaluate the antibacterial efficacy of the TA complexes against a spectrum of bacterial strains, demonstrating notable antimicrobial activity. This exploration contributes to the domain of supramolecular chemistry by not only detailing the synthetic strategy and characterization of the TA complexes but also highlighting their potential applications as antimicrobial agents.

Keywords: Macrocyclic complexes, Thiourea, Adipic acid, FT-IR spectroscopy, NMR spectroscopy, XRD analysis, SEM-EDS, Antimicrobial activity, Supramolecular chemistry

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1. INTRODUCTION

The synthesis and characterization of macrocyclic complexes using thiourea and adipic acid represent a significant area of research in the field of supramolecular chemistry. These macrocyclic complexes, often referred to by their acronym TA, have garnered attention due to their unique structural features and potential applications in various domains, including catalysis, molecular recognition, and as scaffolds for the development of novel antimicrobial agents.^[1] The process of synthesizing these complexes involves a meticulous selection of reagents and conditions to ensure the formation of the desired macrocyclic structure. This study employs a range of analytical techniques, including FT-IR spectroscopy, NMR

spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS), to thoroughly characterize the synthesized compounds. The detailed analysis provides insight into the structural intricacies of these compounds, paving the way for exploring their applications in addressing contemporary challenges in chemistry and biology.^[2]

The experimental journey to synthesize and characterize these macrocyclic complexes begins with the careful selection and preparation of the necessary chemicals, such as thiourea and adipic acid, and employs a variety of sophisticated instruments to elucidate their structural properties. The synthesis process is carefully designed to facilitate the formation of the macrocyclic



compound through a reaction between adipic acid and thiourea under controlled conditions. The resultant compound is then subjected to a series of characterization techniques.^[3]FT-IR spectroscopy reveals the vibrational modes of the molecule, providing evidence of the successful formation of the macrocycle. UV-visible spectroscopy further supports this by showing the electronic transitions within the compound, indicating its potential for further application studies. The crystalline structure is confirmed through XRD analysis, showcasing the ordered arrangement of atoms within the complex. SEM and EDS analyses provide detailed insights into the morphology and elemental composition, respectively, offering a comprehensive understanding of the synthesized macrocyclic complex. These analytical methodologies not only confirm the successful synthesis of the macrocyclic complex but also lay the groundwork for exploring its applications, particularly in the realm of antimicrobial activity, where such compounds hold significant promise.^[4]

2. EXPERIMENTAL STUDY

Most of the standard chemicals like succinimide and thiourea, double distilled water, ethanol, silica gel, TLC sodium hydroxide tablets, and HCl were purchased of sigma Aldrich by commercial sources and utilized without additional purification. The determination of melting points measurements were obtained by using using a digital melting point apparatus (Microsil) with open capillaries and is expressed in terms of degree centigrade (°C).^[5] Perkin Elmer Spectrum IR, Version 10.6.2.1 was used for obtaining FT-IR spectra in transmission mode in a range of MID-IR (4000-400 cm⁻¹). Using TMS as an internal standard, ¹H & ¹³C NMR spectrum were captured with Advance III Bruker 400Mhz apparatus and a "Perkin-Elmer 1800 IR" spectrophotometer. Multipurpose Versatile XRD System (XRD) by using model SmartLab 3kW Rigaku used for XRD analysis. By using 7610F Plus/JEOL model FE- SEM and EDS (for elemental analysis) obtained under high resolution.^[6]

Chemicals/Raw Material Used

Adipic acid (SigmaAldrich), Molecular formula, C₆H₁₀O₄, Molecular wt.

(146.14g/mol), Thiourea (SigmaAldrich), CH₄N₂S, Molecular wt (76.12g/mol) Ethanol, Double distilled water, Adipic acid (3.839gm), Thiourea (1.0gm), Ethanol (50-55 ml), Double distilled water

Synthesis: 3.839 gms of adipic acid was taken and about 15 to 20 ml of ethanol in a 100ml R.B flask was dissolved in it at room temperature. Then, 1.0gms thiourea was taken in a different R.B flask and about 15 ml of ethanol in 100 ml was taken and completely dissolved in it at room temperature. Both the reactant compounds were then mixed in a round "bottom flask" fitted with a reflux water condenser. The contents were heated upto 8-10 hrs at 90-100°C. The reaction mixture was allowed to stand/rest for about 30 minutes at room temperature. The contents of the R.B flask were transferred to a 250 ml beaker, diluted with about 20 ml of water and heated in a water bath till the solution is concentrated to about 2/3 of its original volume. The solution was then filtered and recrystallized from boiling water to remove further impurities. A solid product was sorted out when the reaction had been completed as determined by TLC. The product was cleaned with cold ethanol after being filtered by whatman filter paper.^[7] This crude product was then recrystallized from petroleum & methanol ether (1:1) to produce 3.2 gm of pure product. Let it dry then we used them for further processes. The compound was obtained with a yield of 73% which is highly soluble in water and melting point of the compound is 182 degree celcius (*C). Spectrum: $\nu = 1640-1690$ (C=N), 1760 (strong), 2800-3000 (NH₂).

¹H-NMR: 9.14 (s, 2H, -NH₂), 2.38 (t, 2H, -CH₂-CH₂-C=O), 2.38 (t, 2H, -CH₂-CH₂-C=O), 1.60 (t, 2H, -CH₂-CH₂-CH₂), 1.60 (t, 2H, -CH₂-CH₂-CH₂).

¹³C NMR (400 MHz, DMSO-D₆): 201 (-N-C(=O)), 197.5 (-S-C=O), 163 (-N-C-S), 43.5 (-C-C(=O)), 33.7 (-C-C=O), 25.9 (-C-C-C), 25.8 (-C-C-C).

3. RESULT AND DISCUSSION

Design and synthetic pathway

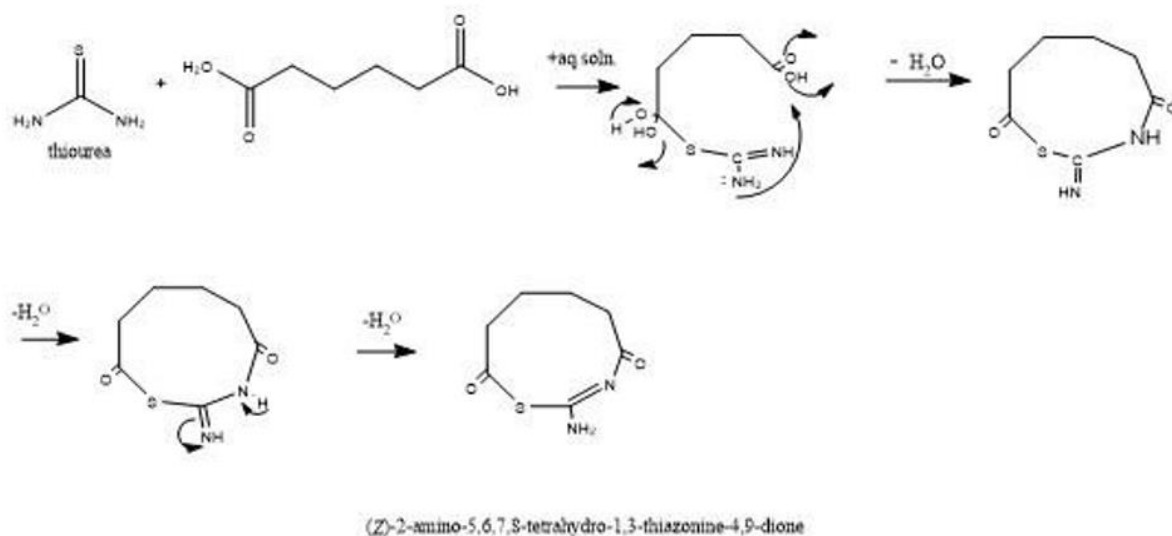
There were certain contradictions in the literature regarding the general reactivity pattern of phthalic acid and thiourea. Based on the commonly accepted consideration and literature data, some probable explanations



were offered. Compounds namely (z)- 2-amino-5,6,7,8-tetrahydro-1,3-thiazonine-4,9-dione Reaction (V) were synthesized by the proposed reactions with phthalic acid and thiourea.

