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Potentiometric Study, DFT Calculations and Thermodynamic Parameters of Complex Formation between Cd(II) and Thiosemicarbazone Ligand

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(E)-N-methyl-2-((E)-1-(2-(p-tolyl)hydrazono)propan-2-ylidene)hydrazine-1-carbothioamide (MTHPH) has been synthesized and characterized using elemental analyses, IR, mass spectra and ^{1}H-NMR measurements. The antibacterial, antifungal and antioxidant activity studies of the synthesized MTHPH ligand was investigated and discussed. Molecular modeling was drawn for MTHPH thiosemicarbazone compound and its molecular parameters were determined. Novel MTHPH ligand protonation constants and its formation constants with Cd(II) ion were determined in 50% DMSO solution at 15 ^{o}C, 25 ^{o}C and 35 ^{o}C. The solution speciation of different species was measured as a function of pH. Calculation and discussion of the thermodynamic parameters were accomplished. Both log K_1 and $-\Delta H_1$, for Cd(II)-complexes have been observed to some degree greater than log K_2 and $-\Delta H_2$, indicating a change in the dentate character of this ligand from tridentate donors in 1:1 chelates to bidentate in 1:2; M:L chelates in addition to steric hindrance induced by entry of 2nd molecule. MTHPH compound may be considered as a good remedy for Cd^{2+} toxicity.

Keywords: Potentiometry; Schiff base; thermodynamics, speciation, Mass spectra, Cadmium.

1. INTRODUCTION

Depending on pH, weak acids and bases ionize to varying degrees in solutions which in turn affecting their availiability to join physical, chemical and biological reactions. The pK_a affects both physicochemical properties like solubility in physiological media, octanol/H_2O and phospholipid bilayer/ H_2O partition coefficients, bilayer membrane permeability, and reaction rates of ionizable drug molecules all can be influenced by pK_a values [1]. Awareness of the pK_a of an orally administered

drug can be used to determine how easily the drug molecule from its prepared tablet dose can be released. Dissolution test is important in design formulations for sparsely soluble or practically insoluble drugs [2-5].

Synthetic chemists use the acid dissociation constants to understand what substances can be used to protonate or deprotonate a compound, to assist a reaction. In biochemistry pK_a helps scientists to understand the activity of enzymes and the stability of proteins.

In pharmacology, ionization of a compound change is physical behavior and affects macro properties like aqueous solubility and lipophilicity. pK_a values are also used to understand more complex ADME (absorption, distribution, metabolism, and excretion) characteristics.

Significant class of compounds, Schiff bases have received much interest in the broad diversity of fields owing to structural changes and exclusive characteristics like preparative accessibility, diverse coordination ability, thermal stability, biological and catalysis properties [6-8]. Schiff bases play a vital role as chelating agents for a huge number of metal ions, since they form stable 5- or 6-membered rings after complexation with the metal ion [9,10].

Based on an exact literature review, it should be observed that most of the above-mentioned studies concern synthesis and physico-chemical properties of the Schiff base complexes in the solid state. Actually, papers dealt with protonation and complexation reactions of Schiff bases in solution are fairly limited although the determination of the equilibrium constants of Schiff bases and their complexes are of paramount importance in understanding complicated biological reactions [11]

The role of coordination agents in heavy metal detoxification is a complex subject that involves cooperation between numerous scientific branches. The primary contribution of chemistry to this subject is to produce both models of coordination and complex formation constants between chelating agents and metal ions, in parliamentary procedure to compare the strength of the formed complexes and their features. Column 12 metal complexes are typically attractive in view of their marked differences in chemical and biological behavior.

Metal ions can lead toxicity in humans. Typical examples being heavy metal poisons like Hg, Cd and Pb. Even vital metal ions, when existing in excess can be harmful.

Cadmium is a very toxic metal ion that poses both human and animal health hazards. Its toxicity is done by its easy localization inside the liver, and then by binding of metallothionein, which eventually forms a complex and is transmitted into the blood stream to be lodged in the kidney and liver. *Itai-itai* disease was one of the diseases caused by chronic cadmium toxicity.

One way for treatment of metal toxicity contains chelation therapy, in which metal-specific chelating agents are administered as drugs to complex and enable excretion of unwanted excess element. The stability constants of metal-chelates are precious measure for designing and choosing of chelators [12].

Thus, it is of paramount importance to find novel compounds that can form stable complexes with Cd, because they can be used as detoxifiers. Referable to the broad scope of pharmacological properties of thiosemicarbazone ligands and their compounds, these compounds can fit also very well for this role. As a part of our studies concerning complex behavior of bio-active ligands [13-18], we have taken into consideration the interaction between (E)-N-methyl-2-((E)-1-(2-(p-tolyl)hydrazono)propan-2-ylidene)hydrazine-1-carbothioamide (MTHPH) with cadmium(II) ion in

solution. Since MTHPH ligand would be used as a model for investigation of the therapeutic effects of chelators in *Itai-Itai* disease.

2. EXPERIMENTAL

2.1. Materials and chemicals used

All chemicals used were of A.R. grade quality. Cd(II) ion solutions were formed by dissolution of $CdCl_2$ salt in deionized H_2O and EDTA titrations were used to calculate their concentrations. NaOH solution was accurately standardized by the standard KH phthalate solution.

2.2. Synthesis

2.2.1. 1-(p-tolylhydrazono) -Propan-2-one (PTHP) compound

The 1-(p-tolylhydrazono) -propan-2-one (PTHP) compound was synthesized according to the method described in the literature [19,20].

2.2.2. Synthesis of (E)-N-methyl-2-((E)-1-(2-(p-tolyl)hydrazono)propan-2-ylidene)hydrazine-1-carbothioamide (MTHPH) thiosemicarbazone compound

1-(p-tolylhydrazono) -propan-2-one (0.1760 g, 1 mmol) in 30 ml ethanol were combined with 30 ml ethanolic solution of N-methylthiosemicarbazide (0.105 g, 1 mmol) and refluxed into hot plate for 3-4 hours. The isolated precipitate was washed with Et_2O and dried overnight under silica gel.

Yield, 74%. Colour, Dark brown. Anal. Calc. for $C_{12}H_{17}N_5S$: C, 54.73; H, 6.51; N, 26.59; S, 12.17. Found: C, 54.69; H, 6.48; N, 25.54; S, 12.13 %. IR (KBr, cm^{-1}): 1494, 1249, 1088, 817 (Thioamide bands), 3434, 3352, 3169 (3NH), 1088 (N–N), 1610 (C=N), 1551 (C=C), 3007 (C-H). MS (m/z): 265 (M^++2, 5.87 %) , 264 (M^++1, 17.92 %) 263 (M^+, 96.95 %), 248 (1.42%), 159 (2.68 %) 118 (3.16 %). ^{1}H NMR (DMSO): 11.32 (s, 1H, NH), 10.31 (s, 1H, NH), 8.33 (s, 1H, NH), 6.96-7.08 (m, 4H, –Ar), 7.53 (s, H, CH=N), 2.05 (s, 3H, $–CH_3$), 2.2 (s, 3H, $–CH_3$). ^{13}C-NMR (DMSO): 11.08, 20.09, 30.77, 112.22, 112.86, 129.61, 130.97, 135.99, 140.98, 142.24, 148.13, 178.09

2.3. Instruments

All the chemicals used have been supplied by Aldrich. CHNS-automatic analyzer, Vario EII-Elementar was used to conduct elemental Microanalysis for C, H, N and S. A Perkin Elmer FTIR, type 1650 spectrophotometer with potassium bromide disc was used to monitor IR spectra. On a spectrophotometer of schimadzu 3101 pc, electronic spectra are recorded. A Bruker ARX-300 instrument was applied to monitor the ^{1}H-NMR spectra. Chemical shifts are recorded in ppm *vs*. TMS using deuterated dimethylsulphoxide (d_6-DMSO) as solvent. Mass spectrometry analyses have been carried out using Shimadzu GCMS-QP1000EX. A Metrohm 848 Titrino supplied with a Dosimat unit

(Switzerland-Herisau) have been utilized for potentiometric titrations. Inside the cell a constant temperature was maintained through circulating bath of H_2O. Based on low solubility for the MTHPH synthesized compound and the possible aqueous solution hydrolysis, all potentiometric measurements were performed in 50% water-DMSO mixture.

2.4. Potentiometric titrations

Through potentiometric technique using the method depicted above in the literature [21], the ligand protonation and formation constants of complexes were estimated. The standard buffer solutions are used for accurately calibrating the glass electrode to NBS standards [22]. Standard solution of 0.05 M NaOH, free from CO_2, is used to titrate all samples in the N_2 atmosphere. The sample solution developed to avoid hydrolysis of the MTHPH compound during titration by mixing equal volumes of DMSO and water. All titrations were carried out at constant ionic strength adjusted at 0.1M using sodium nitrate solution.

As known, the calculated formation constants using a potentiometric method have been carried out using a concentration of hydrogen ion expressed in molarity. Nevertheless, the concentration in pH-meter have been expressed in activity coefficient -log a_{H+} (pH). Thus, Van Uitert and Hass Eq. 1 was used to convert pH-meter readings (B) to $[H^+]$ [23,24]

$$-\log_{10}[H^+] = B + \log_{10} U_H \qquad (1)$$

Where $\log_{10} U_H$ = solvent composition correction factor and the ionic strength read by B. pK_w for titrated samples were estimated as previously described [20]. All precautions and procedures comply with literature requirements [25-28].

