CHAPTER V

Synthesis, physico-chemical characterization and antibacterial activities of newly synthesized amino functionalized thio-modified *β*-cyclodextrin based ligand and its Fe(III) complex

5.1. Introduction

Transition metal complexes with polydentate ligands have played a essential role in chemistry.[1,2] The most common coordinating atoms in polydentate ligands are generally donor hetero atoms, such as nitrogen, phosphine, oxygen, and sulfur.[3,4] Of late, research on metal complexes with nitrogen and sulphur containing ligands have been attracted the chemistry worldwide, because nitrogen and sulphur can have crucial roles in many metallic bio-molecules. Sulfur and nitrogen containing ligands and their transition metal complexes also act as corrosion inhibitors[5, 6] and can be used as extreme pressure lubricant additives.[7] Many of the S, N containing chelating ligands and their metal complexes also possess biological activities such as antifungal,[8] antibacterial[9] and anti humentery[10-16] activities, *etc*. However, poor aqueous solubility of such complexes often restricts their applications regarding their biochemical activities. The solubility of such complexes can be enhanced by tagging of *β*-cyclodextrin moiety[17-19] in the lignd structure and thereby in the complex structure. Therefore in this work synthesis, physico-chemical characterization and antibacterial activities of a new water soluble S, N containing ligand and its Fe(III) complex has been reported.

5.2. Experimental section

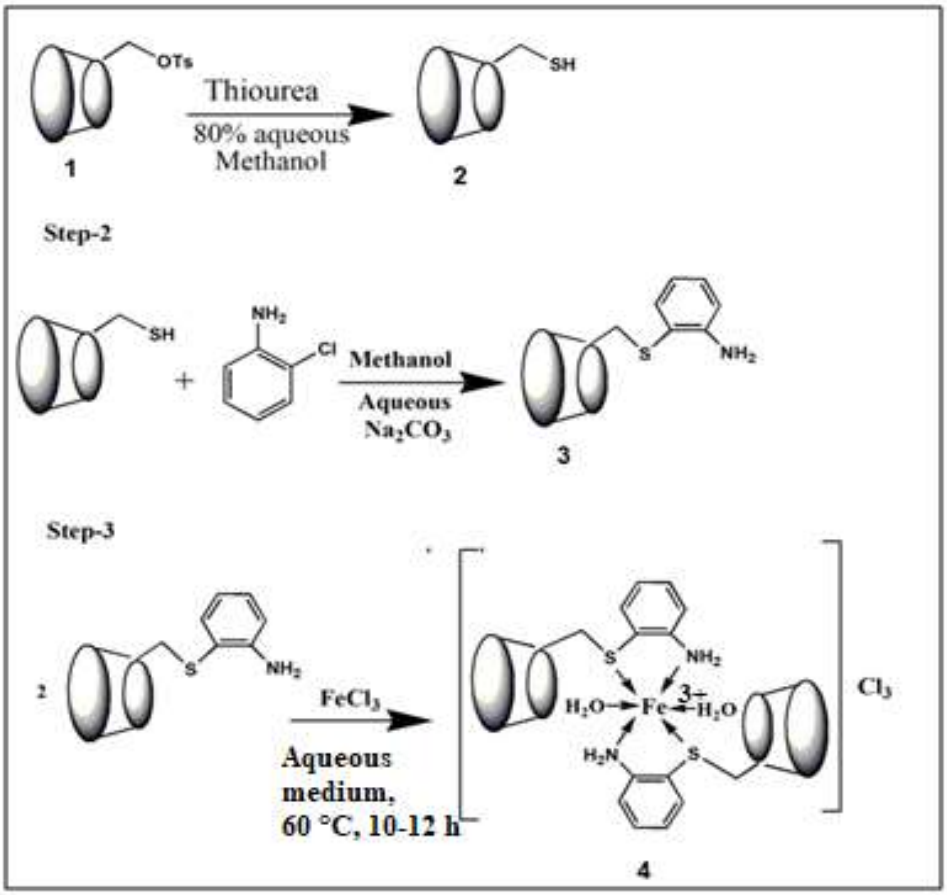
5.2.1. Materials and methods

All the chemicals were obtained from Sigma Aldrich, Germany and were used without further purification. In all experiments bi-distilled water was used. Elemental microanalyses (C, H and N) were performed by using Euro VECTOR EA 3000 analyzer. IR spectra were recorded on Perkin-Elmer Spectrum FTIR spectrometer (RX-1) in the range 4000-400 cm^{-1} at ambient temperature using KBr pellets and UV-Visible spectra were recorded on a JascoV-530 double beam Spectrophotometer using a quartz cell with path length 1 cm equipped with thermostated bath (maintained at 25±0.1 °C) using water and DMSO as solvent reference. ^1H NMR spectra were recorded on a Bruker Advance-II 400 MHz spectrometer by using D_2O and DMSO-d_6 as solvents at room temperature and chemical shifts (δ) were quoted in ppm with

respect to TMS. The ESI-MS of the ligand and complex were measured by the Waters ZQ-40000 instruments. Metal content was determined by AAS (Varian, SpectraAA 50B) by using standard metal-solution from Sigma-Aldrich, Germany. Magnetic susceptibility was measured with a Sherwood Scientific Ltd magnetic susceptibility balance (Magway MSB Mk1) at room temperature. Specific conductance of the synthesized complex was measured in water (at room temperature) using a Systronics (India) conductivity metre (TDS-308). Antibacterial activities (*in vitro*) of the synthesized ligand and the complex were studied by disc diffusion method against four bacteria, *viz.*, gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria.

5.2.2. Synthesis

The synthesis pathway of a thio functionalized β-cyclodextrin based ligand and its Fe(III) complex are shown in Scheme 5.1.



Scheme 5.1. Syntheses of compounds 1, 2, 3 and 4.

5.2.2.1. Synthesis of mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin [*β*-CDOTs, 1]

The method of preparation of Mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin [β-CDOTs, 1] was described in chapter III (3.2.2.1).[18]

5.2.2.2. Synthesis of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2)[15]

2 g of powdered *β* -CDOTs (1) and 2 g of thiourea were dissolved in a 100 mL 80% aqueous methanol and refluxed for 2 days at 60 °C. After refluxing the solution was evaporated in vacuo. 30 mL methanol was added to the residue and stirred for 1 hour. The residue was filtered and dissolved in~34 mL of 10% NaOH and left it for 5 hours at 50-60 °C. The p^H of the solution was maintained to 2 by adding 10% HCl and 2.5 mL trichloroethylene ($CHCl_3$) and was stirred overnight. The obtained precipitate was filtered and washed with water several times. The trichloroethylene ($CHCl_3$) was removed in vacuo and repeated recrystalization from water results the compound 2.