Even though mechanistic aspects of the reaction for the synthesis of (z)-2-amino-5,6,7,8-tetrahydro-1,3-thiazonine-4,9-dione Reaction (V) was investigated in the current

study, the presence of substituent in the product indicates the formation of derivative as an intermediate product.^[8] Also, the attempts to isolate the intermediate were not successful. The plausible mechanism for the formation of (z)-2-amino-5,6,7,8-tetrahydro-1,3-thiazonine-4,9-dione reaction (V) is shown below:



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Scheme 1 Synthetic pathway of adipic acid and thiourea.

It was clear from the finding that the reaction had a similar pattern to the proposed reactions. In order to broaden the research scope, it was considered useful to examine the influence of varying solvent on the reaction. Accordingly, various tests were performed on the substrate thiocarbamide with CH₃CN, i-BuOH, n-BuOH, i-PrOH, n-PrOH, and MeOH. The response didn't always exhibit the same pattern. Nevertheless, one product was obtained with solvent ethanol. The result of this reaction is summarized in the spectrum (IR, ¹H, ¹³C NMR) analytical data of all the newly synthesized substances which were used to confirm their structural details. The required product for the present research was prepared by the reflux condensation with the ethanol with a highly accurate technique for sealing heat by silica gel with TLC done and excess ethanol was evaporated with the help of recrystallization.^[9]

3.1 CHARACTERIZATION

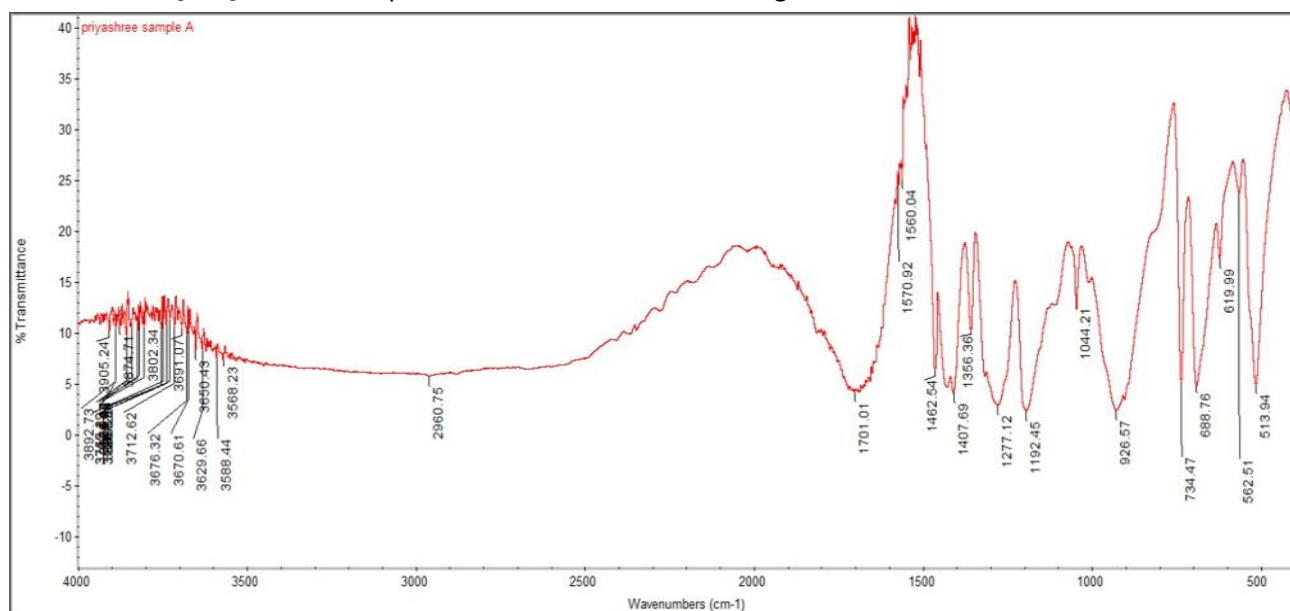
FTIR Study

The spectra of adipic acid and thiourea exhibited several absorption peaks. There was a broad absorption band due to the composition of stretching nodes. Symmetric and asymmetric stretching vibration of N-H occurred in the region of 2960cm⁻¹.

Thiourea included "two amines (NH₂), each of which is projected to give rise to stretched vibrations" between 3350 and 3200cm⁻¹ and 3300 and 3080cm⁻¹, respectively. The unequal stretching vibration of the NH₂ group peaked in intensity at a low 3312cm⁻¹ and was IR active.^[10] The bending vibrations vibration of the NH₂ group was determined to be the faint intensity peak seen at around 3215cm⁻¹ in the FTIR spectra. Thiourea's most notable vibrations are the N-C-N stretching vibrations, both asymmetric and symmetric which commonly occur between 1380 and 1300 cm⁻¹ and 1190 and 1140 cm⁻¹, respectively. The asymmetric stretching mode of the N-C-N was detected as a faint "intensity

peak” in the FTIR spectra, located at 1379cm⁻¹. Weak intensities of the symmetric stretching vibration of the N-C-N group were seen in the FTIR spectrum from 1167 to 1180cm⁻¹, respectively. Most NH₂ group deformation vibrations occur between 1701 and 1570cm⁻¹[153]. The FTIR spectra of the

TA compound showed a modest intensity peak at 1624cm⁻¹ associated with this vibration.^[11] In the FTIR spectra, the TG compound produced both moderate and strong intensity peaks at 1462cm⁻¹, where the C=S stretching vibration can be seen below in figure 6.1 below.



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Fig. 1: IR Spectra of template mediated synthesized macrocyclic complex of adipic acid and thiourea (TA)

UV Visible Study

The area, as well as lower cut-off wavelength, are two highly important information complex concerning formed crystals that are provided by transmittance spectral analysis. The TA templates that had been produced was evaluated for absorbance and transmittance spectra. As shown in Fig. 2, the obtained findings showed that TA crystal had good transparency with effectiveness of more than 65 percent in the 225– 800 nm range. Around 800 nm, a sharp drop in transmittance was seen, which was attributed to the thiourea molecule's C-N stretching vibration. It was interesting to note that the TA (thiourea adipic acid) template had a wavelength of 225nm because of the π - π^* transition. Compounds containing hetero-particles like nitrogen, oxygen, etc. need “dousing” when they have excess of electrons and also have non-holding electrons. When the mixtures are separated in DMSO, unusual split bundles at 298 nm and 275 nm be recorded, which may be logically deduced from the n^* transition of

the carbonyl companion and the n^* development of the carbonyl affiliation and the aromatic ring, respectively.^[12]