Titrating (40 cm^3) ($1.25x10^{-3}$ M) MTHPH thiosemicarbazone solution with standard sodium hydroxide solution estimated the protonation constants of the compound thiosemicarbazone. Cd(II) complex formation constants were determined by titration (40 cm^3) of $CdCl_2$ ($1.25x10^{-3}$ M) + MTHPH ($1.25x10^{-3}$ M / $2.5x10^{-3}$ M). Eqs (2) and (3) were defined to evaluate the equilibrium constants from titration data where M, L and H depict Cd(II), MTHPH and H^+ respectively.

$$p(M) + q(L) + r(H) \rightleftharpoons [M_p(L)_q(H)_r] \qquad (2)$$

$$\beta_{pqr} = \frac{[M_p(L)_q(H)_r]}{[M]^p[L]^q[H]^r} \qquad (3)$$

2.5. Processing of data

Computerized method have become very important procedures in the calculation of complex formation equilibriums. MINIQUAD-75 program is one of the most beneficial and familiar interactive programs for calculation of equilibrium constants [29]. Species distribution diagrams for the studied samples were given by the SPECIES program [30].

2.6. Molecular modeling studies

DFT analyses have been completed in Materials Studio packages [31] using the $DMOL^3$ software [32-34] was done. Calculations for DFT semi-core pseudopods (dspp) were created with dual numerical base sets and polarization properties (DNP) [5. The RPBE model is focused on the (GGA) generalized gradient approximation as the best functional approximation [36,37].

2.7. Biological activity

2.7.1. In vitro antimicrobial activity

Antibacterial activity of MTHPH thiosemicarbaone compound was checked using the disc diffusion process [38,39]. Aerobic G^+ bacteria: *Staphylococcus aureus, Bacillus subtillis, Steptococcus faecalis* and G^- aerobic bacteria: *Pseudomonas aereuguinosa, E. coli, Neisseria gonorrhoeae* are among the bacterial strains that were used in the present study. Additionally, two fungal strains *(Aspergillus flavus, Candida albicans)* were checked. Stock solutions of novel synthesized compounds were prepared in DMSO. 100 µl of each of the synthesized thiosemicarbazone compound was inserted into discs (0.8 cm) and then they were allowed to dry. The discs were completely saturated with the synthesized compounds. The discs were then placed at least 25 mm from the edge into the upper layer of the medium. The disks were then gently placed on the surface of the same plate. At 37 ^oC for 72 hours, the plate was then incubated, and the clear area of inhibition was examined. The inhibition zone (an area where there is no growth around the discs) was eventually determined by the ruler millimeter.

2.7.2. In vitro antioxidant activity

Free radical scavenging action of the synthesized thiosemicarbazone compounds were analyzed by the 1,1- diphenyl-2-picrylhydrazyl (DPPH) assay [40] using ascorbic acid as standard material. Using Thermo Scientific Evolution 201 UV-Visible Spectrometer, the absorbance of the sample, blank and control were measured at 517 nm in the dark at room temperature. The procedure was performed three times.

The % of antioxidant activity was determined as follows:

$$\text{Antioxidant activity percent} = 100 - [\{(Abs_{sample} - Abs_{blank}) \times 100\}/Abs_{control}] \quad (4).$$

3. RESULTS AND DISCUSSION

3.1. Characterization of MTHPH thiosemicarbazone compounds

Condensation of the 1-(p-tolylhydrazono)-Propan-2-one compound with

N-methylthiosemicarbazide readily gives rise to the corresponding MTHPH-thiosemicarbazone compound. The isolated MTHPH compounds is air stable and insoluble in H_2O, but easily soluble in

C_2H_5OH, DMF or DMSO. Different analytical tools were utilized to identify the structure of prepared compounds. The results from the basic analysis are well in line with the calculated results for the proposed formula.

3.2. IR spectrum

Absence of the ν(>C=O) and emergence of new band at 1618 cm^{-1} that refers to ν(C=N) stretching vibration [41] supporting condensation reaction and formation of MTHPH compound. Thiosemicarbazone compounds can exhibit thione ↔ thiol tautomerism [42] as a result of the presence of a thiol amide -NH-C=S linkage but ν(S-H) absorption band in the region 2500-2600 cm^{-1} was absent with an appearance of ν(C=S) band at 798 cm^{-1} indicating presence of the MTHPH compound as thione form in the solid state. For MTHPH thiosemicarbazone compound, vibrational bands with the wave numbers of 3012 cm^{-1} (ν_{C-H}, Ar-H), 1615 cm^{-1} ($\nu_{C=N}$), 1548 cm^{-1} ($\nu_{C=C}$), 1082 cm^{-1} (ν_{N-N}) were detected. In the MTHPH thiosemicarbazone compound spectra, the bands observed in the range 1499, 1250, 1080, 798 cm^{-1} are attributed to the bands of thiomide, I, II, III and IV, consecutively [43].

3.3. NMR spectrum

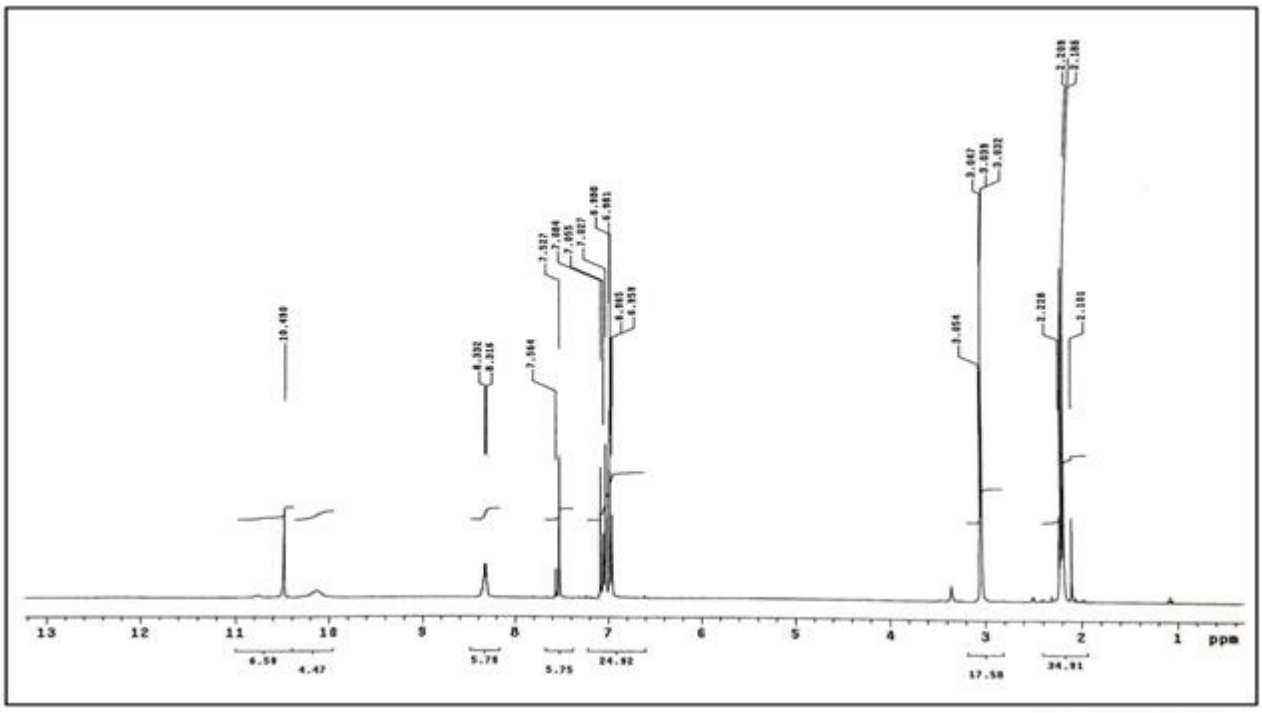


Figure 1. ^{1}H-NMR spectra of MTHPH compound.

The ^{1}H-NMR spectra of the MTHPH compound (Fig. 1) in DMSO-d_6 shows no resonance at approximately 4.0 ppm due to -SH proton resonance [43], whereas the presence of a peak at 10.77 ppm (signal field of existence of the NH group next to C=S) suggests that they remain in the thione form even in a polar solvent like DMSO. Methine proton of the characteristic azomethine group (CH=N) for

MTHPH compound was observed at δ = 7.36 ppm due to the azomethine proton clearly indicates the Schiff-base formation. Signals of the aromatic protons appear at 6.91-7.11 ppm. Methyl group was observed as a singlet signal at δ = 2.05-2.21 as usual [44],