Color: White; Yield (56%); IR, KBr cm^{-1}: 2562, 1650, 1365-1200 cm^{-1}; ^1H NMR (400 MHz, DMSO-D_6, 25 °C): 5.95-5.86 (m, 14H, OH2 and OH3 CD), 4.92-4.85(m, 7H, H1 CD), 4.59-4.51 (m, 6H, OH6 CD), 4.35 (m, 2H, H6/ CD), 3.98 (m, 1H, H5/ CD), 3.71-3.61 (m, 25H, H3, H5 and H6 CD), 3.43-3.34 (m, H2, H4 overlap with water), 2.14 (s,1H,SH), ppm;[18,20] Anal. Calcd for $C_{42}H_{70}O_{34}S$: C, 43.82; H, 6.12; O, 47.25; S, 2.78. Found: C, 43.28; H, 5.87; O, 46.86; S, 2.27. m/z (ESI): calculated 1151.02 found 1152.24 $[M+H]^+$.

5.2.2.3. Synthesis of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3)

A mixture of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2) (344.7 mg) and *o*-chloroaniline (38.25 mg) was dissolved with 70 mL of aqueous Na_2CO_3 (p^H 10) containing 20 mL ethanol and stirred under nitrogen for 2 days at room temperature. The p^H of the solution was adjusted to 3 by adding 1N HCl and concentrated it in vacuo to~ 40 mL. The solution was stirred for 1 day after adding 5 mL trichloroethylene. The precipitate formed was collected with trichloroethylene using a separating funnel. After evaporation of the trichloroethylene *in vacuo* the solid product was obtained and purified by repeated recrystalization.

Color: White; Yield (78%); IR cm^{-1}: 1612, 1262, 753 cm^{-1}; ^1H NMR (400 MHz, D_2O, 25 °C): δ = 7.53 (m,1H, Ph), 7.28 (m, 1H, Ph), 7.12 (m, 1H, Ph), 6.91 (m, 1H, Ph), 5.93-5.89 (m, 14H, OH2 and OH3 CD), 4.71-4.64 (m, 7H, H1 CD), 4.45-4.38 (m, 6H, OH6 CD), 4.25 (s, 2H, NH_2), 4.11 (m, 2H, H6/ CD), 3.89 (m, 1H, H5/

CD), 3.78-3.64 (m, 25H, H3, H5 and H6 CD), 3.54-3.41 (m, H2, H4).;UV: 206 nm, 312 nm; Anal. Calcd for $C_{48}H_{75}NO_{34}S$: C, 46.41; H, 6.08; N, 1.12; O, 43.79; S, 2.58. Found: C, 46.03; H, 5.19; N, 0.97; O, 43.24. m/z (ESI): calculated 1242.13, found 1242.63 $[M]^+$.

5.2.2.4. Synthesis of Fe (III)-complex of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (4)

A aqueous solution of 0.1 mmol (27.03 mg) $FeCl_3.6H_2O$ was added drop wise to a stirred aqueous solution of 2 mmol (248 mg) of ligand (3) at room temperature and refluxed for 10-12 h at 60 °C. The solution was then concentrated upto the volume of 15 mL. The precipitate was obtained by addition of acetone (100 mL) was filtered. The obtained solid was dried under vacuum. The prepared compound is air stable and water soluble.

Color: Purple; Yield (80%); IR cm^{-1}, KBr: 840, 735, 525, 466; UV: 232 nm, 370 nm, 524 nm; Anal. Calcd for $C_{84}H_{142}N_2O_{70}S_2Cl_3$ Fe: C, 39.94; H, 5.66; N, 1.10; O, 44.31; S, 2.53, Fe, 2.21. Found: C, 39.42; H, 5.11; N, 0.94; O, 44.07; S, 2.06; Fe, 1.93. m/z (ESI): calculated 2525.80, found 2525.54 $[M]^+$.

5.2.3. Antibacterial activity of the synthetic compounds

The method of performing antibacterial activity following well diffusion method with the complexes has been described in chapter II (section 2.10).

5.3. Results and discussion

Mono-6-deoxy-6-mercapto-β-cyclodextrin (2) was synthesized from mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin [*β*-CDOTs, 1]. After synthesis of the marcapto modified *β*-cyclodextrin (2), it is allowed to react with *o*-chloroaniline which produce mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3), then, it was allowed to react with $FeCl_3.6H_2O$ in aqueous methanol to give amino functionalized marcapto modified *β*-cyclodextrin based Ferric complex (4) (Scheme 5.1). The structure of Fe(III) complex was confirmed by various analytical and spectroscopic method, such as elemental analysis, FTIR, ^{1}H NMR, UV-visible spectroscopy, ESI-MS and molar conductance measurement.

5.3.1. FTIR spectra

The FTIR spectrum of compounds 1 and 2 are shown in (Figure 5.1 and Figure 5.2). The characteristic absorption peaks at 1646 (C=C), 1366 (SO_2 assym), 1153 (SO_2 sym) cm^{-1} in the IR spectrum of 6-OTs-*β*-CD (1)[23] corresponding to sulfonic acid group, have disappeared in the IR spectrum of compound 2. On the other hand, a

characteristic absorption peak of 2562 cm^{-1} appears in the spectrum of 2. The band at 2562 cm^{-1} corresponds to SH stretching vibrational bands suggesting that *β*-cyclodextrin has been thiolated.[20] Again characteristic absorption band at 1200-1055 cm^{-1} appears due to antisymmetric glycosidic (C–O–C) and C-O vibrations in polysaccaride.[24] The band at 1650 cm^{-1} can be attributed to C-C stretching of polysaccharide.