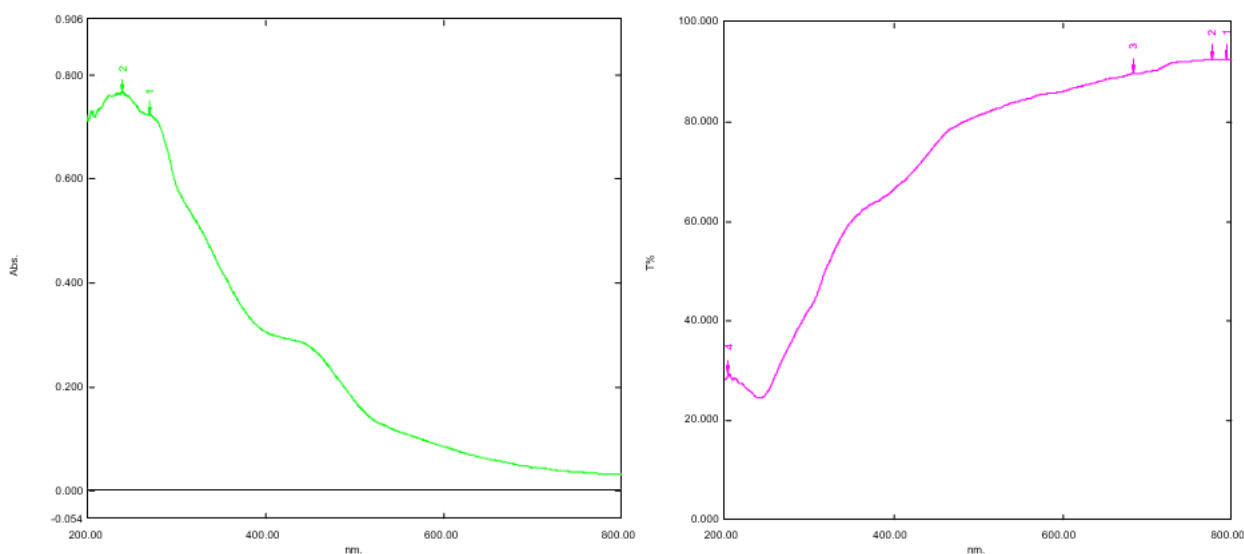


Fig. 2: Absorption and Transmittance Spectra of template mediated macrocyclic synthesized complex of adipic acid and thiourea

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XRD Study

The X-Ray diffraction pattern of TS (thiourea adipic acid) crystal "is recorded using a Rigaku MiniFlex2 goniometer X-Ray powder diffractometer by $\text{CuK}\alpha$ ($\lambda=1.5406 \text{ \AA}$) radiation. The sample is scanned for a 2θ range of $10^\circ - 80^\circ$ at a scanning rate of $1^\circ/\text{minute}$. All the observed reflections were included and which is shown in Fig. 3. The sharp and well defined Bragg's peaks at specific 2θ angle confirm the high crystallinity and purity of TS crystals.^[13] The two highest peaks intensity of 45000 cps was recorded at 18.5° and 28.5° of 2θ . The second highest peaks of 15000 cps was recorded at 25.5° of 2θ .

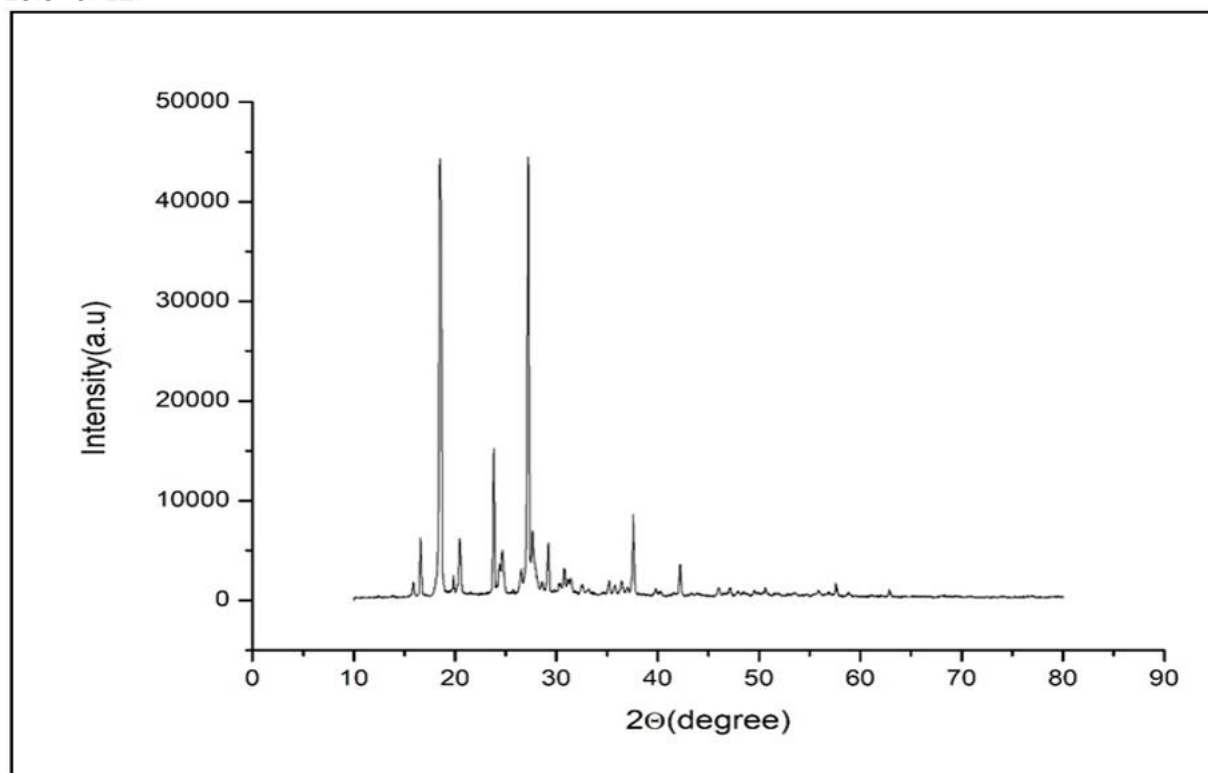


Fig. 3: X-Ray Diffraction pattern of template mediated macrocyclic synthesized complex of adipic acid and thiourea (TA)

NMR Study

¹H-NMR Study

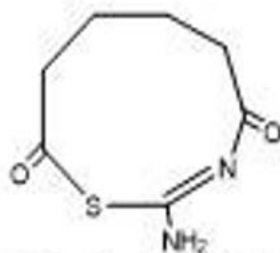
DMSO-d₆ was used to capture the spectrum, while the most significant resonance signals. Seven distinct proton group signals seen across the spectrum. Protons of CH amines showed up as a singlet at 9.50 ppm.^[14] This chemical contains NH groups that may be assigned to amines and amides, as well as vinyl CH, aromatic CH, methine CH, and methyl groups. ¹H-NMR: 9.50(s,2H,-NH₂), 2.38(t,2H,-C=O), 2.38(t,2H,CH₂-C=O), 1.60(t,2H,-CH₂-CH₂-CH₂), 1.60(t,2H,-CH₂-CH₂-CH₂).

¹³C-NMR Study

In DMSO-d₆, a recording of the ¹³C-NMR spectra of the synthesised chemical was made. The spectrum on the resonance signal can be noticed. The carbon atoms of the ring have been assigned chemical shifts of 201(-N-C(=O)), 197.5(-S-C=O), 163(-N-C-S), 43.5(-C-C(=O)), 33.7(-C-C=O), 25.9(-C-C-C), 25.8(-C-C-C) ppm, respectively. The ¹³C chemical shifts of the compound have been allocated in comparison to the assignments that are available for the individual components of compounds.

¹H-NMR: 9.14 (s,2H, -NH₂), 2.38 (t,2H, -CH₂-CH₂-C=O), 2.38 (t,2H, -CH₂-CH₂-C=O), 1.60 (t,2H, -CH₂-CH₂-CH₂), 1.60 (t,2H, -CH₂-CH₂-CH₂).

¹³C NMR (400 MHz, DMSO-D₆): 201 (-N-C(=O)), 197.5 (-S-C=O), 163 (-N-C-S), 43.5 (-C-C(=O)), 33.7 (-C-C=O), 25.9 (-C-C-C), 25.8 (-C-C-C).