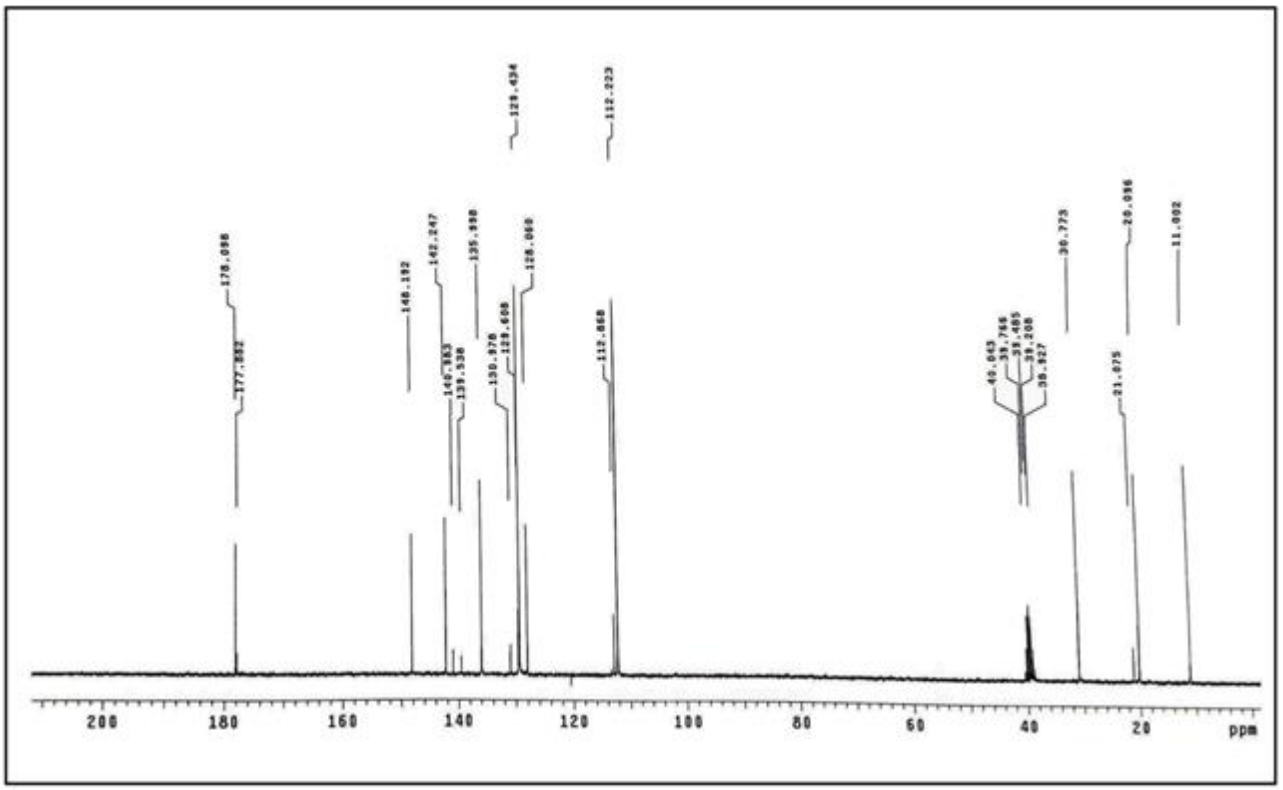
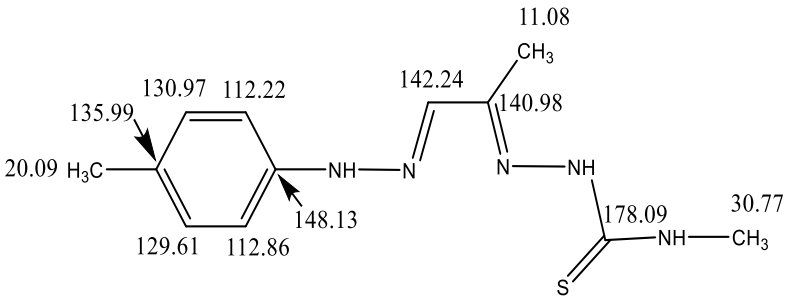


Figure 2. ^{13}C-NMR spectra of MTHPH compound.

The ^{13}C NMR spectra of the MTHPH compound (Fig. 2) was carried out in DMSO-d_6. The singlet peaks at 11.0, 21.07 and 38.6 ppm are due to methyl (-CH_3) carbons. The spectrum of the MTHPH ligand, display phenyl ring carbons (C=C) at 112.86-148.13 ppm, the singlet peaks at 140.98 and 142.24 ppm corresponding to the azomethine carbon of MTHPH compound. The signal for the carbon atom of C=S was detected at 178.09 as given in scheme 1.



Scheme 1. ^{13}C-NMR signals of MTHPH compound.

3.4. UV–Vis spectrum

Electronic spectrum of MTHPH ligand (Fig. 3) shows two absorption bands. The first band at 33020 cm^{-1} was assigned to $\pi \rightarrow \pi^*$ and the second one at 26830 cm^{-1} is due to $n \rightarrow \pi^*$ transition. Always $\pi \rightarrow \pi^*$ transitions occurs at higher energy than $n \rightarrow \pi^*$ transitions [45].

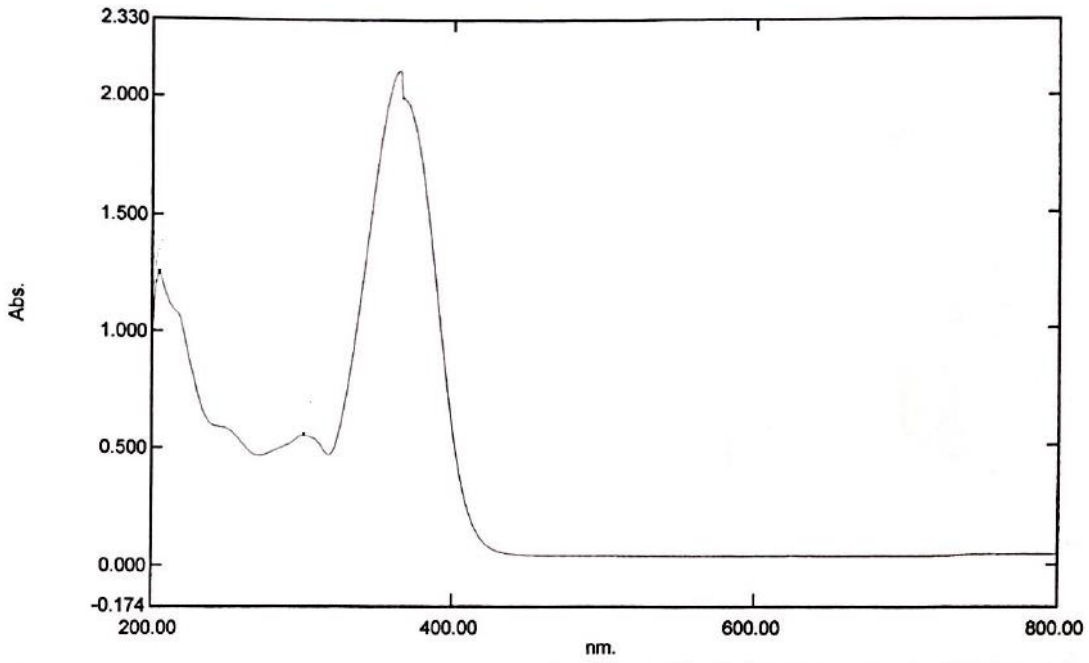


Figure 3. UV-Vis spectra of MTHPH compound.

3.5. Mass spectrum

The proposed formulae can be further proven by mass spectroscopy. In addition to a number of peaks that are attributive to the different fragments of the MTHPH compound, the electron mass impact spectrum of the MTHPH support the assumed formulation by displaying a peak at 263 which correspond to $C_{12}H_{17}N_5S$ compound formula. These data suggests that a ketone PTHP group is condensed with the amino group of N-methylthiosemicarbazide and agree very well with the molecular formulation proposed for MTHPH compound.

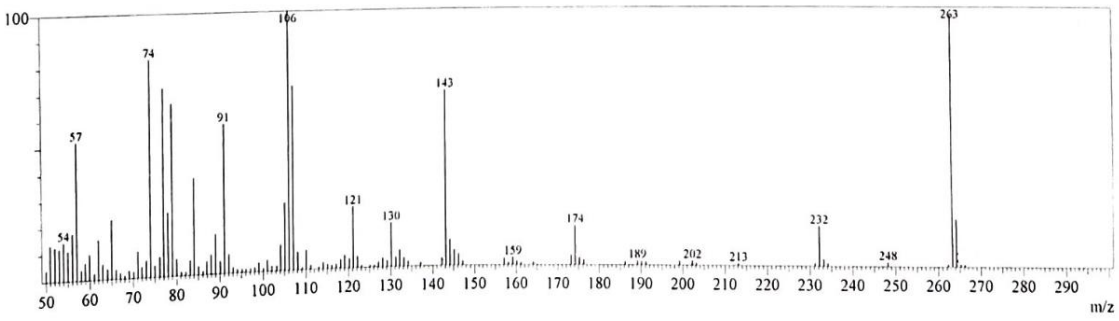
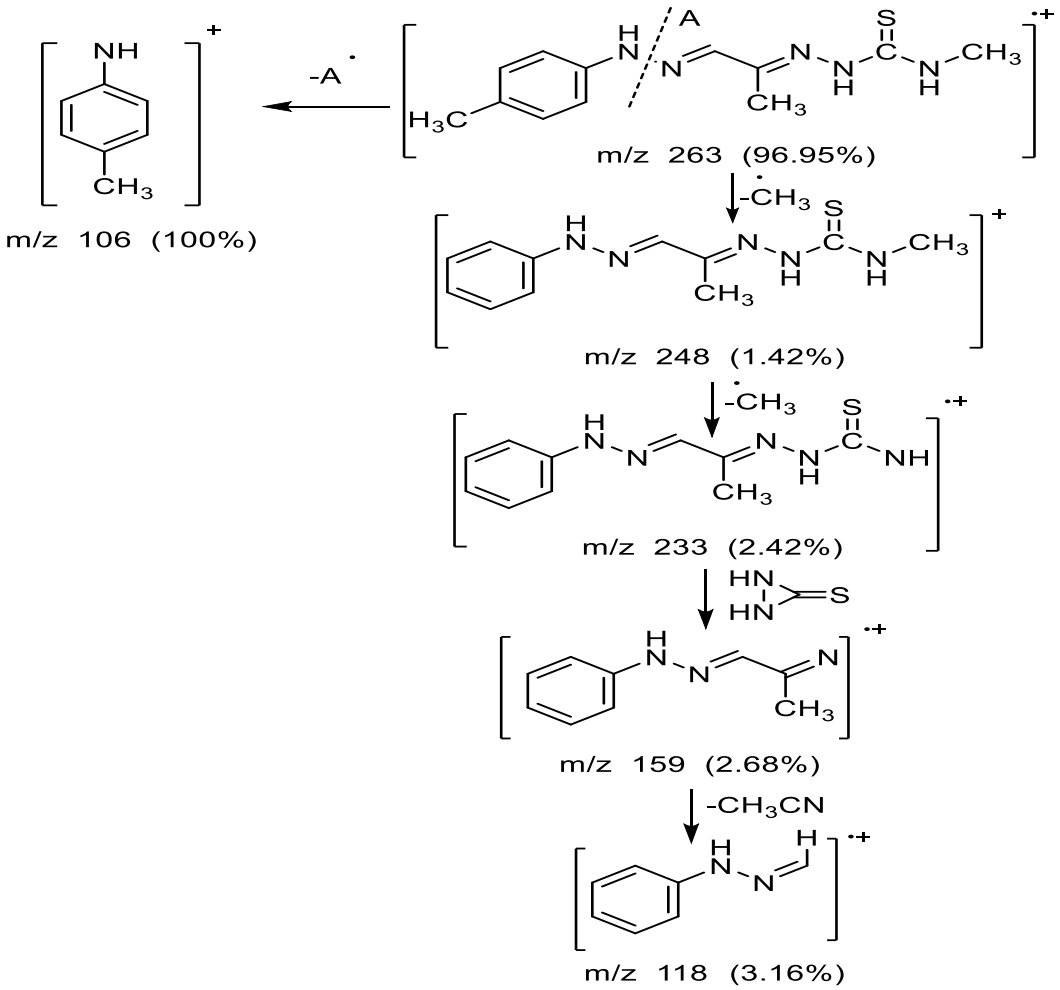