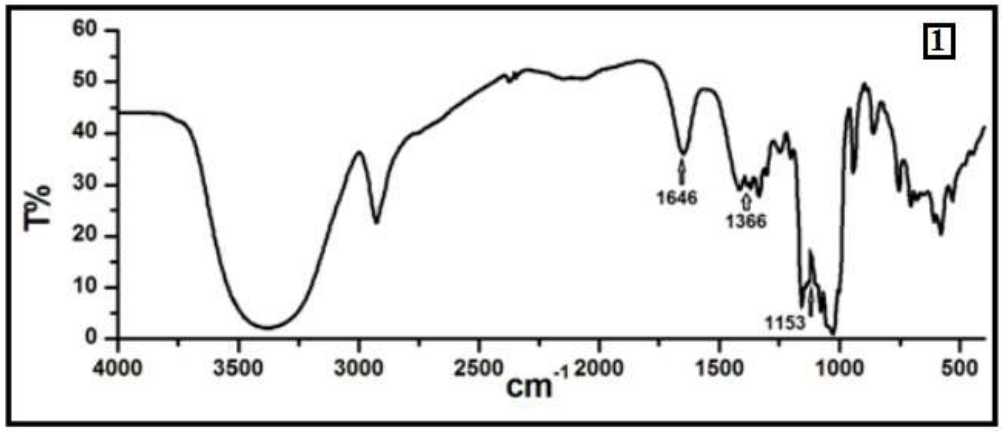


Fig 5.1. FTIR spectrum of Mono-6-deoxy-6-(p-tosylsulfonyl)-β-cyclodextrin [β-CDOTs, 1].

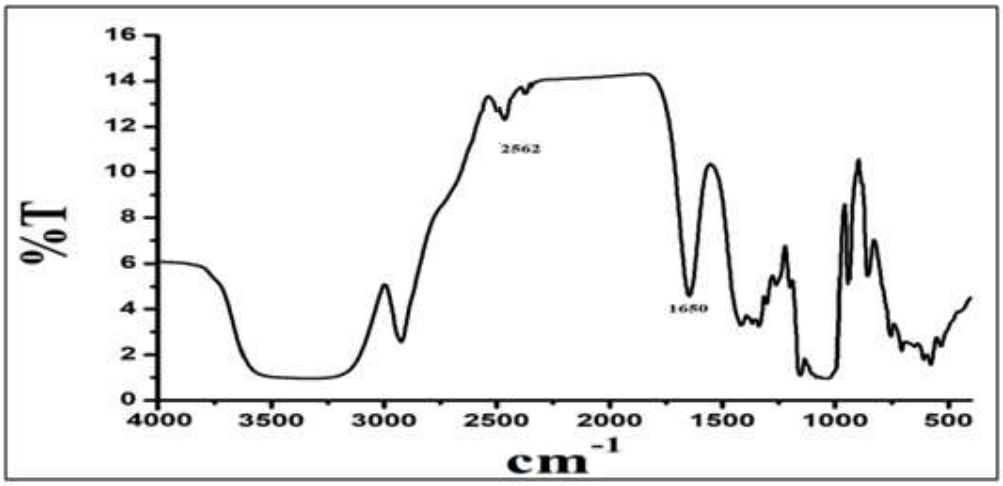


Fig 5.2. FTIR spectrum of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2).

In the FTIR spectrum of 3 (Figure 5.3), the new three characteristic absorption peaks appeared at 1612 (N-H bending), 1262 (C-N stretching) and 753 (N-H wagging) cm^{-1} and the absorption peak at 2562 cm^{-1} in compound 2 disappeared. The results showed that the hydrogen atom of thiol group has been substituted by the aniline group.

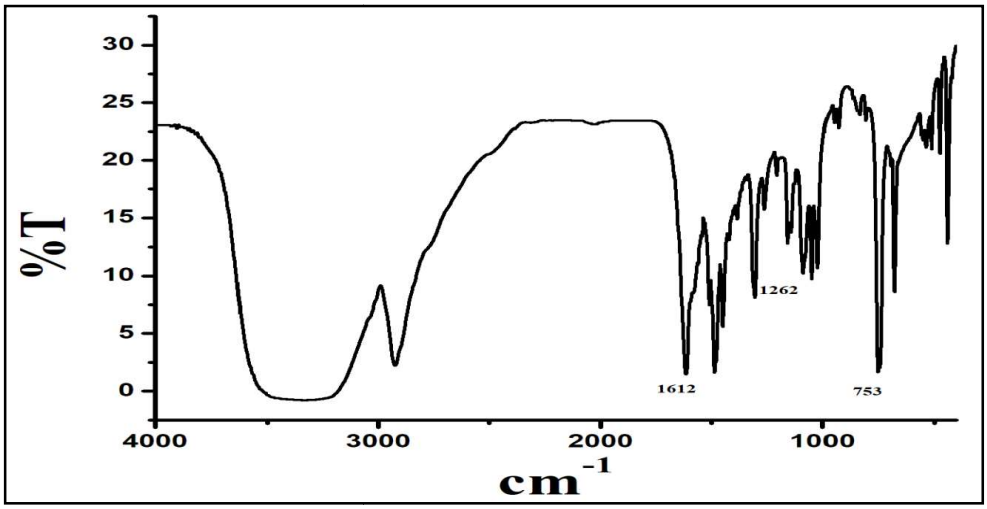


Fig 5.3. FTIR spectrum of mono-6-deoxy-(o-aminobenzylthio)-β-cyclodextrin (3).

In the IR spectra of 4 (Figure 5.4), the bands that appeared at 840 cm^{-1} and 735cm^{-1} were assigned for the rocking and wagging vibration of coordinated water molecule.[18] The band observed at 466 cm^{-1} was attributed to ν(M-S) and the band observed at 525 cm^{-1} was attributed to ν(M-N) vibrations for the complex 4.[25]

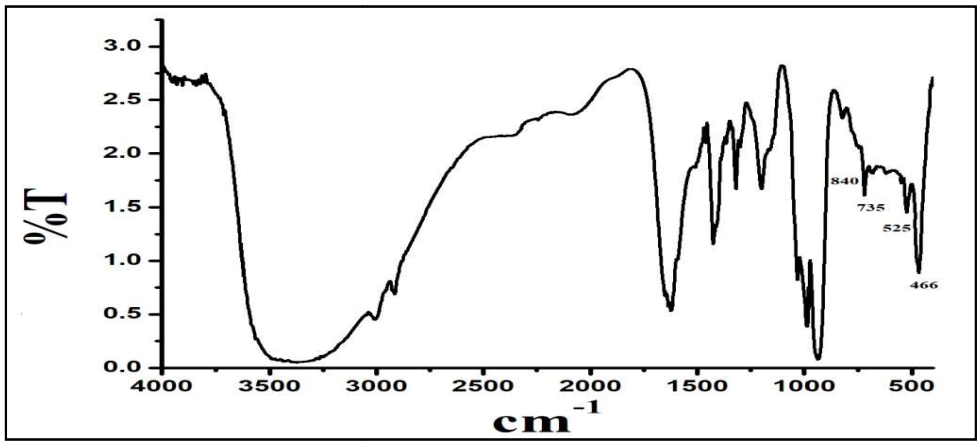


Fig 5.4. FTIR spectrum of Fe(III) complex of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (4).