(Z)-2-amino-5,6,7,8-tetrahydro-1,3-thiazonine-4,9-dione

3.2

Results and discussion of templates mediated macrocyclic compounds with heavy metal sulphates

3.2.1 Material and methods

Without further purification, all of the drugs were used in the tests. All of the solutions were made using de-ionized water. Heavy metal remedies were created using a final concentration of 200mg/L and additional dilutions of 10 mg/L, 50mg/L, 75mg/l, 100mg/l, and 150mg/L. These solutions were provided by "Qualigens fine chemicals," Maharashtra, India (given test 99 percent). It was of no use to further dilute the aqueous system as its pH was already 5.0. The acidity of Cu(II), Ni(II), Pb(II), and Zn(II) solutions was adjusted by adding drops of 0.1M NaOH solution to study them at different pH levels.^[15]

The chemical powder (0.15 average build) was mixed with 100 ml of the wet heavy metal preparations at a continuous speed of 200 rpm in a shaking water bath set at a comfortable temperature of 70 degrees Celsius in order to perform metal removal studies in cuvettes (NSW, Mumbai). After filtering, EDS (EDS Spectroscopy) was used to determine which metal was left in the solution. With the use of eq-1, how much of the death rock was absorbed by the compound per gramme of material can be determined.

$$q = \frac{Co - Ce}{M} \quad (1)$$

Where Co and Ce denote the initial and final heavy metal contents in mg/L before and after adsorption, respectively, and M denotes the quantity of absorbent in g obtained for a 1L heavy metal liquid. Adsorption is expressed as a percentage using the eq-2.^[16]

$$\text{Adsorption\%} = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

All batch experiments that looked at the effects of things like pH, temp, etc. were done in triplicate with a + 0.5% error ratio. The following were just the experimental circumstances that led to the selection of several batch trials: (Table-5.1)

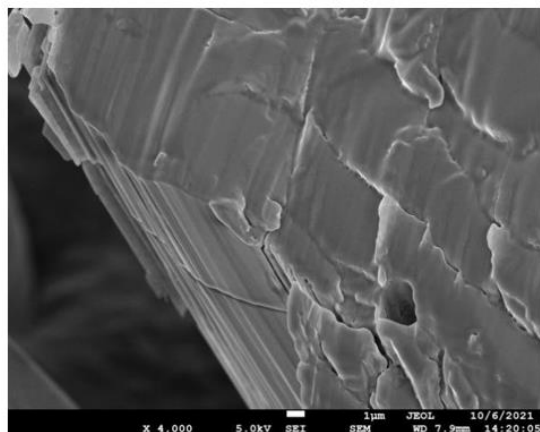
Table 1 Experimental conditions for batch experiments

Amount of compound(gm)	0.2,0.4,0.6,0.8,1.0,1.2
Initial metal concentration(mg/L)	10,50,75,100,150 and 200
“Adsorption temperature(*C)	10,20,30,40,50,60,70 and 80
Agitation time (min.)	10,20,30,40,50,60,70,80,90,100,110 and 120”
pH(initially)	2.0,3.0,4.0,5.0,6.0,7.0 and 8.0

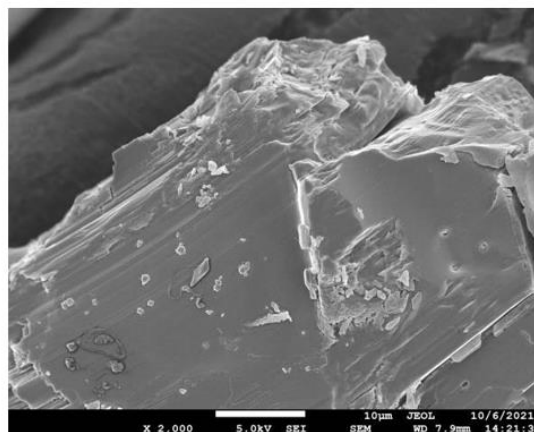
By starting with six different concentrations of initial metal ions (10-200mg/L; see Table-5.1), we were able to optimise the process of determining the optimal dosages of HM ions for absorption of Cu(II), Pb(II), Ni(II), and Zn(II) ions.

SEM

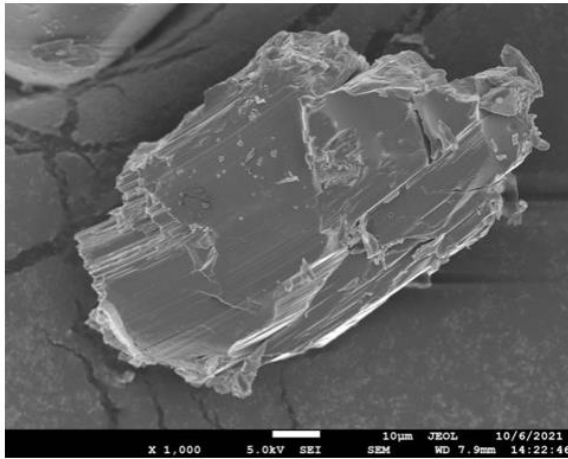
The SEM images of template mediated synthesized complex of adipic acid and thiourea at 1000X magnification prior to adsorption are shown in Fig. 4 (a-d) and afterward of surface assimilation of metals in Fig. 5 (e-i) observed at different orientation of the miscible blend of compounds or more in Fig 5.5 using SEM analysis.^[17] There are noticeable changes to the surface morphology of the synthesized complex as well as the creation of discrete aggregates on the synthesized complex surface following metal removal via adsorption showing that the biomaterial surface following metal removal via adsorption various dimension Fig. 5. showing that the heavy metal (II) sulphates was an assemblage of fine particles (needle like structure due to crystals and like cavities due to templates but irregular. The particles were found to be of various dimensions consisting of steps and voids on the external surface. Interaction of heavy metal (II) sulphates has resulted in the formation of flake-like deposition on its surface.



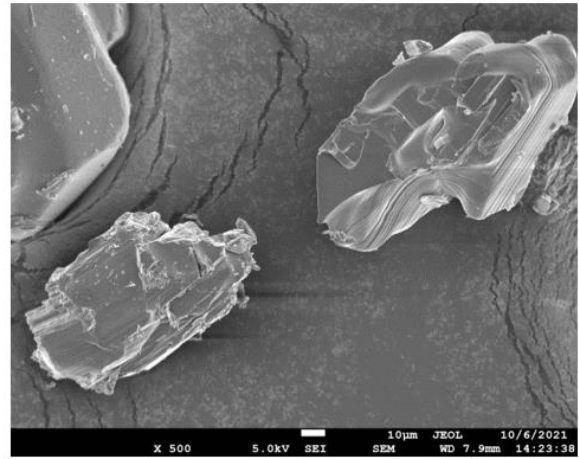
(a) At magnification $\times 4,000$



(b) At magnification $\times 2,000$



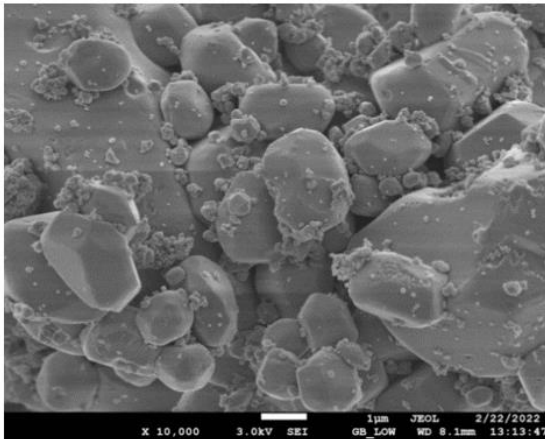
(c) At magnification $\times 1,000$



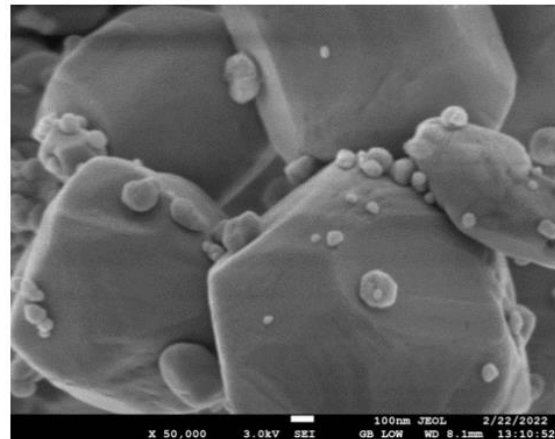
(d) At magnification $\times 500$

Fig. 4: SEM (a-e) images of template mediated macrocyclic complex of adipic acid and thiourea before adsorption.

