1.1 Introduction

During the 20th century inorganic chemistry has been greatly enriched by the continuing development of coordination chemistry and the entry of new thinking from an organic perspective. The two important aspects of life, the ability to capture solar energy and the ability for controlled release of that energy have been contributing much for the development of this perspective. The catalysts controlling such activities are enzymes which control the synthesis and degradation of biologically important molecules. Most of the enzymes depend on a metal ion for their activity. There has been a remarkable growth in the understanding of biological systems containing transition metal ions [1]. Custom design of complexes with organic chelating ligand systems will require newer diversified donor systems. The new coordination compounds [2] with redox-
tunable properties point to the need of including more electronegative nitrogen, oxygen and sulfur atoms in the ligand structure. Determination of structure of many metalloproteins has further emphasized the importance of ONS donors. The development of advanced spectral characterization techniques for calculating spectroscopic parameters, accurately predicting structures and understanding chemical reactivity blessed this situation.

1.2 Thiosemicarbazones

In this context coordination complexes of heterodentate ligands [3-5] has been a subject of interest to many researchers and thiosemicarbazones are a class of heterodentate ligands with NS donor groups. They are obtained by the condensation of the appropriate thiosemicarbazide with an aldehyde or ketone. The structural features include an azomethine group and a thioamide group as shown in the structure below.

![Thiosemicarbazone Structure](image)

R¹, R²: H, alkyl, aryl or heterocyclic
R³, R⁴: H, alkyl, aryl, heterocyclic or part of a cyclic system

When R² is heterocyclic like pyridine an additional functionality also is included to give NNS donors. When R³ and R⁴ of the thioamide group are part of a cyclic system a ring incorporated thiosemicarbazone is formed. Such pendant arm containing thiosemicarbazones are found to have interesting structural and biological properties.

The acid character of N²H is another feature of thiosemicarbazone which allows the donor site to be either neutral or anionic. When coordinated as anionic ligands the conjugation is extended throughout the skeleton. It has
been proposed that extended conjugation enhances biological activity [6]. The structure-activity correlation studies of heterocyclic thiosemicarbazones [7] by Wilson et al found that the activity is affected by changing the S of the thiocarbonyl group, parent aldehyde or ketone, N⁴ substituent and the position of attachment of aldehyde or ketone. The molecular features essential for such activities is ascertained by designing synthetic routes to modify, replace or substitute the derived thiosemicarbazone ligand.

1.3 Thiosemicarbazones of dialdehydes and diketones

Thiosemicarbazones of dialdehydes and diketones have been area of interest since a new kind of proligands highly polydentate in nature is obtained. Ketoaldehydes, dialdehydes or diketones when condensed with appropriate thiosemicarbazide in 1:2 ratio will give bis(thiosemicarbazones). If R² group is alkyl, they can be tetradeutate and if heterocyclic, they can be pentadentate due to an additional functionality. Bis(thiosemicarbazones) (Fig. 1.1) was first synthesized by Bahr fifty-six years ago [8]. It has been found that synthesis of aryl substituted bis(thiosemicarbazone) proligands if not carefully controlled may lead to the formation of cyclised products [9].

![Fig. 1.1 General structure of a bis(thiosemicarbazone).](image)

The parent carbonyl compound and the thiosemicarbazide if taken in 1:1 ratio monothiosemicarbazones as shown in Fig. 1.2 (a) are formed. Monothiosemicarbazones can give rise to SNNO or SNN donor sites depending on the conditions of complexation. Complexes of monothiosemicarbazones [10]
were first isolated along with the synthesis of Cd(II) complexes of bis(thiosemicarbazones).

Fig. 1.2 (a) 2,6-diacetylpyridine mono(thiosemicarbazone). (b) Diacetyl bis(thiosemicarbazone) (c) 2,6-diacetylpyridine bis(thiosemicarbazone)

Diacetyl bis(thiosemicarbazone), 2,6-diacetylpyridine bis(thiosemicarbazone) and 2,6-diacetylpyridine mono(thiosemicarbazone) (Fig. 1.2) are some examples for these type of compounds. In the case of heterocyclic bis(thiosemicarbazones) the ligand can be dianionic which results in a highly delocalized system on extended conjugation as shown in Fig. 1.3. Some of the compounds are found to be showing fluorescence.