5.3.2. NMR spectra

^{1}H NMR spectra of the mono-tosyleted β-cyclodextrin (1), mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2), and mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3) were recorded in D_2O and DMSO-D_6 (Figures 5.5 - 5.7). The ^{1}H NMR spectrum of the metal complex could not be obtained, as it is paramagnetic. ^{1}H NMR spectrum of the compound 2 shows that the peak at δ ≈ 2.14 ppm for –SH group.[20] The signals of the H6 protons with a thiol group (H6'a. H6'b) appear at 0.74 ppm lower field than

those of H6 protons of other rings of *β*-cyclodextrin suggesting the formation of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2).[18] The peak of –SH proton in the ^{1}H NMR of the compound (2) get disappeared in the ^{1}H NMR spectrum of the compound (3) and signals of the aromatic protons appeared as multiplets in the range of $\delta \approx$6.91-7.53 ppm and $-NH_2$ group appeared as a singlet in 4.25 ppm which supports the fact of replacement of the thiol proton with aromatic amine in the compound 3.[18]

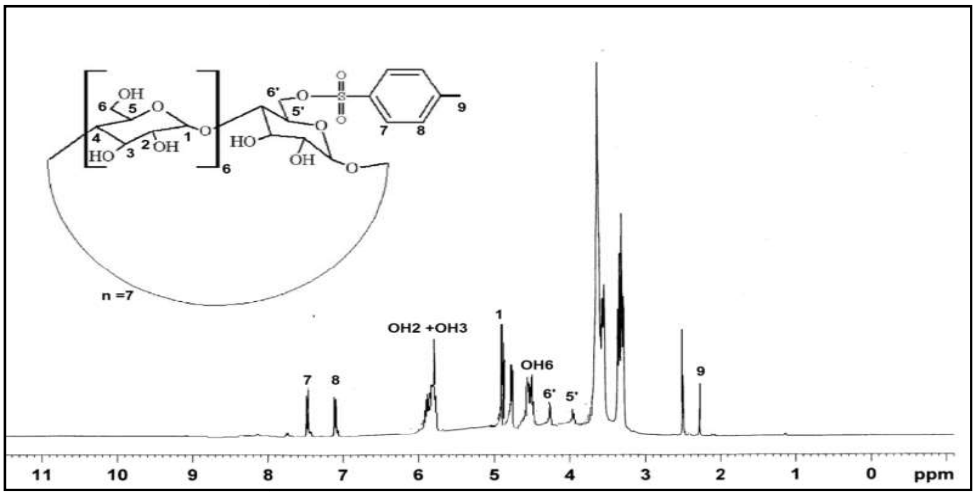


Fig 5.5. ^{1}H NMR of Mono-6-deoxy-6-(p-tosylsulfonyl)-*β*-cyclodextrin [*β*-CDOTs, 1].

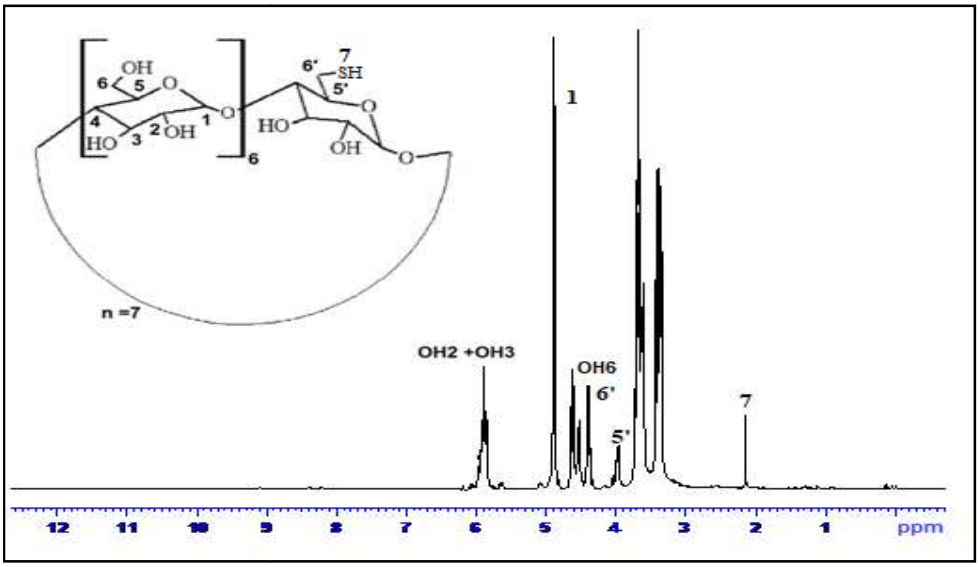


Fig 5.6. ^{1}H NMR of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2).