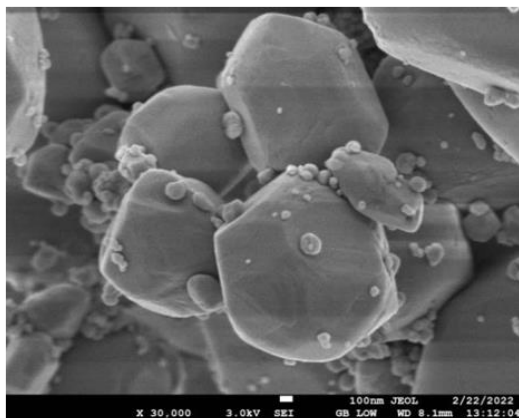
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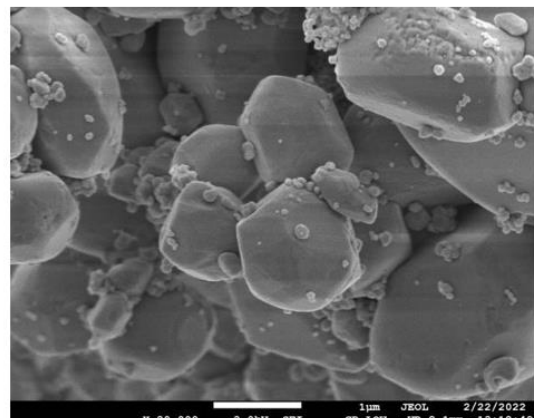
(e) At magnification $\times 10,000$



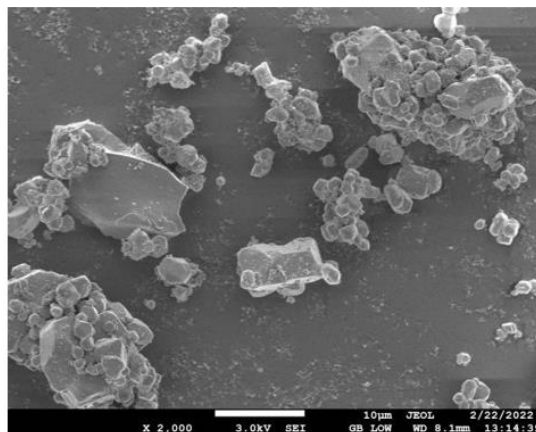
(f) At magnification $\times 50,000$



(g) At magnification $\times 30,000$



(h) At magnification $\times 20,000$



(i) At magnification $\times 2,000$

Figure.5.5.: SEM (e-i) images of template mediated macrocyclic complex of adipic acid and thiourea after adsorption.

EDS Study

This chemical test uses the technique of Energy-dispersive X-ray spectroscopy, to examine the chemical compounds from the desired sample of Thiourea. The reason behind the operating principles that helps the EDS technique is to measure the capacity of high-energy “electromagnetic radiation”. This technique helps to eject the core electrons from the atom of the compound Theoria. This technique follows the theory known as Moseley’s law, which can help to determine the direct correlation between the frequency of the and to measure the correlation of the light and the atomic number of the atom of the surface structure. Using EDS spectroscopy, this study has tried to remove the electrons from the system, which can help to release the energy. However, this study preferred to use EDS spectroscopy to do the analysis based on the energy spectrum to assess the

abundance of the particular elements in the specific complex named PTUNi complex.^[18]

Based on the discussion, in this section, it can be stated that the use of the SEM can help to offer a clear as well as high-resolution process to identify the texture of the specific complex named PTUNi. In addition, the use of SEM and EDS can help to examine the molecular structure of the specific complex. With the help of these two spectroscopies, this study has measured the molecular structure of the Pb sulfate from the process of making Theoria from Phthalic acid. However, in this sense, this study has chosen the two techniques of SEM and EDS to produce the magnified image for analyzing the complex formed in this process of microanalysis based on the Phthalic acid from Thiourea. These two processes have helped to determine the presence of Lead(II) sulfate from the complex formed from the Phthalic acid.

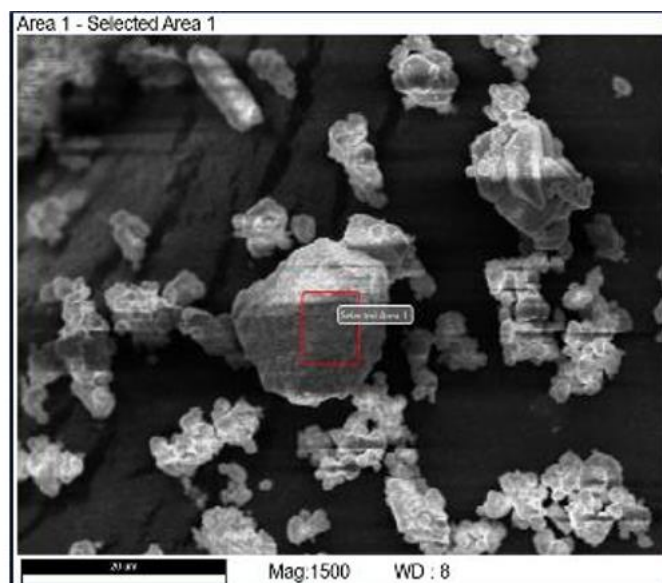


Fig. 6(a)

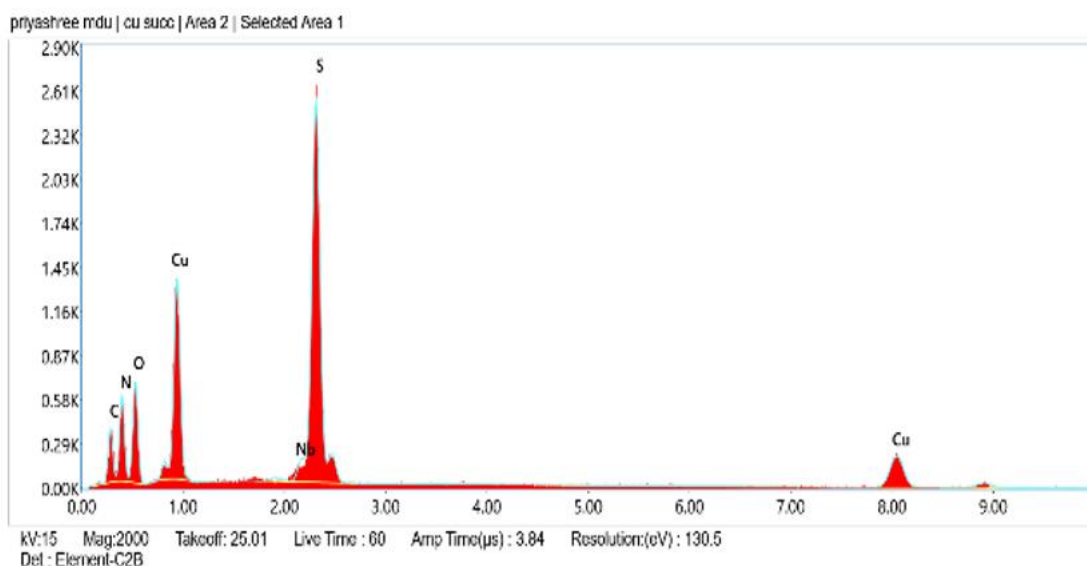


Fig. 6(b)

Fig. 6 (a&b) : EDS analysis of the % adsorption of M (II) sulphates with template mediated macrocyclic complex of succinimide and thiourea.