Figure 4. Mass spectra of MTHPH compound.

The mass spectra curve is given in Fig. 4 and the fragmentation pattern of MTHPH compound is given in Scheme 2.



Scheme 2. Mass fragmentation pattern of MTHPH compound.

3.6. Molecular modeling

Without a crystal structure, energy minimization were conducted using DFT semi-core pseudopod calculations utilizing DMOL^3 software [32-34] in the Materials Studio package to achieve the molecular compound conformation [31].

Table 1. The calculated quantum chemical parameters for MTHPH compound.

Quantum parameters	**Theoretical data**
Total energy	-61764.4[a]
Binding energy	-3453.7
Electronic energy	-404014.1
Isolated Atomic Energy	-58310.3
Core–core interaction	342250.0
Heat of formation	114.1

Dipole moment	6.71 Debyes
E_{HOMO}	-8.571 eV
E_{LUMO}	-0.934 eV
ΔE	7.637
I	8.571
A	0.934
χ	4.753
η	3.819
S	0.262
ΔN_{max}	-2.245
ω	2.957
ω^-	5.811
ω^+	1.059

[a] Energy unit: (kcal/mol)

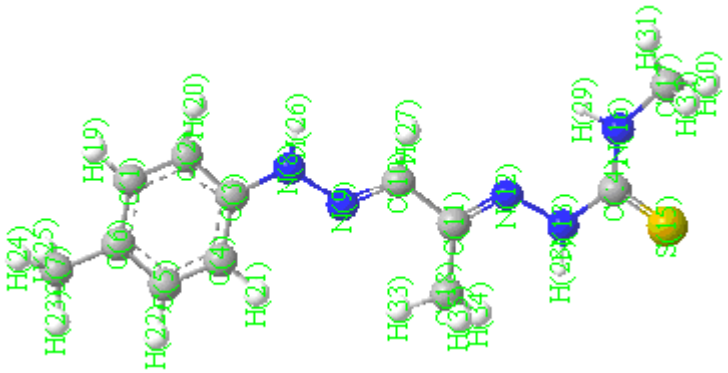


Figure 5. Optimized structure of MTHPH compound.

Calculations are used to achieve quantum chemical specifications of synthesized compounds, like high-occupied molecular orbital energy (E_{HOMO}), lower unoccupied molecular orbital (E_{LUMO}) (Table 1). The MTHPH compound molecular structure was given in Fig.5.

3.6.1. Bond length and angles

Table 1S lists the ligand bond lengths and bond angles of the MTHPH compound. C-S and N-C(S) bond lengths are 1.697 Å and 1.354 Å respectively.

3.6.2. Molecular parameters

Quantum parameters like E_{HOMO}, $E_{LUMO,}$ in addition to specific parameters like ionization potential (IP), absolute softness (σ), absolute hardness (η), electron affinity (EA), separation energy (ΔE), absolute electronegativity (χ), global softness (S), electrophilicity (w), electron accepting power (w^+), electron donating power (w^-) and additional electronic charge (ΔN_{max}) [46-50 have been

computed according to Eqs 5-14 as shown below [46-51]. The inverse of the global hardness is called the softness σ [52].

$\chi = - 1/2 (E_{LUMO}+E_{HOMO})$ (5)

$IP = -E_{HOMO}$ (6)

$\eta = 1/2 (E_{LUMO}-E_{HOMO})$ (7)

$S= 1/2\ \eta$ (8)

$\Delta N_{max} = -IE/\ \eta$ (9)

$\sigma = 1/\eta$ (10)

$EA = -E_{LUMO}$ (11)

$\omega = IE^2 /2\ \eta$ (12)

$\omega^- = (3*IE+EA)^2 /16\ (IE-EA)$ (13)

$\omega^- = (IE+3*EA)^2 /16\ (IE-EA)$ (14)

From the obtained data, we can deduce each of the following:

a) HOMO and LUMO are called the frontier molecular orbitals (FMOs). The HOMO acts as an electron donor, since it is the highest energy orbital containing electrons while the LUMO acts as the electron accepter, since it is the lowest energy orbital that can accept electrons. The negativity of HOMO and LUMO indicates the stability of the studied compounds [51].

b) When HOMO energy decreases, the molecule's ability to donate electron decreases while high HOMO energy means that the molecule is an efficient donor of electrons. The greater donation function of the compound is a significant parameter for formation of charging transfer complex between the compound and its bio-target [52,53].

c) Hard nucleophiles have a low energy HOMO, soft nucleophiles have a high energy HOMO, hard electrophiles have a high energy LUMO and soft electrophiles have a low energy LUMO.

d) Absolute hardness (η) and softness (σ) are essential characteristics in calculation of molecular stability and reactivity. The hard molecules have a large energy space with less reactivity ($E_{HOMO}–E_{LUMO}$), whereas the soft molecules have a smaller energy space and a greater reactivity. Thus, energy gap is an index of stability and can be used to measure the chemical reactivity and kinetic stability of the molecule [53-56].

e) When HOMO energy decreases, the molecule's ability to donate electron decreases while high HOMO energy means that the molecule is an efficient donor of electrons.

f) The energy gap ($\Delta E = E_{HOMO} -E_{LUMO}$) for MTHPH compound is 2.19. This large ΔE assumes automatic high excitation power for the title compound, high stability and great chemical hardness.

g) Binding energy value of the free MTHPH ligand is -3453.7 kcal / mol.

3.7. Antibacterial and antifungal activities:

Antimicrobial activities of MTHPH compound were screened versus three Gram-positive (*Staphylococcus aureus, Bacillus subtilli, Steptococcus faecalis),* three Gram-negative bacteria *(Pseudomonas aereuguinosa, E. coli, Neisseria gonorrhoeae)* and two fungal strains *(Aspergillus flavus, Candida albicans)* (Table 2). The following observations were deduced:

a) The investigation of the biological action of MTHPH compound on Gram-negative and Gram-positive bacteria revealed that MTHPH was inactive against the tested *Streptococcus faecali*s and *Pseudomonas aereuguinosa* organisms.

b) MTHPH compound displayed a moderate to weak activity versus *Staphylococcus aureus* and *Bacillus subtillis* as gram positive bacteria and *Escherichia coli* as gram-negative bacteria.

c) According to results of the antifungal activity screening, the MTHPH compound possess no antifungal activity *versus* both *Candida albicans* and *Aspergillus flavus* fingi.

Table 2. Antibacterial and antifungal activities of the synthesized MTHPH compound.

Compound	Gram positive			Gram negative			Fungi	
	Staphylococcus aureus	Bacillus subtillis	Steptococcus faecalis	Pseudomonas aereuguinosa	E. coli	Neisseria gonorrhoeae	Aspergillus flavus	Candida albicans
MTHPH	10	10	0	0	12	9	-	-
Ampicillin	21	26	27	26	25	28	-	-
Amphotericin	-	-	-	-	-	-	16	19

Ampicillin: Standard antibacterial agent, *Amphotericin*: Standard antifungal agent

3.8. Antioxidant activity

Recently, there is an increasing concern about the use of antioxidants for medicinal purposes. Therefore, antioxidant drugs are used for the prevention and/or cure of diseases, which are directly linked to absence of the organisms' antioxidant capability. DPPH$^{\bullet}$ is a stable free radical which is frequently used to detect radical-scavenging activity [57] in chemical analysis in comparatively little time with respect to other methods [58,59]. Antioxidant activity of the compounds depends on their ability to donate electrons or hydrogen radicals release to DPPH, thus they converted to stable diamagnetic molecules [60]. Accordingly, the interaction of antioxidants with DPPH$^{\bullet}$ radical causes reduction of its absorbance because of purple to yellow color discoloration.