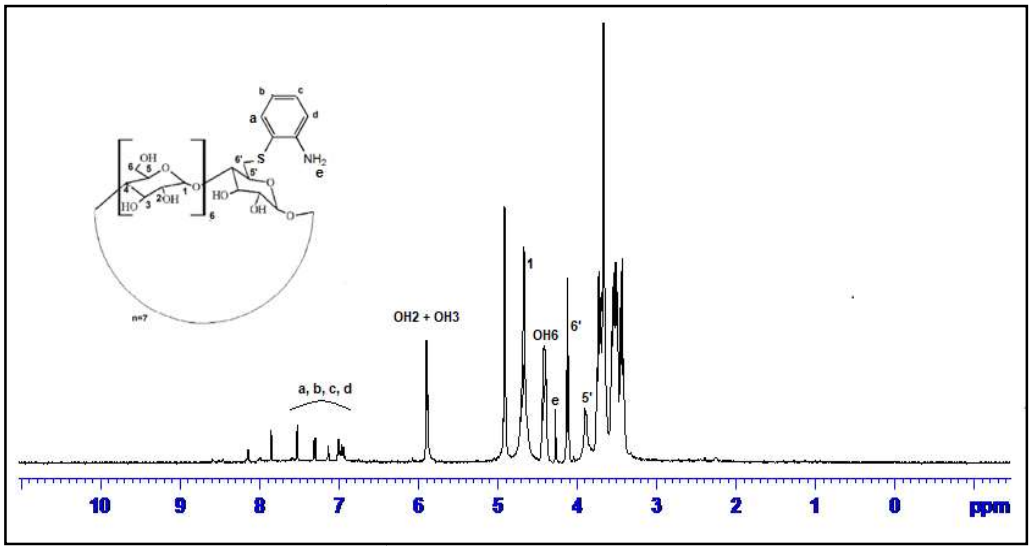


Fig 5.7. ^{1}H NMR of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3).

5.3.3. UV-visible spectra and magnetic moment studies

UV-VIS spectra of the mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3) and Fe (III) complex of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (4) were recorded in water (Figures 5.8 and 5.9).

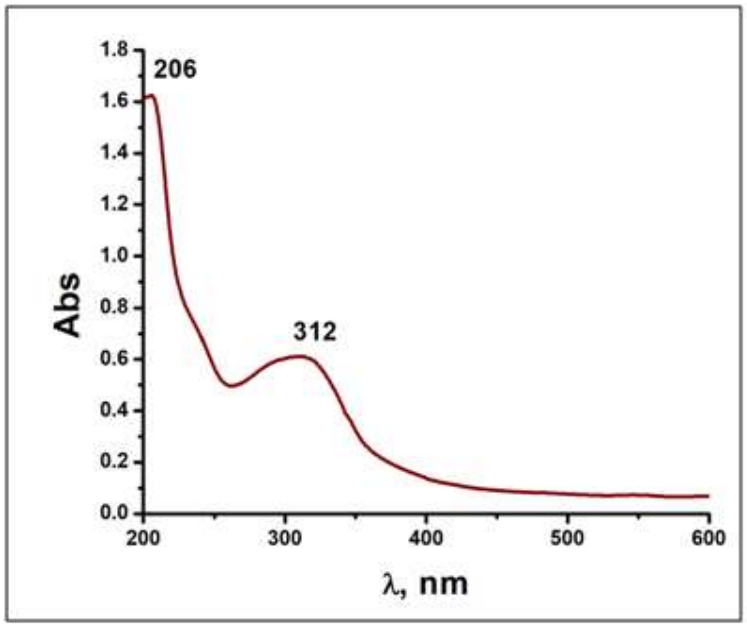


Fig 5.8. Absorption spectrum of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3).

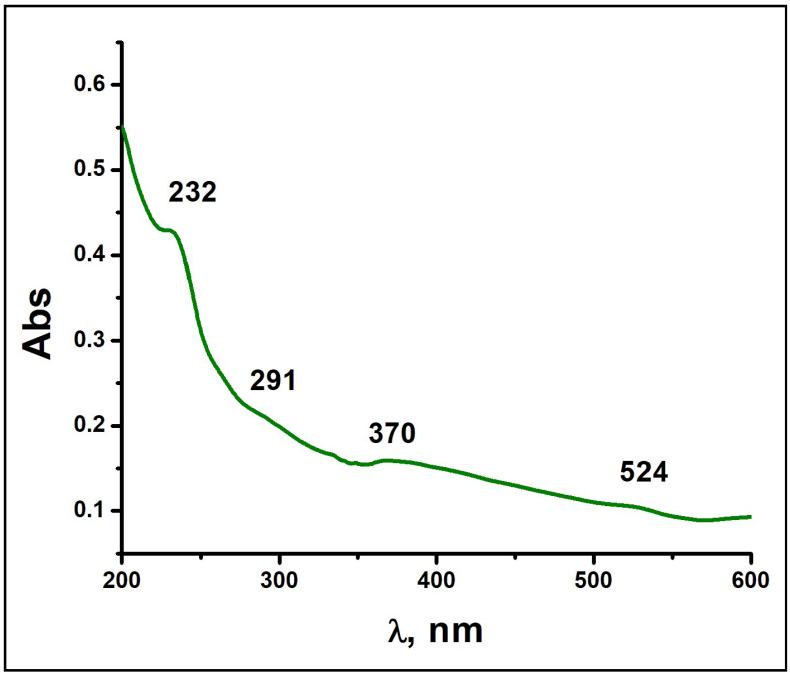


Fig 5.9. Absorption spectrum of Fe(III) complex of mono-6-deoxy-(*o*-aminobenzylthio)-β-cyclodextrin (4).

The compound 3 shows two bands at 312 and 206 nm due to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. In Fe(III) complex (4) the band gets shifted from 206 nm to 232 nm (bathochromic) and thus suggested the coordination of the metal ion (Fe^{3+}) with ligand. Disappearance of the peaks at 312 nm in the complexe compared to that of ligand suggested the coordination of Fe with N-atom of the ligands. The peaks appearing at 370 nm for the complex (4) can be ascribed to MLCT/LMCT transition. However, in several spin equilibrium systems, the high spin (S = 5/2) form has been characterized by transition at 555-500 nm and the low-spin (S = 1/2) form by transition at 714-625 nm.[26] From the spectral study of the Fe(III) complex it can be seen that the complex exhibit one band at 524 nm which can be assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$ transition characteristic of octahedral structure.[26] So, the Fe-complex has high spin octahedral geometry.

From the magnetic moment calculation of the synthesized Fe(III) complex it was found that the complex was paramagnetic. The magnetic moment value (μ_{eff}) is 5.88 B.M. at room temperature. This value indicates high-spin octahedral structure for the Fe(III) complex.