Regarding the components OK, CuK Mol Pb M UV transmittance 7.99% probability that the weight is less than CuK 1.3 higher Pb M, 67.6 higher atomic percentage OK28.0 and Cu K is 1.3 higher percentage error Cu K 36.4, Pb M 5.9 and UV transmittance are the respective values. 1.1802 CuK has a higher element frequency while 1.0000 has a lower frequency as shown in Fig. 6. In the OK picture element, CuK Mol Pb M kv 15 mag. 2000 no starting point 8/25 real-time 60 minutes amp. Time (µs) 3.84 Resolution (ev) 130.5 where pb is 20.0 k from the interval to point 0.00K.

Table 2: EDS analysis of the % adsorption M (II) sulphates in template mediated complex of adipic acid and thiourea.

eZAF Quant Result - Analysis Uncertainty: 8.26 %						
Element	Weight %	Atomic %	Error %	R	A	F
Pb M	67.6	20.0	5.9	0.7583	0.6624	1.0069

Cu M	1.3	1.3	36.4	0.8879	0.9382	1.1802
Ni M	9.5	2.8	10.9	0.7572	0.6525	1.0138
Zn M	1.8	0.6	21.7	0.7548	0.6144	1.0143

Pharmacology

On the basis of the findings gathered from a variety of different chemical and physical experiments, the antibacterial studies for adipic acid and thiourea are as follows:

The TA Compound and its Metal Complexes Exhibit Antibacterial Activity

Studies were conducted on the antibacterial activity of TA as well as its metal complexes, such as Coll and Nill. By using a two-fold serial micro dilution method, these complexes were put through in vitro testing against three different bacterial species, including *Staphylococcus aureus* (gramme +ve), *Escherichia coli* (gramme - ve), and *Pseudomonas aeruginosa* (gramme -ve). The minimal inhibitory concentration, also known as the MIC, and the minimum bactericidal concentration, also known as the MBC, are both stated in micro litres.

Order of Activity

SA : $ZnSO_4.TA.2H_2O = CuSO_4.TA.2H_2O = NiSO_4.TA.2H_2O = PbSO_4.TA. 2H_2O > TA$

E.coli : $ZnSO_4.TA.2H_2O > CuSO_4.TA.2H_2O > NiSO_4.TA.2H_2O = PbSO_4.TA. 2H_2O, TA$

PA : $ZnSO_4.TA.2H_2O > CuSO_4.TA.2H_2O = NiSO_4.TA.2H_2O, TA > PbSO_4.TA.2H_2O$

The $[ZnSO_4.TA. (H_2O)_2]$ complex has the greatest antimicrobial efficacy against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The Nickel sulphate compound of TUA has the lowest level of antimicrobial action against *Pseudomonas aeruginosa*. When compared to the antibacterial activity shown by the metal complexes, the ligands have a relatively little amount of this activity.^[19]

According to the Health Protection agency (WOAH) and the Food and Nutrition Organization (FAO), the spread of germs, which are impervious to several treatments, is a major threat to human and animal research. Developing resistance to antibiotics in bacteria is neither rare nor unprecedented. However, the problem is becoming more difficult due to the emergence of unique evolving resistance phenotype among many infectious agents and even innocuous species. An in-vitro antibiotics susceptibility test may provide a good indication of how an organism will respond to antimicrobial therapy in a sick host. These epidemiological surveillance data provide the backbone for determining which first-line treatment is the most effective and for tracking the emergence and spread of resistant bacterial strains and resistance

patterns across different bacterial species. Multiple Antibiotic Susceptibility Testing (AST) methods may determine whether a bacterium is sensitive to a certain antimicrobial. When deciding on a method, several factors are considered. These include the technique's precision, reliability, cost, ease of automation, flexibility, usefulness, and even the user's own preferences.

Reference strains recommended by the CLSI for AST profiling are as follows:

- *Bacillus subtilis*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

There are two types of approach for pharmacological studies and we used the Disk and well diffusion approach.

(a) Disk and well diffusion approach

The terms "disc filtration" and "well" refer to

the process of introducing a predetermined concentration of an antibiotic agent into a solid culture medium that has already been implanted with a liquid culture of such desired inoculum using discs, strips, or tablets. The effectiveness of disk/well diffusion is dependent on the identification of an inhibition zone whose size is directly related to the degree to which the disk's resident bacteria are sensitive to the antibiotic. An antibacterial gradient is generated as an antimicrobial agent diffuses into a seeded culture media. The inhibition zone is defined as the range of antibiotic concentration where further growth of the test bacterium is no longer inhibited. The minimum effective concentration (MIC) of the antimicrobial disc against the test microorganisms is proportional to the diameter of the minimum inhibitory concentration around the disc. In general, minimal antibiotic is required to halt an organism's growth if the zone of inhibition is wider. Of course, this assumes that the antibiotic has a high enough concentration and diffusivity in the disc.

Disk/well diffusion experiments that depend only on the presence or absence of an inhibition zone, without also taking the size of the inhibition zone into account, are not accepted by the AST approach.

- Consideration for the use of the disk/well diffusion method
- The disk/well diffusion procedure is simple, repeatable, and equipment-free.

Benefits include

- low cost
- simplicity of altering test antimicrobial disk as needed,
- The disks/well should be distributed evenly, no closer than 24mm from the center to avoid the overlap of zones.
- Always a secondary culture having turbidity equal to 0.5 macfar land or OD600 is equal to 0.08 to 0.12 and use the culture within 15 min.
- A stock solution of each compound was prepared using a solvent such as dimethyl sulphoxide (DMSO). A blank paper disc/well was loaded with 20ul

of the prepared stock solutions to obtain the desired drug conc per disk.

- Zone of inhibition measured in mm and compared with the CLSI guidelines, to categorize the isolate as sensitive(S), Resistant(R), or intermediate (I) activity against the particular antibiotic.
- In the case of a new anti-microbial agent the zone will be compared with the currently available broad-spectrum antibiotics such as ampicillin and amoxicillin.

Test Protocol

- Overnight growth culture of pure bacterial isolate inoculated in nutrient broth (secondary culture), incubated at 37°C shaking until turbidity matches 0.5 MacFarland.
- Once the turbidity matched 0.5 MacFarland swab the culture back and forth on the nutrient agar plate and let the plate dry for 10 min.
- After that, test antimicrobial agent disks were placed on the plate and incubated at 37°C overnight.
- The zones of inhibition on the plates were examined after incubation.

(b) Broth dilution technique

The minimum inhibitory concentration (MIC), usually denoted in units of micrograms per millilitre ($\mu\text{g/ml}$) or milligrammes per litre (mg/l), of an antibiotic is determined by broth dilution methods. The phrase "broth dilution" refers to a technique that involves testing several doses of an infectious drug in a liquid state against even a bacterial isolate at a known, optimal concentration. Microdilution allows the broth dilution operation to be performed with as little as 2 ml of broth in a tube. The "real" MIC is found somewhere between the tested concentrations in the test that inhibits bacterial growth and the next least intensity in the test. Therefore, it is reasonable to presume that the inherent variance in MIC studies that use a series many dilutions was a dilution. Both quality check reference creatures and the interpret requirements for a given antimicrobial drugs combination should be included in the

antimicrobial ranges. When compared to the dilution approach for testing bacteria for resistance to antibiotics, the agar disk/well diffusion technique seems to be less precise and numeric. Because of the importance of accuracy and reliability in these laboratories' procedures, quality control organisms are important.

Test protocol

Each tube carrying 1ml of the antimicrobial agent in the dilution series (a positive control tube comprising only broth) should have 1ml of the adjusted inoculum added to it within 15 minutes after the inoculum has been standardized to 0.5 McFarland.