Table 3. DPPH activity

Compound	**Concentration (µg/ml)**	**DPPH scavenging activity (%)**
MTHPH	50	89.11
	100	89.24
	150	90.87
	200	91.72
Ascorbic acid	50	96.8
	100	97.09
	150	97.6
	200	97.8

DPPH = 1,1-diphenyl-2-picrylhydrazyl

Maximum absorption in ethanol for stable DPPH• radical was 517 nm. Consequently, the reduction capacity of DPPH radicals was determined by decreasing of its absorption at 517 nm by antioxidants [61]. Free radical scavenging activity of the MTHPH thiosemicarbazone increased with increasing concentration. Though, the steady increase in the activity in all the cases was observed with increase in test compounds concentrations.

Based on the data obtained (Table 3) from this study, this MTHPH compound is free radical inhibitor or scavenger and can limit free radical damage in the human body. Antioxidant activities of MTHPH compound is due to the existence of $(C=N)_{azomethine}$, SH, and NH groups [62].

3.9. Antioxidant activity and quantum molecular parameters of the synthesized MTHPH compound

The electron releasing affinity of the molecule can be predicated on the basis of quantum molecular parameters from the values of E_{HOMO} and E_{LUMO}. The HOMO and LUMO orbital energies are associated with free radical scavenging activities of the antioxidants [63,64]. Thus, E_{HOMO} measures electron-donating character of a compound (ionization potential), while, E_{LUMO} measures its electron-acceptor character (electron affinity). MTHPH acts as a powerful antioxidant with similar activity to the standard antioxidant ascorbic acid with low energy gab (7.637) reflecting its high electron-releasing affinities [65].

3.10. Equilibrium Studies

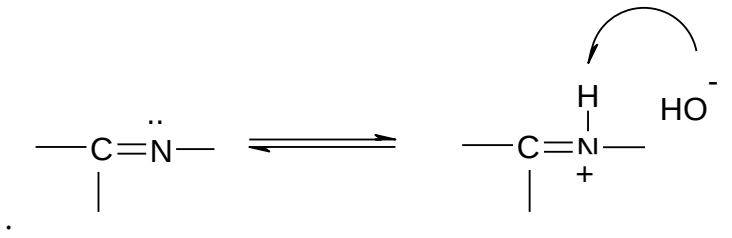
The protonation constants of MTHPH ligand are calculated. This MTHPH ligand behaves as a triprotic as shown by Eqs. 15-17.

$$L^- + H^+ \rightleftharpoons HL; \qquad K_1 = \frac{[HL]}{[L^-][H^+]} \qquad (15)$$

$$HL + H^+ \rightleftharpoons H_2L^+; \qquad K_2 = \frac{[H_2L^+]}{[HL][H^+]} \qquad (16)$$

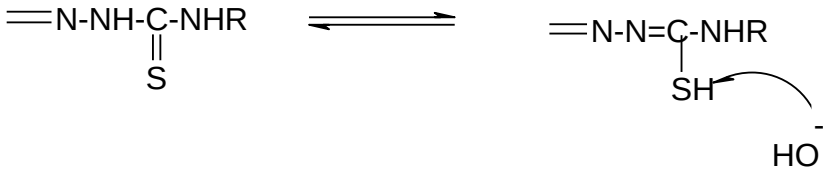
$$H_2L^+ + H^+ \rightleftharpoons H_3L^{2+}; \qquad K_3 = \frac{[HL^{2+}]}{[H^+][H_2L^+]} \qquad (17)$$

We can conclude that the 1st and 2nd deprotonation constants correspond to the deprotonation of the two N-imino sites in MTHPH ligand as shown in scheme 3.



Scheme 3. Possible deprotonation pathway of the imino groups.

While the 3rd deprotonation constant correspond to the thiolate group site in MTHPH ligand as shown in scheme 4. However, a similar conclusion was obtained in literature [66].



Scheme 4. Possible deprotonation pathway of the thiolate group.

The log $K_{N\text{-imino}}$ values ranges from (3.07-3.62) are similar to those found in the literature for the imino group (4.40) [67]. The log K_{SH} value ranges from (7.96–8.35) are similar to those described in the literature for the thiolate group (5.5-9.0) [68].

The study of protonation equilibria for the MTHPH ligand under study cannot be performed in aqueous solution because it is insoluble in H_2O. DMSO solvent has been widely used for potentiometric studies of both protonation and formation equilibria. The mixture DMSO-water 50 %:50 % was best chosen solvent to give soluble and stable Schiff base solution. The use of these mixed solvents has some advantages over pure DMSO that are:-

(i) Pure DMSO is very hygroscopic and determining its H_2O content is problematic [65] which in turn would extensively affect results reproducibility.

(ii) A mixture of 50%:50% DMSO-water has only small hygroscopic nature.

(iii) Compatibility of this mixture with the standard glass electrode, so pH measurements can be done in a comparable way to that of a purely aqueous solution. Conversely, using pure DMSO is not 'potentiometric favourable..

(iv) Acidity range of this mixture (pK_w = 15.52±0.1) is large [43,69] which enables the study of the deprotonation equilibria of weak acids that could hardly be studied in pure H_2O [43,69].

Herein, three protonation constants were calculated for MTHPH ligand and the SPECIES program was utilized to give the distribution of MTHPH ligand species as a function of pH (Fig.6). The species distribution graph is a good tool for obtaining complete picture about the concentration of each species present as a function of pH. It enables us to obtain the best conditions for preparation a solid complex as pH, concentration and ligand:metal ratio. At low pH, (MTHPH) exists initially in a fully protonated form with maximum percent of 100% as H_3L below pH < 2. On addition of base, pH value increases so the (H_3L) species loses its first proton from an imino group to form (H_2L), which is the predominant species at pH = 3.3. As conditions become more alkaline, the second proton released from the second imino group begins deprotonation to HL ligand reaching a maximum percent of 99.5 % at pH = 5.6. Further increase of pH is accompanied by release of the third proton from forming the fully deprotonated ligand L with maximum percent species 99.4% at pH = 10.2.

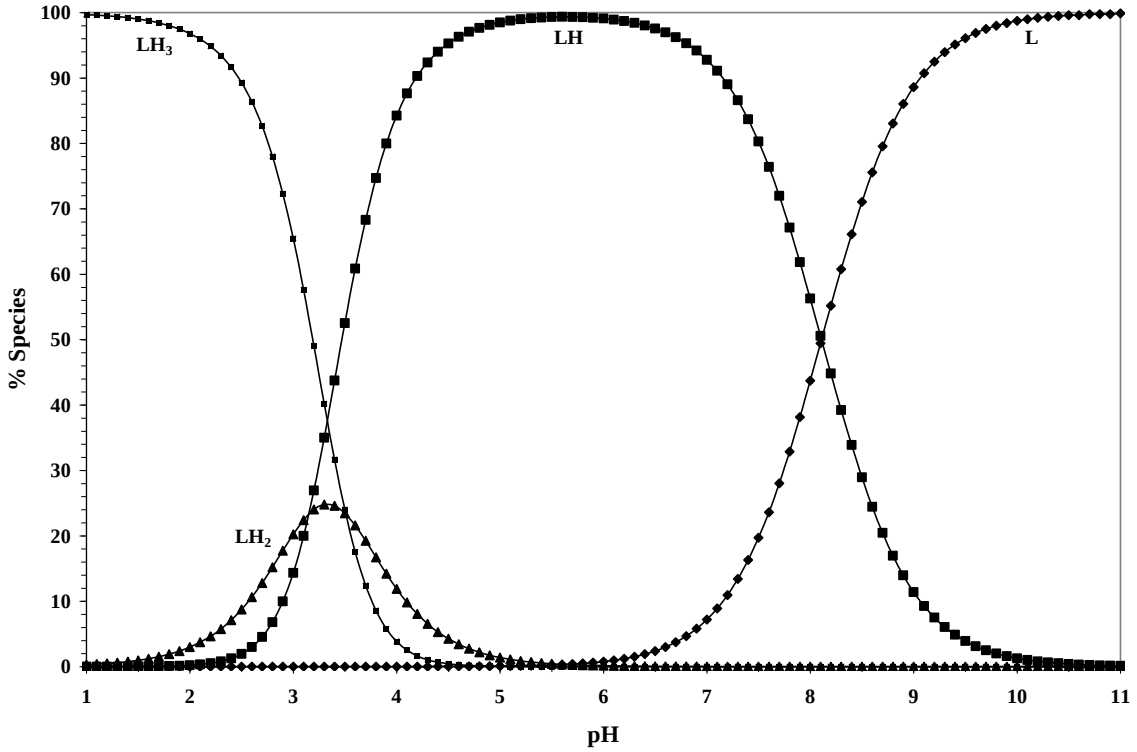


Figure 6. Concentration distribution diagram of various species as a function pH in the MTHPH system (at concentration of MTHPH = 1.25 M and T = 25 ^{o}C).