5.3.4. ESI-MS

The ESI-MS spectra of the compound 2, 3 and 4 were recorded and shown in figure (Figures 5.10 - 5.12). The compound 2 and 3 shows m/z peak at 1152.24 and 1242.63 corresponds to $[M + H]^+$ and $[M]^+$, respectively. In Fe(III) complex (4) the new peak appeared at 2525.54 $[M]^+$ which represents molecular weight of complex. Therefore these results were in good agreement with the respective structures as already revealed by the elemental and other spectral analyses.

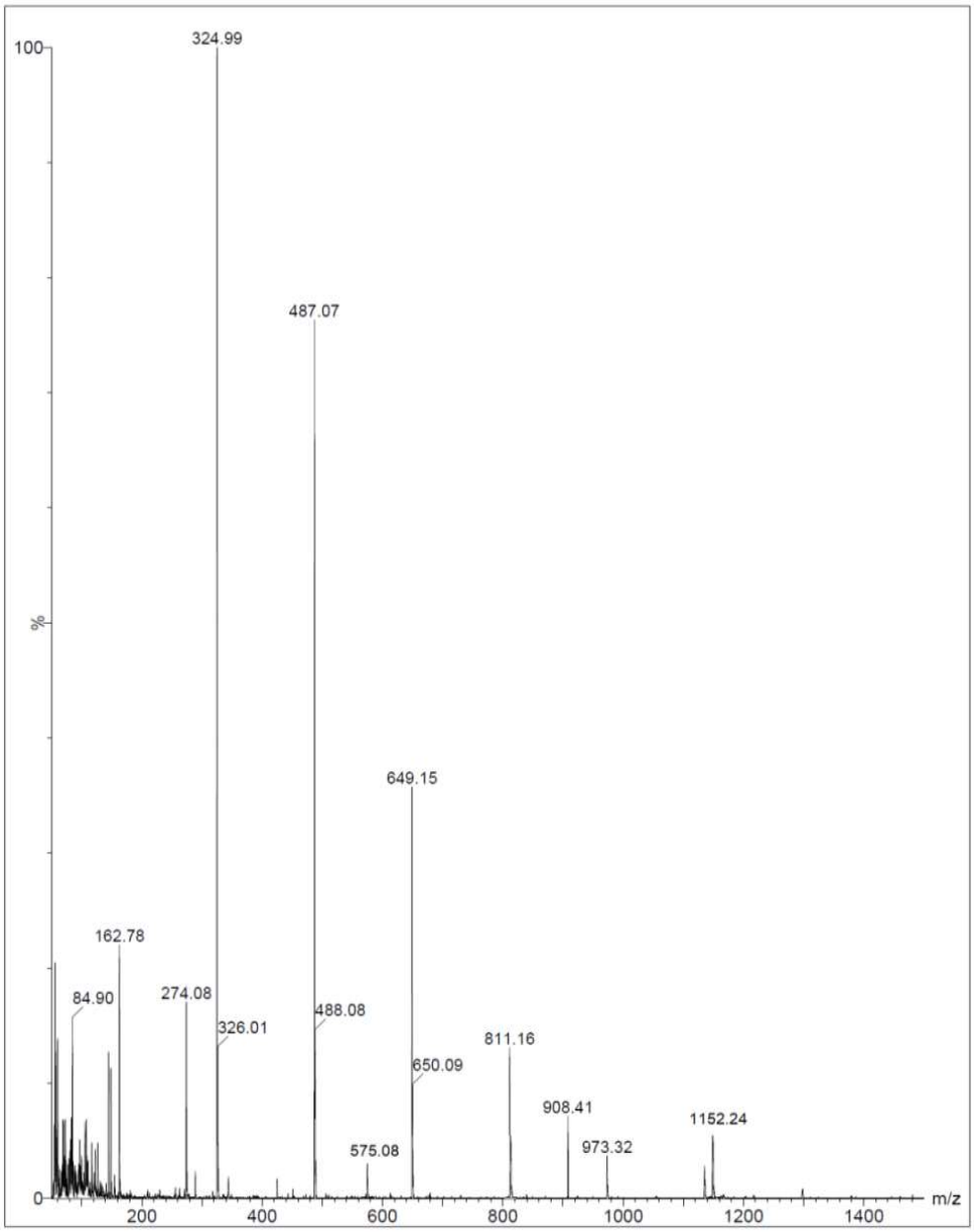


Fig 5.10. ESI-MS spectrum of mono-6-deoxy-6-mercapto-*β*-cyclodextrin (2).