Fig. 1.3 The extended conjugation of 2,6-diacetylpyridine bis(thiosemicarbazone) on enolization.

1.4 Ring incorporated thiosemicarbazones

Biological activity of thiosemicarbazones is found to be related to the substituent at 4N position. Studies of these compounds have been done by incorporating different rings. NMR studies show that they exist in chloroform
solution as mixture of isomers. However few crystal structures have been solved for those compounds. Structural studies and coordinating properties of hexamethylenimine, pentamethylenimine, tetramethylenimine and morpholino group substituted thiosemicarbazones have been done. Though they have not found to change the number of donor groups, the activity of the compound is affected with ring incorporation. The studies made by de Souza et al. [11] show that 2,6-diacetylpyridine bis(3-hexamethyleniminhthiosemicarbazone) show that the structure is almost planar except for the hexamethyleneimine rings which are tilted in opposite directions from the plane of the molecule. It is found to possess a bifurcated $E'$ structure similar to 2-acetylpyridine-3-hexamethyleniminhthiosemicarbazone.

1.5 Isomerism of thiosemicarbazones

Heterocyclic $^4N$-substituted or ring incorporated thiosemicarbazones have been characterized in three isomeric types $Z$, $E$ and $E'$. With respect to the azomethine bond it is the $Z$-isomer of 2-acetylpyridine-$^4N$-methylthiosemicarbazone (Fig.1.4a) which makes it possible to be involved in H-bonding and a six membered ring is formed by pyridyl nitrogen N1, N2 and N3 [12]. 2-Acetylpyridine-$^4N$-ethylthiosemicarbazone (Fig. 1.4b) is the $E$ form with respect to azomethine bond.

![Fig. 1.4](image)

**Fig. 1.4** $Z$, $E$ and $E'$ forms of 2-acetylpyridine-$^4N$-substituted thiosemicarbazones.
In the $E'$ form, 2-acetylpyridine-3-hexamethyleneiminylthiosemicarbazone (Fig. 1.4c) the N$_3$ hydrogen has moved to N$_2$, bonded to both N$_1$ and thione sulfur giving a bifurcated H-bonding. This has been supported by single crystal X-ray studies in which the bond lengths are found to be 1.21 Å for N$_2$–H, 2.61 Å for N$_1$–H and 1.82 Å for S–H [11].

In case of 2,6-diacetylpyridine bis(3-hexamethyleneiminylthiosemicarbazone) [11] as noted above the crystal structure reported is $E'$ bifurcated structure. Whereas in case of 2,6-diacetylpyridine bis($^4$N-ethylthiosemicarbazone), a symmetric structure with the two arms disposed on either side of the pyridine ring [13] and a solvated form [14] are reported.

The stereochemistry adopted by thiosemicarbazones while interacting with transition metal ions depend on the denticity and the charge on the ligand. This in turn depends on the thione $\leftrightarrow$ thiol equilibrium. As a result depending on the preparing condition, a neutral, dianionic or monoanionic complex can be formed. The steric effects of various substituents on the thiosemicarbazone backbone, additional interactions such as intramolecular hydrogen bonding also plays a role in stereochemistry.

1.6 Versatile chelating modes and geometry

Usually pentadentate ligands can form square pyramidal or trigonal bipyramid geometry in complexes. Many of the compounds which appear to be five coordinate on close examination are found to be tetrahedral or octahedral geometry. If electrostatic forces alone are the forces operating in bonding five coordinate complexes are found to disproportionate into four and six coordinate species. As far as the stability is concerned mostly the compounds are considered as distorted SP, distorted TBP or highly distorted structures i.e., something between SP and TBP. Proligands of 2,6-diacetylpyridine initially synthesized were hydrazones [15-19] and semicarbazones [20,21]. A number of transition
metal complexes [5] synthesized were found to be heptacoordinate adopting a pentagonal- bipyramid geometry along with anions or water molecules as coligands resulting in the formation of a neutral complex [22-25]. Mn(II) [26], Fe(II) [27], In(III) [28], Sn(IV) [29], and mononuclear Zn(II) [30] were found to be heptacoordinate. It was convinced that the equatorial positions were occupied by pentadentate ligand system and the axial positions by coligands. In case of deprotonated zinc complex of 2,6-diacetylpiperidine bis(2'-pyridylhydrazone) the two arms of same molecule was coordinating to two zinc centers with bridging by the central pyridine ring [15]. Binuclear Zn(II) complexes of bis(thiosemicarbazones) were found to show \{6+6\}, \{6+4\}, \{4+4\} and \{5+5\} coordination geometries [31-34]. Binuclear Zn complex synthesized from a dialdehyde with \{5+5\} coordination was found to adopt a trigonal bipyramid geometry [35]. Ni(II) complex of 2,6-diformylpyridine bis(4N-dimethylthiosemicarbazone) prefer a square planar geometry by excluding azomethine N and thiolato S of one of the arm and including hydrazinic N [23]. Planar Pd(II) and Ni(II) complexes of 1-phenylglyoxal bis(4N-diethylthiosemicarbazone) [36] have been reported to have coordinated in the same way. Bis(thiosemicarbazonato) Cd(II) complex reported is a sulfur bridged box dimer [14] in which each Cd(II) center is distorted pentagonal bipyramidal.