3.11. Species distribution curves

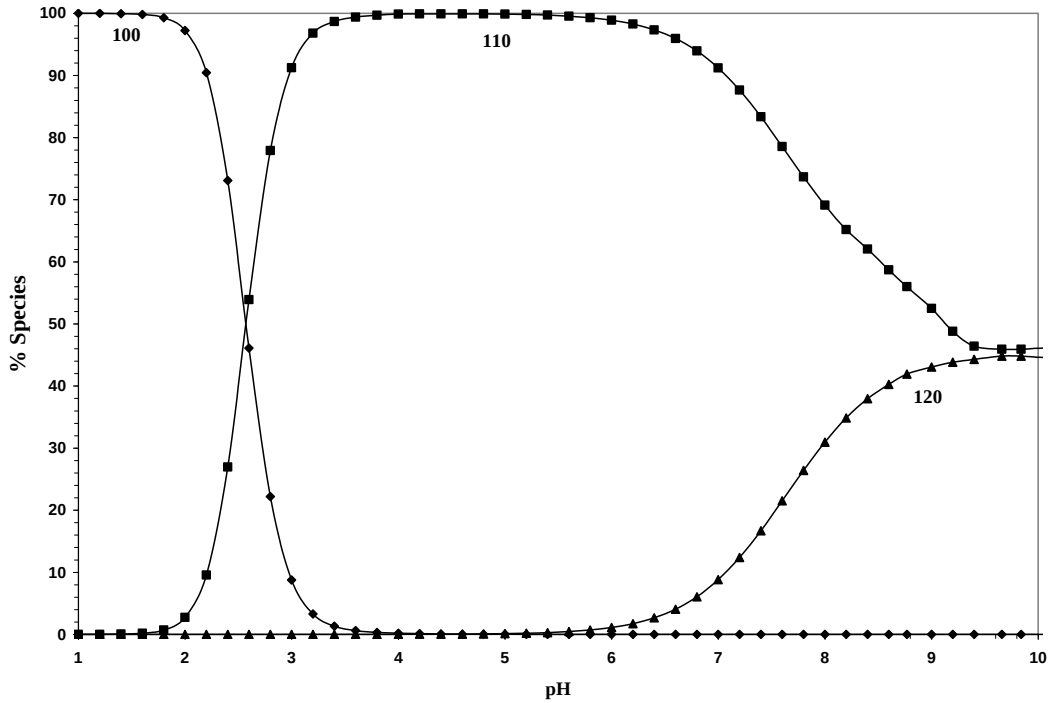


Figure 7. Concentration distribution diagram of various species as a function pH in the Cd(II)-MTHPH system (at concentration of Cd(II) = 1.25 and MTHPH = 2.50 M and T = 25 ^{o}C).

The calculation of equilibrium complex concentrations of Cd(II) with MTHPH as a function of pH provides a useful picture of metal(II) binding in the biological system. As a representative example of metal complexes, Fig. 7 showed a concentration distribution diagram for the complex Cd(II)-MTHPH. The 110 complex species of MTHPH with Cd(II) begins to form in acidic pH range and reaches a constant concentration of 99.9 % at pH = 5.0, whereas $Cd(MTHPH)_2$ complex species reaches a maximum concentration of 45 % at pH 9.8.

3.12. Thermodynamics

The data derived for ΔH^o, ΔS^o and ΔG^o associated with protonation of MTHPH and Cd(II)-complex were calculated from temperature dependence data tabulated in Tables 4-5.

Table 4. Protonation constants for MTHPH ligand and stability constants for Cd(II)complexes with MTHPH ligand at different temperatures.

Reaction	p	q	r	log β (±σ)[a]		
				15 °C	25 °C	35 °C
L + H = HL[a]	0	1	1	8.35(0.07)	8.11(0.06)	7.96(0.07)
L + 2H = H_2L	0	1	2	11.59(0.04)	11.26(0.05)	11.03(0.04)
L + 3H = H_3L	0	1	3	15.21(0.05)	14.77(0.03)	14.46(0.03)
Cd + L = CdL_2	1	1	0	9.98(0.08)	9.84(0.04)	9.72(0.05)
Cd + 2L = CdL_2	1	2	0	13.11(0.05)	12.92(0.04)	12.75(0.06)

[a](σ) is the standard deviation; Definitions of stability constants: $\beta_{110} = \frac{[ML]}{[M][L]}$; $\beta_{120} = \frac{[ML_2]}{[M][L]^2}$; (L = MTHPH thiosemicarbazone ligand); Charges are omitted for clarity.

Table 5. Stepwise stabilityc for the complexes of MTPHP with Cd(II) metal ion in 50% DMSO-H_2O (V/V) solution.

Temp.(°C)	$\log K_1$(±σ)[a]	$\log K_2$(±σ)	$\log K_1$- $\log K_2$
	CdL	CdL_2	Cd(II) complex
15	9.98(0.08)	3.13(0.05)	6.86
25	9.84(0.04)	3.08(0.04)	6.76
35	9.72(0.05)	3.03(0.06)	6.68

[a](σ) is the standard deviation; K_1= [CdL]/[Cd][L]; K_2= $[CdL_2]$/[CdL][L]; (L = MTPHP thiosemicarbazone ligand).

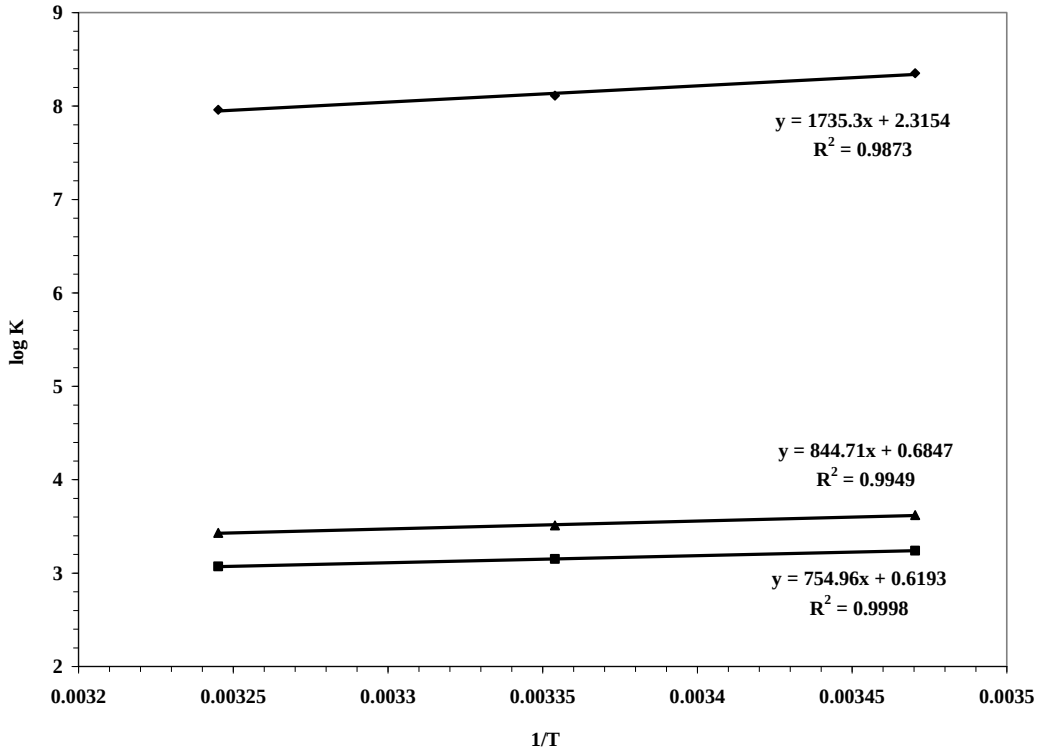


Figure 8. Effect of temperature on the protonation constant of MTHPH compound.

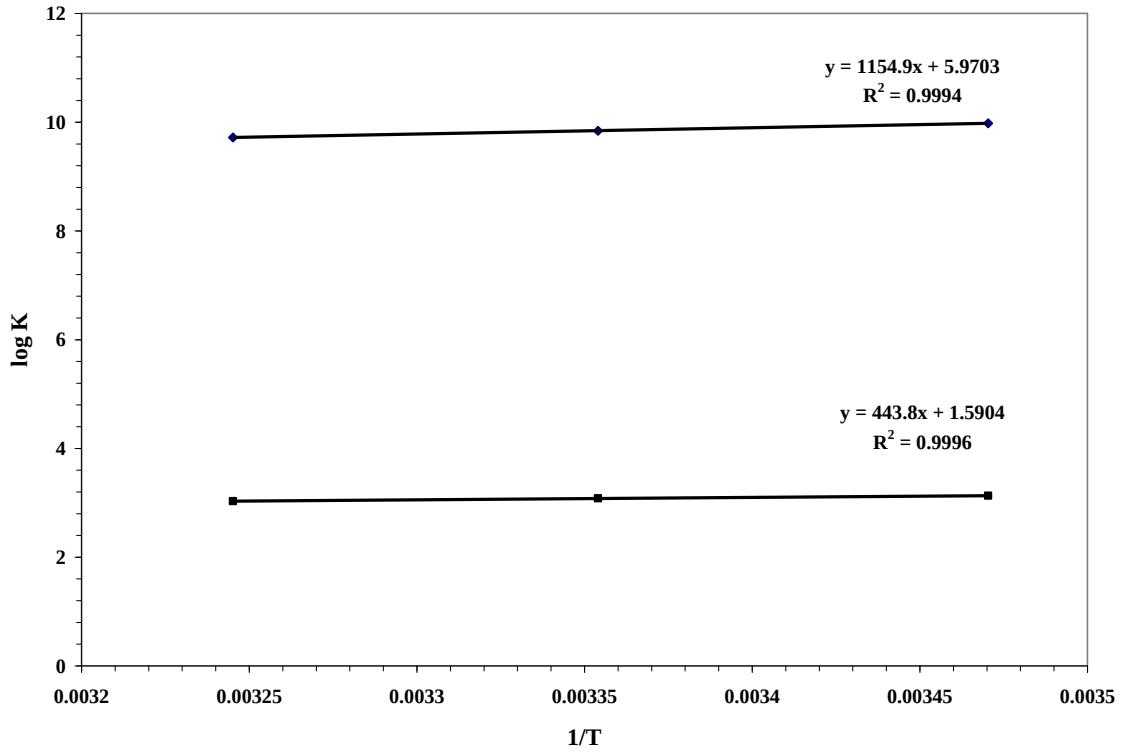


Figure 9. Effect of temperature on the formation constant of Cd(II)-MTHPH complexes

The value of (ΔH) for ligand proronation or complexation process was determined from the

plot slope (log K vs. 1/T) (Figs. 8 ,9) via graphical representation of Van't Hoff equation

$$-2.303\ R\ T \log_{10} K = \Delta H^o - T\ \Delta S^o \quad (18)$$

Or

$$\log K_{10} = -(\Delta H^o/2.303R)(1/T) + \Delta S^o/R \quad (19)$$

With the well-known relations (6) and (7), from the values of free energy change (ΔG) and enthalpy change (ΔH), one can infer the entropy change (ΔS):

$$\Delta G^o = -2.303\ RT \log_{10} K \quad (20)$$

$$\Delta S^o = (\Delta H^o - \Delta G^o) / T \quad (21)$$

Where R = 8.314 J K^{-1} mol^{-1}, K is protonation constant of MTHPH or its complex stability constant, and T is Kelvin temperature.