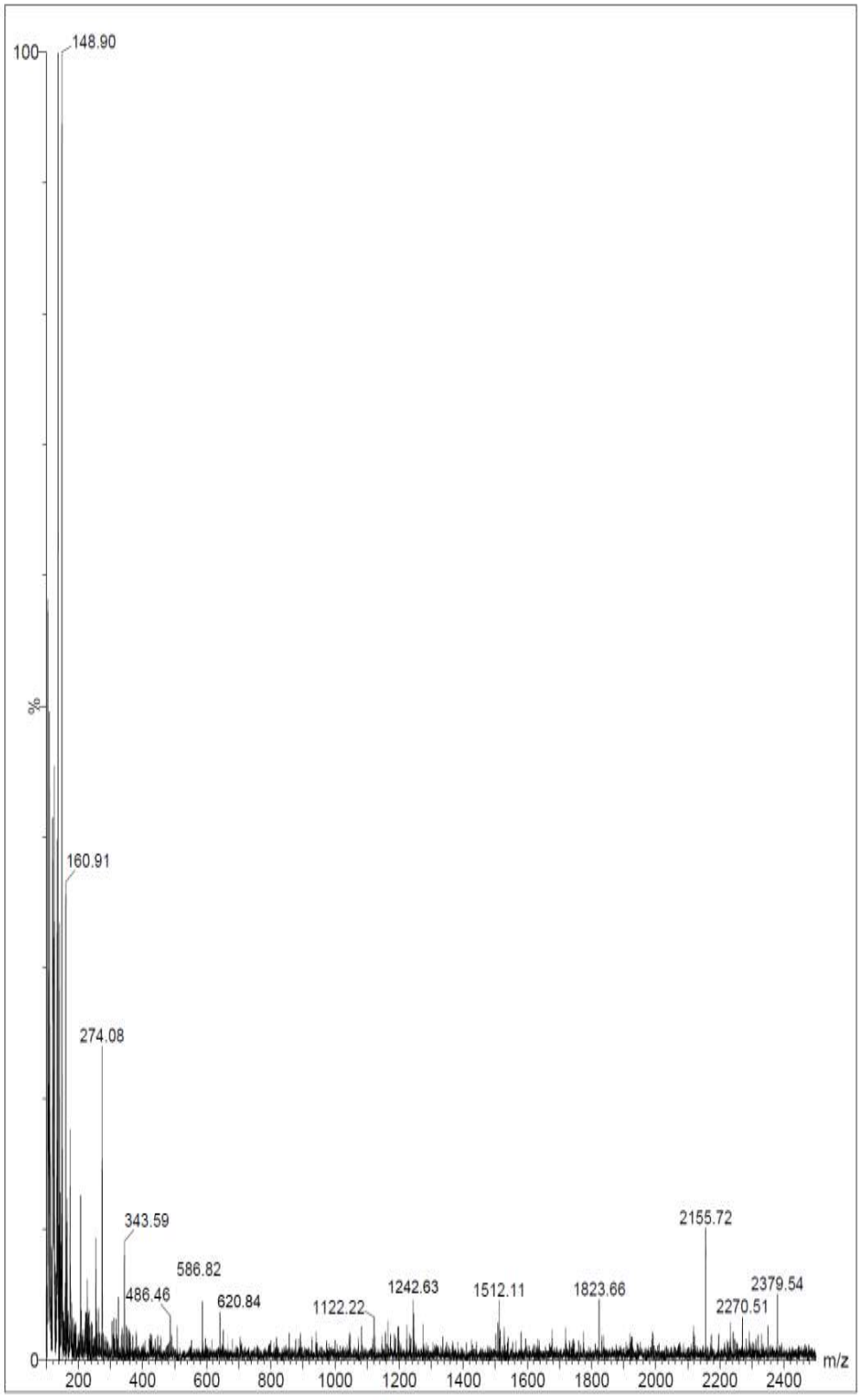


Fig 5.11. ESI-MS spectrum of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (3).

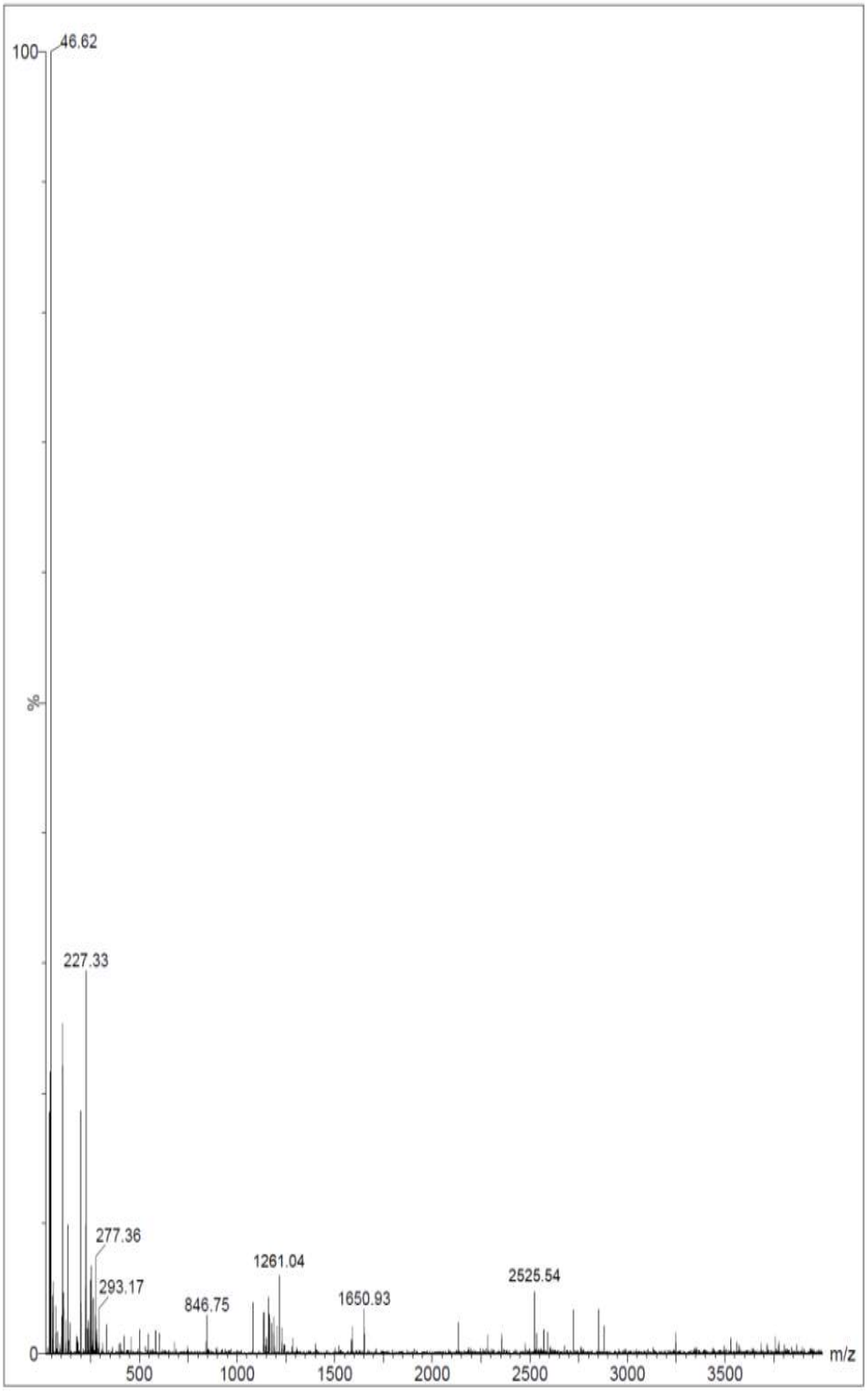


Fig 5.12. ESI-MS spectrum of Fe(III) complex of mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (4).