Incubate the inoculated tubes at appropriate conditions in an ambient air incubator.

The growth endpoints for each set of tests are calculated by comparing the growth in the antimicrobial agent-containing growth tubes with the growth in the growth-control tubes (without the antimicrobial).

3.3 INOCULUM PREPARATION

Bacterial

- Prepare the inoculum by creating a broth suspension after secondary inoculation of an overnight grown culture in the nutrient broth.
- To produce turbidity that meets the 0.5 McFarland turbidity requirement, modify the suspension. As a consequence, the suspension has a

CFU ("Colony Forming Unit") content of 1 to 2×10^8 /ml.

- 0.5 McFarland standard along with inoculum tube should be compared to a card with a black line and white background for contrast.
- Dilution in a broth of the appropriate inoculum suspension should take place ideally within 15 min of preparation.
- Culture preparation technique is different for each culture while the test protocol remains the same.

3.4 IN VITRO ANTIBACTERIAL ACTIVITY

The effectiveness of the newly synthesised chemical in preventing the development of *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was evaluated using the agar well diffusion technique. All of the compounds showed antibacterial action against "Gram-positive" bacteria like *S. aureus* and *B. subtilis*.

According to the data in the table below, only the positive controls significantly inhibited the growth of the test microorganisms, whereas the negative standards had no impact at all. Antibiotics streptomycin, ciprofloxacin were used as gold standards against which the effectiveness of the title compounds may be compared.

Table 3 : In vitro antimicrobial activity of template mediated macrocyclic complex with Cu(II) sulphates through agar well diffusion method

Templates	E.coli	Staphylococcus	pseudomonas
ATCu (ug/ml)	Diameter of growth of inhibition zone (mm) ^a	Diameter of growth of inhibition zone (mm) ^a	Diameter of inhibition zone (mm) ^a
25	15	27	23
50	20	20	20
75	25	26	27
100	30	30	30

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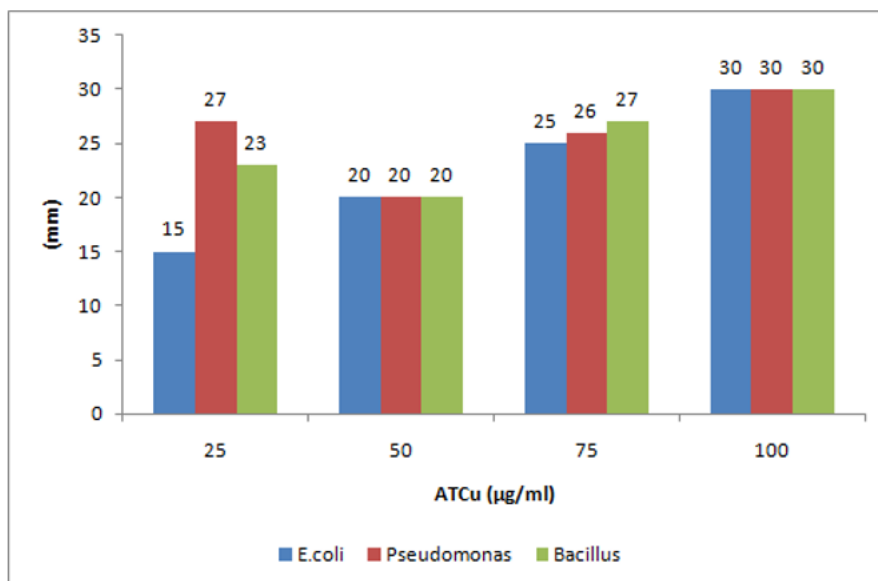


Fig. 7: Comparisons of diameter of growth of inhibition zone (mm) of template mediated macrocyclic complex with Cu(II) sulphates

Anti-microbial activity of template mediated macrocyclic complex with Cu(II) sulphates

All the tested derivatives were approved for anti-bacterial action as opposed to gram-positive bacteria and gram-negative bacteria. The synthesized derivatives displaying inhibition zone in Fig. 7, vary in the middle of 10mm to 30 mm for B.subtilis and S.aureus. On the report of the zone of inhibition generated, template mediated complex seems to be very effective with the highest rate of inhibition which is 30 mm in Bacillus, as opposed to S.aureus and the zone

varies in the middle of 15 mm to 20 mm for E.coli and P.aeruginosa. On the report of inhibition generated, template mediated macrocyclic complex with Cu(II) sulphates was estimated to be very effective with the higher rate of inhibition which is 30 mm as opposed to E. coli. MIC (minimum inhibitory concentration) of templated complex was estimated that displayed in primary screening. Templated complex shows good activity for both gram-positive (B.subtilis, S.aureus) and gram-negative strains (E.coli, P.aeruginosa).^[20]

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Table 4: In vitro antimicrobial activity of template mediated macrocyclic complex with Zn (II) sulphates through agar well diffusion method

Templates	E.coli	Staphylococcus	pseudomonas
ATZn (µg/ml)	(Diameter of growth of inhibition zone (mm)a	(Diameter of growth of inhibition zone (mm)a	(Diameter of growth of inhibition zone (mm)a
25	...	23	..
50	...	26	..
75	...	24	..
100	...	27	..



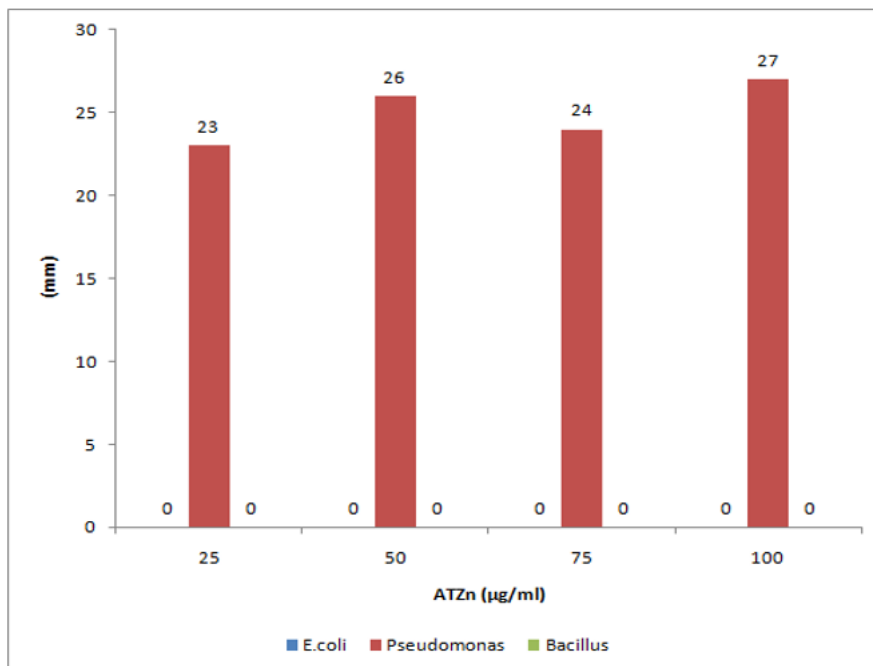


Fig. 8: Comparisons of diameter of growth of inhibition zone (mm) of template mediated macrocyclic complex with Zn (II) sulphates

Anti-microbial activity of template mediated macrocyclic complex with Zn (II) sulphates

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All the tested derivatives were approved for anti-bacterial action as opposed to gram- positive bacteria and gram-negative bacteria. The synthesized derivatives displaying inhibition zone in Fig. 8, vary in the middle of 10mm to 30 mm for B.subtilis and S.aureus. On the report of the zone of inhibition generated, template mediated complex seems to be very effective with the highest rate of inhibition which is 27 mm in P.aeruginosa, as opposed to S.aureus and the zone varies in the middle of 00 mm to 00 mm for E.coli and Bacillus. On the report of inhibition generated, template mediated macrocyclic complex with Zn(II) sulphates was estimated to be very effective with the higher rate of inhibition which is 27 mm as opposed to E. coli. MIC (minimum inhibitory concentration) of templated complex was estimated that displayed in primary screening. Templated complex shows no activity for both gram- positive (B.subtilis, S.aureus) and good for gram- negative strains (P.aeruginosa).