The main reasons for the protonation constant determination can be explained as follows:

(1) The pH and ratio of the various forms of a substance can be determined using its protonation constants.

(2) Very useful in preparation of newly synthesized compounds. The suggested structure can be reliable where protonation constants are theoretically well calculated according to the experimental values.

(3) Because different types of substances have different UV spectrums, quantitative spectrophotometric analysis can be performed by choosing the appropriate pH value. To choose the pH values, the known protonation constants are required.

(4) Preparation of buffer solutions at different pH values requires determination of protonation constants [43,70],

(5) Therefore, the measurements of the stability constants for the complicated formation of bioactive ion compounds include protonation constants to be determined.

Additionally, their protonation constants are used for calculating the stability constants of the dynamic M-bio-active compounds formation [71].

(6) The equilibrium constants of certain compounds must be understood to measure concentration of each ionized species at pH which is important to understand their physiochemical behavior [71].

Table 6. Thermodynamic parameters for MTPHP protonation in 50% DMSO-H_2O (V/V) solution.

Parameter[a]	**Reaction**								
	L + H = HL[a]			L + 2H = H_2L			L + 3H = H_3L		
	15°C	25 °C	35 °C	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C
-ΔG	46.07	46.30	46.97	19.97	20.04	20.24	17.88	17.98	18.11
-ΔH	33.23			16.17			14.45		
ΔS	44.32			13.09			11.85		

*Δ*G:Gibbs energy/kJ.mol^{-1}; ΔH: Enthalpy/kJ.mol^{-1}; ΔS: Entropy/J.mol^{-1}.K^{-1}.

Table 6 describes the measured thermodynamic functions can be discussed as:

1) The corresponding thermodynamic processes for the protonation reactions are:

i) Neutralization reaction is an exothermic process;

ii) Ions desolvation is an endothermic process;

iii) Structure alteration and alignment of H-bonds around the free and protonated ligands.

2. Protonation constants values decrease with rise of temperatures indicating rising of acidity as the temperature rises.

3. Negative ΔH^o for protonation of MTHPH ligand indicates that this process is exothermic followed by heat release.

4. Positive entropy of MTHPH's protonation reaction indicates increased disorder due to desolvation processes and breakdown of H-bonds.

Table 5 includes the stepwise stability constants of the complexes formed at various temperatures. Such values decrease and confirm with increasing temperature that the phase of complexation is preferred at low temperature. These results provide the following findings:

1-Negative values of ΔH^o indicate that the coordinating process is exothermic assuming that the metal-ligand bonds are strong and complexity reactions are favored at low temperatures.

2- Negative ΔG^o values of complexation indicate the spontaneity of coordination process.

3- ΔG^o and ΔH^o values for 1:1 complexes are more -ve than that of 1:2 complexes, Table 7.

Table 7. Thermodynamic parameters for Cd(II)-MTPHP complexes in 50% DMSO-H_2O (V/V) solution.

Parameter[a]	**Reaction**					
	Cd + L = CdL			Cd + 2L = CdL_2		
	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C
-ΔG	55.06	56.17	57.35	17.27	17.58	17.88
-ΔH	22.11			8.50		
ΔS	114.31			30.44		

[a] *Δ*G:Gibbs energy/kJ.mol^{-1}; ΔH: Enthalpy/kJ.mol^{-1}; ΔS: Entropy/J.mol^{-1}.K^{-1}.

4-This can be attributed to both steric hindrance caused by the addition of 2nd ligand and the charge neutralization concept.

5-The electrostatic attraction in the 1:1 complex is greater than in the 1:2 complex due to the 1:1 complex is formed by reaction of dipositively charged metal ion and mononegatively charged ligand anion; while the 1:2 complex is created by reaction of monopositively charged 1:1 complex and mononegatively charged ligand anion.

6- The ΔS^o values for all investigated complexes are positive indicating that entropy increase results from the release of bound solvent molecules on coordination is greater than the decrease resulting from the coordination process itself. This occurs because the solvent molecules organized in an orderly manner around the ligand and metal ion have a random coordination configuration. This is referred to as increase in entropy for configuration.

4. CONCLUSION

The condensation reaction of 1-(p-tolylhydrazono)-Propan-2-one (PTHP) with N-methylthiosemicarbazide in the molar ratio (1:1) provided the corresponding (E)-N-methyl-2-((E)-1-(2-(p-tolyl)hydrazono)propan-2-ylidene)hydrazine-1carbothioamide (MTHPH) compound. Potentiometric studies have shown that MTHPH forms complexes 1:1 or 1:2 with Cd(II) ion. The log K_1 and $-\Delta H_1$, for Cd(II)-MTHPH thiosemicarbazone complexes are somewhat larger than log K_2 and $-\Delta H_2$, indicating a change in the dentate character of this ligand from tridentate (SNN-donors) in 1:1 chelates to bidentate (SN-donors) in 1:2; M:L chelates in addition to the steric hindrance generated by the entry of a second molecule. The formation of the metal complexes has been found to be spontaneous, exothermic and entropically favorable. The molecular properties of the structures such as hardness, the highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO-LUMO) gap, bond lengths and bond angles for MTHPH thiosemicarbazone have been investigated by means of DFT calculations. MTHPH compound may be considered as a good antidote for Cd^{2+} ion toxicity as it forms a highly stable complex with it i.e., MTHPH compound can be used as a model for therapeutic agent for *Itai-Itai* disease.

SUPPLEMENTARY DATA

Table 1S. Selected bond lengths (Å) and bond angles (Å) for the MTHPH ligand.

Atoms	**Actual**	**Optimal**	**Atoms**	**Actual**	**Optimal**
Bond length			**Bond angle**		
C(18)-H(35)	1.101	1.113	H(35)-C(18)-H(34)	107.642	109
C(18)-H(33)	1.099	1.113	H(35)-C(18)-C(11)	111.403	110
C(17)-H(32)	1.104	1.113	H(34)-C(18)-H(33)	106.836	109
C(17)-H(31)	1.098	1.113	H(34)-C(18)-C(11)	111.491	110
C(17)-H(30)	1.099	1.113	H(33)-C(18)-C(11)	112.463	110
N(16)-H(29)	0.996	1.022	H(32)-C(17)-H(31)	109.013	109
N(16)-C(17)	1.469	1.460	H(32)-C(17)-H(30)	107.779	109
C(14)-N(16)	1.379	1.369	H(31)-C(17)-H(30)	108.561	109
N(13)-H(28)	1.002	1.012	N(16)-C(14)-S(15)	125.768	124.3
N(12)-N(13)	1.392	1.392	C(18)-C(11)-C(10)	121.290	121.4
C(11)-C(18)	1.492	1.497	H(26)-N(8)-N(9)	113.926	113

C(10)-H(27)	1.105	1.100	H(25)-C(7)-H(24)	107.499	109
N(8)-H(26)	1.010	1.050	H(25)-C(7)-H(23)	107.645	109
C(7)-H(24)	1.099	1.113	H(25)-C(7)-C(6)	111.260	110
C(7)-H(23)	1.098	1.113	H(24)-C(7)-H(23)	107.511	109
C(6)-C(7)	1.485	1.497	H(24)-C(7)-C(6)	110.494	110
C(4)-H(21)	1.097	1.100	H(23)-C(7)-C(6)	112.225	110
C(3)-N(8)	1.449	1.462	C(7)-C(6)-C(5)	120.802	121.4
C(2)-H(20)	1.097	1.100	C(7)-C(6)-C(1)	119.851	121.4
C(2)-C(3)	1.402	1.420	C(5)-C(6)-C(1)	119.345	120
C(1)-H(19)	1.096	1.100	H(22)-C(5)-C(6)	119.795	120
			H(22)-C(5)-C(4)	119.462	120
			H(21)-C(4)-C(5)	119.342	120
			H(21)-C(4)-C(3)	121.031	120
			N(8)-C(3)-C(4)	121.901	120
			C(4)-C(3)-C(2)	119.911	120
			H(20)-C(2)-C(3)	121.039	120
			H(20)-C(2)-C(1)	119.129	120
			H(19)-C(1)-C(6)	119.610	120
			H(19)-C(1)-C(2)	119.849	120
			H(22)-C(5)-C(4)	119.462	120