5.3.5. Molar conductance

The synthesized Fe(III) complex was dissolved in water and molar conductance of 10^{-3} mol dm^{-3} complex solutions was measured at room temperature. The conductance value of the Fe(III) complex (4) was 412 Ω^{-1} cm^2mol^{-1} indicating its 1:3 electrolytic behaviour.[27]

5.3.6. Antibacterial activity

The synthetic compounds were tested against gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria. As the ligand and its Fe(III) complex are having biologically active donor sites (N and S) it was very much expected that they would be active against the selected bacteria under trial. But the synthesized ligand and its Fe(III) complex did not show any significant antimicrobial activities against the selected gram positive and gram negative bacteria as per as their respective zones of inhibition are concerned (shown in Figure 5.13). Hence, on the basis of the results obtained it is very much evident that although the synthesized compounds are very much air and moisture insensitive and highly soluble in water they are not having any potentiality to inhibit the bacterial efflux systems and enhancing the antibiotic activity.

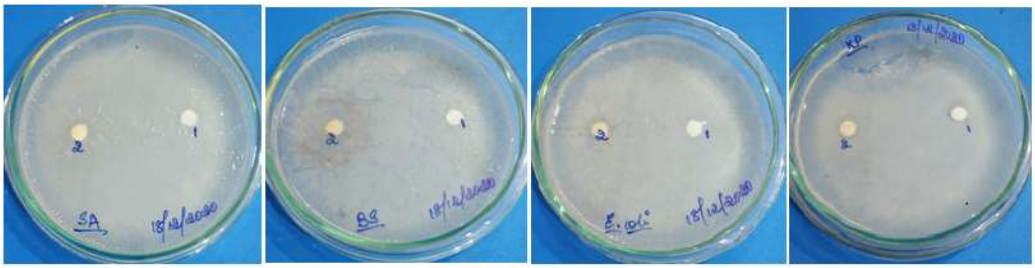


Fig 5.13. Antibacterial activity of ligand (3) (denoted as 1 in above pictures) and Fe(III) complex (4) (denoted as 2 in above pictures) against gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria.

5.4. Conclusion

In summary, a water soluble octahedral Fe(III) complexes of thio modified *β*-cyclodextrin based amino ligand, *viz.*, mono-6-deoxy-(*o*-aminobenzylthio)-*β*-cyclodextrin (4*)* have been synthesized. FTIR spectrum of the Fe(III) complexes confirmed that the ligand coordinates the metal ion *via* the sulfur and nitrogen atoms of the *β*-cyclodextrin moiety and amino groups, respectively of the two ligand molecules and two H_2O occupied the fifth and sixth coordination site. The ESI-MS study suggested that it was a 1:2 metal-ligand complex. The synthesized compounds

do not show any potentiality to inhibit the bacterial efflux systems and enhancing the antibiotic activity

References

[1] S. F. Kettle, *A Physical Inorganic Chemistry: A Coordination Chemistry Approach*, **1996**, Springer, Berlin, Heidelberg.

[2] G. A. Lawrance, *Introduction to Coordination Chemistry*, **2009**, Wiley, Chippenham,.

[3] T. R. Cook, P. J. Stang, *Chem.Rev*., **2015**, *115*, 7001.

[4] H. J. Lu, X. P. Zhang, *Chem. Soc.Rev*., **2011**, *40*, 1899.

[5] M. M. Singh, R. B. Rastogi, B. N. Upadhyay, M. Yadav, *Materials Chemistry and Physics*, **2003**, *80*, 283.

[6] R. B. Rastogi, M. M. Singh, M. Yadav, K. Singh, *Indian Journal of Engineering and Materials Sciences*, **2003**, *10*, 155.

[7] R. B. Rastogi, M. Yadav, A. Bhattacharya, *Wear*, **2002**, *252*, 686.

[8] H. Singh, L. D. S. Yadav, S. B. S.Mishra, *Journal of Inorganic and Nuclear Chemistry*, **1981**, *43*, 1701.

[9] K. S. A. Melha, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **2008**, *23*, 493.

[10] C. C.William, C. J. Lock hart, F. H. Musa, F. H., *J. Chem. Soc, Dalton Trans*, **1986**, *47*, 53.

[11] S. H. Naji, H. A. Fathel, F. H. Musa, *J. Al – Mustansiriya . sci*, **2008**, *19*, 59.

[12] S. H. Naji, *J. Al – Mustansiriya . sci*., **2008**, *19*, 53.

[13] W. K. Muhdi, F. H. Musa, *J. of AL-Nahrain Univ*, **2004**, *7*, 86.

[14] H. A. Mohamad, *PhD thesis Education college – IBN–AL –Haitham – Baghdad Univ*, **2006**.

[15] F. H. Musa, W. K. Mahdi, *J. IBN – AL – Haitham,* **2003***, 16*, 77.

[16] F. H. Musa, A. A. AL-Rawi, B. M. Serhan, *J. Chem*., **2000**, *26*, 725.

[17] A. Das, D.K. Mishra, B. Sinha, *J. Coord. Chem*., **2017**, *70*, 3035.

[18] A. Das, S. Dutta, B. Sinha. *J. Coord. Chem.*, **2018**, *71*, 3731.

[19] K. Fujita, T. Ueda, T. Imoto, I.Tabushi, N. Toh, T. Koga, *Bioorganic chemistry*, **1982**, *11*, 72.

[20] J. Wang, L. T. Kong, Z. Guo, J. Y. Xua, J. H. Liu, J. Mater. Chem.*,* **2010**, *20*, 5271.

[21] J. W. Snyder, R. M. Atlas, **2006**, *Handbook of media for clinical microbiology*, CRC Press.

[22] C. Perez, M. Pauli, P. Bazerque, *Acta. Biol. Med. Exp.*, **1990**, *15*, 113.

[23] M. Raoov, S. Mohamad, Md. R. Abas, *Int. J. Mol. Sci*., **2014**, *15*, 100.

[24] H. Dernaika, S. V. Chong, C. G. Artur, J. L. Tallon, *Journal of Nanomaterials,* **2014**, doi:10.1155/2014/207258

[25] P. B. Pansuriya, M. N. Patel, *Journal of Enzyme Inhibation and Medicinal Chemistry*, **2008**, *23*, 230.

[26] F. Shabani, A. Saghatforoush, S. Ghammamy, *Bull. Chem. Soc. Ethiop*, **2010**, *24*, 193.

[27] I. Ali, W. A. Wani, K. Saleem, *Synthesis And Reactivity In Inorganic, Metal-Organic And Nano- Metal Chemistry*, **2013**, *43*, 1162.