Table 5: In vitro antimicrobial activity of template mediated macrocyclic complex with Ni (II) sulphates through agar well diffusion method

Templates	E.coli	Staphylococcus	pseudomonas
ATNi (µg/ml)	(Diameter of growth of inhibition zone (mm)a)	(Diameter of growth of inhibition zone (mm)a)	(Diameter of growth of inhibition zone (mm)a)
25	..	23	..
50	..	26	..
75	..	24	..
100	..	27	..



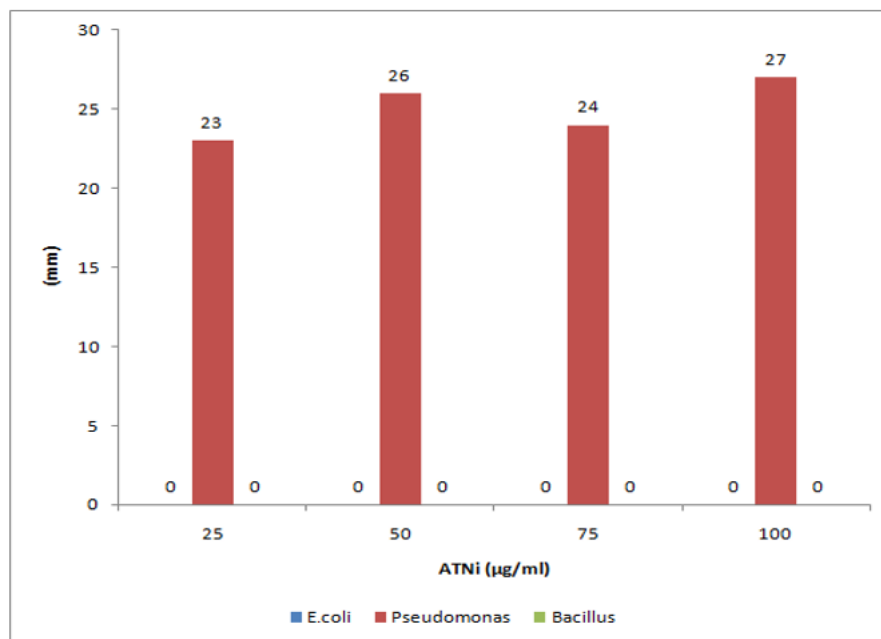


Fig. 9: Comparisons of diameter of growth of inhibition zone (mm) of template mediated macrocyclic complex with Ni (II) sulphates
Anti-microbial activity template mediated macrocyclic complex with Ni (II) sulphates

All the tested derivatives were approved for anti-bacterial action as opposed to gram-positive bacteria and gram-negative bacteria. The synthesized derivatives displaying inhibition zone in Fig. 9, vary in the middle of 10mm to 27mm for *B.subtilis* and *S.aureus*. On the report of the zone of inhibition generated, template mediated complex seems to be very effective with the highest rate of inhibition which is 27 mm in *P.aeruginosa*, as opposed to *S.aureus* and the zone varies in the middle of 00 mm to 00 mm for *E.coli* and *Bacillus*. On the report of inhibition generated, template mediated macrocyclic complex with Ni (II) sulphates was estimated to be very effective with the higher rate of inhibition which is 27 mm as opposed to *E. coli*. MIC (minimum inhibitory concentration) of templated complex was estimated that displayed in primary screening. Templated complex shows no activity for both gram-positive (*B.subtilis*, *S.aureus*) and good for gram-negative strains (*P.aeruginosa*).

When compared to the commercial streptomycin ciprofloxacin, which showed a max inhibition zone of 30 mm against *E. coli*, *Pseudomonas*, and *Bacteroides*, the derivative Cu Adipic acid thiourea showed 27 mm. The

series as a whole saw a wide variety of MIC values, from 16 to 256 g/ml, among the many “chemical substances” tested. The *Escherichia coli* bacteria were immune to all of the chemicals.

3.5 BIOLOGICAL ASSAY

Test microorganisms

The focus was on three bacterial species because of their possible role they may play as disease-causing agents in human medicine. Three bacterial strains were used to assess the effectiveness of the antibacterial: one gram-positive (“*Bacillus subtilis* MTCC 121”) and two gram-negative (*Escheria coli* MTCC 16520 and *Pseudomonas aeruginosa* (MTCC 741)). Microbial Culture Farm Collection was done at IMTECH in Chandigarh from where all of the microbes came from (or MTCC for short). Subcultures of yeast were kept alive on malt yeast agar, whereas those of bacteria were kept alive on nutrient agar.

The agar culture diffusion technique was used to analyse the antibacterial potency of newly synthesised medications. Microorganism concentrations were calculated using the 0.5 McFarland standard, which visually represents a microbiological solution with 1.5×10^8 cfu/ml. After allowing 20 ml of agar material to sink into each PETRI plate for 15 minutes, 100µl of

the bacterial isolates were added. Wells were drilled using a sterile 8 mm cork borer, and 100µl volumes holding 2.0mg/ml of each compound reconstitution in DMSO (Dimethylsulphoxide) were applied to seeded agar medium. Each plate was incubated at 37 degrees Celsius for 24 hours. Each facility's antimicrobial activity was determined quantitatively by using a zone reader ("Hi Antibiotic zone scale") to quantify the area where growing suppression was seen in relation to the test organisms.

The agar well diffusion technique was used to examine the antibacterial activity of the recently synthesised 3-aminobenzo[e] [1,3] thiazepine-1,5-dione against three different bacterial species namely: *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. All of the substances that were put through this study's rigorous testing process had distinctive qualities. As the positive controls had a significant inhibitory impact on the test microorganisms, whereas the negative controls had no such effect. Antibiotics ciprofloxacin (a common commercial option) and streptomycin were used to evaluate how the substance in question performed, it displays the "in vitro antibacterial activity" (as assessed by the agar well diffusion technique) of several synthesised chemical compounds. Negative activity values (including good diameter, 8 mm) are averages of three replicates based on the highest inhibitory activity as shown against Gram-positive bacteria, which demonstrated a region of inhibition of 27 mm when compared to that of the commercial antibiotics' streptomycin and ciprofloxacin. MICs for the whole battery of compounds tested against Gram-positive bacteria varied between 16 and 256 g/ml. It was found that the compound with the lowest MIC against *B. subtilis* (16 g/ml) was also the overall most effective compound overall (32 g/ml). Despite repeated chemical treatments, the gram-negative bacteria remained unaffected.

4. CONCLUSION

The synthesis and detailed characterization of template-mediated macrocyclic complexes using thiourea and adipic acid (TA) presented in this study underscore the potential of these

compounds in supramolecular chemistry and as promising antimicrobial agents. The systematic approach to synthesis, followed by rigorous analytical characterization, has validated the formation of the TA complexes with distinct structural features. The study's findings from FT-IR, NMR, XRD, and SEM-EDS analyses provide a deep insight into the molecular architecture and surface morphology of the complexes, confirming their successful synthesis and high purity. The antimicrobial evaluation of the TA complexes against various bacterial strains has unveiled their potential as effective antibacterial agents, highlighting their significance in addressing the ongoing challenge of bacterial resistance. This research not only contributes to the field of chemistry by expanding the understanding of macrocyclic complexes but also opens avenues for further exploration into their applications in medicine, particularly in developing new antimicrobial compounds. The promising results advocate for continued investigation into the structural modification of these complexes to enhance their efficacy and specificity as antimicrobial agents, potentially leading to novel therapeutic options against resistant bacterial infections.

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