References

1. A. Avdeef, Drug ionization and physicochemical profiling. In: R. Mannhold, (ed.). Drug Properties: Measurement and Computation, Wiley-VCH, Weinheim, (2007) 55- 83.
2. A. Avdeef, D. Voloboy, A. Foreman, Dissolution and solubility. In: B. Testa, H. van de Waterbeemd, (eds.). Comprehensive Medicinal Chemistry II, Elsevier, Oxford, UK, (2007) 399.
3. A. Avdeef, O. Tsinman, *J. Pharm. Res., 25* (2008) 2613.
4. K. Tsinman, A. Avdeef, O. Tsinman, D. Voloboy, *J. Pharm. Res.,* 26 (2009) 2093.
5. J.H. Fagerberg, O. Tsinman, K. Tsinman, N. Sun, A. Avdeef, *J. Mol. Pharm*., 7 (2010) 1419.
6. Y. Jia, J. Li, *J. Chem. Rev.*, 115 (2015) 1597.
7. C. Millan, E. Cavalli, T. Groth, K. Maniura-Weber, M. Zenobi-Wong, *Adv. Health. Mater*., 4 (2015) 1348.
8. A. Gorczyński, M. Zaranek, S. Witomska, A. Bocian, A.R. Stefankiewicz, V. Kubicki, P, Pawluć, *Catal. Commun.,* 78 (2016) 71.
9. B. Dash, P. K. Mahapotra, D. Panda, J. M. Pattnaik, *J. Indian Chem. Soc*., 61, (1984) 1061.
10. J. Casaszar, J. Morvay, and O. Herczeg, *Acta Phys. Chem*., 31(1985) 717.
11. H. Sigel, Metal ions in biological systems, New York; Marcell Dekker, 1978, Vol 5.
12. W. D. Kim, D. C. Hrncir, G. E. Kiefer, , A. D. Sherry, *J. Inorg Chem*., 34 (1995) 2225.
13. A.A. El-Sherif, M. M. Shoukry and R. van Eldik, *J. Chem. Soc. Dalton Trans.,* 1425-1432 (2003).
14. A.A. El-Sherif and M. M. Shoukry, *J. Coord. Chem*., 58 (2005)1401.
15. A.A. El-Sherif, *J. Solution Chemistry*, 35 (2006) 1287.
16. Ahmed Fetoh, Kareem A. Asla, Ahmed A. El-Sherif, H. El-Didamony, Gaber M. Abu El-Reash, *J.*

Molecular Structure, 1178 (2019) 524.
17. Ahmed A. El-Sherif, Elbastweesy R Elbastweesy, Gaber M. Abu El-Reash, Mutlaq S. Aljahdali, *Int. J. Electrochem. Sci.*, 14 (2019) 7241.
18. Ahmed A. El-Sherif, Mohamed M. Shoukry, Mohamed M. A. Abd-Elgawad, *J. Solution Chemistry,* (2013) 42:412.
19. N. Rabjohn, Organic Synthesis, Collective Volume 4, John Wiley and Sons Inc., 1963.
20. A.A. El-Sherif, *Inorg. Chim. Acta,* 362 (2009) 4991.
21. A.A. El-Sherif, *J. Solution Chem*., 39 (2010) 131.
22. Bates, R.G., Determination of pH, Theory and Practice, 2nd edn. John Wiley and Sons, New York (1975).
23. E.M. Woolley, D.G Hurkot, L.G. Heplerm, *J. Phys. Chem*., 74 (1970) 3908.
24. G.L. Van Uitert, C. G. Hass, *J. Am. Chem. Soc*., 75 (1971) 451.
25. E.P.Serjeant,: Potentiometry and potentiometric titrations. Wiley, New York (1984).
26. A. Golcu, M. Tumer, H. Demirelli, R.A. Wheatley, *Inorg. Chim. Acta,* 358 (2005) 1785.
27. A.E. Martell, R. J. Motekaitis, The Determination and Use of Stability Constants. VCH, Weinheim (1988).
28. M. Meloun, J. Havel, E. Högfelt, Computation of Solution Equilibria: A Guide to Methods in Potentiometry, Extraction and Spectrophotometry; Ellis Horwood Limited: Chichester, Wiley, New York (1988).
29. P. Gans, A. Sabatini, A. Vacca, *Inorg. Chim. Acta,* 18 (1976) 237.
30. Pettit, L., University of Leeds, Personal Communication.
31. Materials Studio (Version 5.0), Copyright 2009. Accelrys software Inc., San Diego, USA.
32. B. Delley, *Int. J. Quantum Chem*., 69 (1998) 423.
33. Delley, B.: From molecules to solids with the $DMol^3$ approach. *J. Chem. Phys.,*. 113, (2000) 7756-7764.
34. A. Kessi, B. Delley, *Int. J. Quantum Chem*., 68 (1998) 135.
35. W.J. Hehre, L. Radom, P.V.R. Schlyer, J.A. Pople, Ab Initio Molecular Orbital Theory, Wiley, New York, (1986).
36. B. Hammer. LB. Hansen, J.K. Nrskov, *Phys. Rev.,* B 59 (1999) 7413.
37. A. Matveev, M. Staufer, M. Mayer, N. Rösch, *Int. J. Quantum Chem*., 75 (1999) 863.
38. C. Janiak, S. Deblon, H.P. Wu, M.J. Kolm, P. Ku¨ flers, H. Piotrowski, P. Mayer, *Eur. J. Inorg. Chem*., (1999) 1507.
39. A. M. Rayan, M.M. Ahmed, M.H. Barakat, A.T. Abdelkarim , A.A. El-Sherif, J. Coord. Chem., 68(4) 678.
40. S. Wang, Y. Cui, R. Tan, Q. Luo, J. Shi, Q. Wu, *Polyhedron,* 13 (1994) 1661.
41. M.P. Swami, D. Gupta, M. Mohan, A.K. Srivastava, *Prog. Natl. Acad. Sci. India* A, 50(3) (1980) 176.
42. M.R.P. Kurup, M. Joseph, *J. Synth. React. Inorg. Met. Org. Chem*., 33 (2003) 275.
43. M. Aljahdali, A. A. EL-Sherif, *Inorg. Chim. Acta,* 407 (2013) 58
44. C. Janiak, S. Deblon, H.P. Wu, M.J. Kolm, P. Ku¨ flers, H. Piotrowski, P. Mayer, *Eur. J. Inorg.,. Chem., (*1999) 1507.
45. V. Philip, V. Suni, M. R. P. Kurup, *Polyhedron,* 25 (2006) 1931.
46. R.G. Pearson, *J. Org. Chem*., 54 (1989) 1423.
47. R.G. Parr, R.G. Pearson, *J. Am. Chem. Soc*., 105 (1983) 7512.
48. P. Geerlings, F. De Proft, W. Langenaeker, *J. Chem. Rev*., 103 (2003) 1793.
49. R.G. Parr, *J. Am. Chem. Soc*., 121 (1999) 1922.
50. P.K. Chattaraj, S. Giri, *J. Phys. Chem., A* 111 (2007) 11116.
51. G. Speie, J. Csihony, A.M. Whalen, C.G. Pie-pont, *J. Inorg. Chem*., 35 (1996) 3519.
52. S. Sagdinc, B. Koksoy, F. Kandemirli, S.H. Bayari, *J. Mol. Struct*., 917 (2009) 63.
53. J.I. Aihara, *J. Phys. Chem*. A 103 (1999) 7487.

54. R.G. Pearson, Hard and Soft acids and bases, Dowden, Hutchinson and Ross, Stroudsburg, PA, 1973.
55. A. F. Kareem, A. Asla, A.A. El-Sherif, H. El-Didamony, G.M. Abu El-Reash, *J. Molecular Structure*, 1178 (2019) 524
56. M. B. Ferrari, S. Capacchi, G. Pelosi, G. Reffo, P. Tarasconi, R. Al-bertini, S. Pinelli and P. L. Helicin, *Inorg. Chim. Acta*, 286 (1999) 134.
57. P.D. Duh, Y.Y. Tu, G.C. Yen, *Lebensm.-Wis. Technol*., 32 (1999) 269.
58. J.R. Soares, T.C.P. Dinis, A.P. Cunha, L.M. Almeida, *J. Free Radical Research*, 26(5)(1997) 469.
59. P.D. Duh, Y.Y. Tu, and G.C. Yen, *Lebensmittel-Wissenschaft und-Technologie*, 32(5) (1999) 269.
60. J.R. Soares, T.C.P. Dins, A.P. Cunha, L.M. Ameida, *J. Free Radical Res*., 26 (1997) 469.
61. B. Matthäus, *J. Agricultural and Food Chemistry*, 50(12) (2002) 3444.
62. M-Hsiu Shih, Fang-Y. Ke, *Bioorg. Med. Chem*., 12 (2004) 4633.
63. B.P. Bandgar, S.S. Gawande, R.G. Bodade, N.M. Gawande, C.N. Khobragade, *Bioorg. Med. Chem*., 17 (2009) 8168.
64. K. Tuppurainen, S. Lotjonen, R. Laatikainen, T. Vartiainen, U. Maran, M. Strandberg, T. Tamm, *Mutat Res*., 247 (1991) 97.
65. K.M. Honório, A.B.F. Da Silva, *Inter. J. Quant. Chem*., 95 (2003) 126.
66. M. A. Hassan, A. El-Roudi, M. T. J. Quenawy, *J. Pharm. Sci*., 34 (1993) 253
67. T. Gunduz, E. Kilic, F. Koseoglu and E. Canel. *Anal. Chim. Acta*, 282 (1993) 489.
68. F.G. Bordwell, D.L. Hughes, *J. Organic Chemistry*, 47(17) (1982) 3224
69. A.A. El-Sherif, M.M. Shoukry, M.M.A. Abd-Elgawad, *J. Solution Chem*., 42 (2013) 412
70. H. Rossotti, The Study of Ionic Equilibria, Longman, London, 1987.
71. H. Sigel, R.B. Martin, *Chem. Rev*., 82 (1982) 385.