Copper complexes of bis(thiosemicarbazones) are found to be having versatile geometries in which a very interesting trinuclear complex is also reported [37]. Schematic representation of the coordination modes of different thiosemicarbazones (Fig. 1.5) shows versatile possibilities. The structures of some of the complexes (Fig. 1.6 – 1.9) show chelating rings in all of them. The stability of them can be accounted by the five membered fused chelating rings formed in all of them. Schematic representation of some zinc complexes are also shown in Fig. 1.10 and Fig. 1.11.
Diorganothallium(III) complexes of 2,6-diacetylpypidine monothiosemicarbazone were prepared to study the coordinating behaviour of monothiosemicarbazone and found to be tetracoordinating [38]. Ru(II) complex of a monothiosemicarbazone was found to be tridentate coordination through NNS donor sites occupying a meridional plane [24]. The Cd(II) complex [10] of 2,6-diacetylpypidine monothiosemicarbazone is also reported in which a pentagonal bipyramid geometry is found. Such geometry is evolved along with coligands.

Fig. 1.5 Schematic representation of coordination modes of (a) alkyl bis(thiosemicarbazone) (b) heterocyclic bis(thiosemicarbazone) (c) Tetradentate heterocyclic monothiosemicarbazone (d) meridional tridentate heterocyclic monothiosemicarbazone.
Fig. 1.6 Mononuclear pentadentate Cu(II) complex.

Fig. 1.7 A trinuclear Cu(II) complex with a pentadentate ligand.

Fig. 1.8 A dinuclear Zn(II) complex with bridging pyridine rings.
Fig. 1.9 A square planar Ni(II) complex in which hydrazinic N is coordinated.

Fig. 1.10 Schematic stereo representation of [6+4] zinc(II) complex.

Fig. 1.11 Schematic stereo representation of [5+5] zinc(II) complex.
1.7 Applications

Thiosemicarbazones and their complexes have been studied for a considerable period of time for their versatile properties like redox nature, biological activity etc. Traces of interest date back to the beginning of the 20th century but the first reports on their medical applications began to appear in the fifties as drugs against tuberculosis and leprosy. In Open Crystallography Journal Pelosi has made a review on structure activity study on thiosemicarbazones and complexes [39-43].

1.7.1 Biological activity

Heterocyclic thiosemicarbazones, a class of compounds possessing a wide spectrum of medicinal properties have been studied for activity against bacterial and viral infections, tuberculosis, leprosy, coccidiosis and malaria [44-48]. They have been investigated for superoxide dismutase-like radical scavengers [49]. Commercialization of methisazone, an antiviral agent resulted in Maboran. Recently Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) has been developed as an anticancer drug and reached clinical phase II [39].

1.7.2 Analytical applications

The analytical applications of these compounds extend in the microestimation of steroid ketones [50], di-2-pyridylketone thiosemicarbazone for estimation of Fe, 2-acetylpyridine thiosemicarbazone for Au(III) and several metals like copper, tin, zinc etc. In most of the cases the estimation is done spectrophotometrically [51].

1.7.3 Enzyme modelling

Transition metal complexes of ligands containing N/S donor centers are found to constitute the active centers of several metalloenzymes such as
hydrogenases, xanthine oxidase and nitrogenase [52]. Hence bis (thiosemicarbazones) are used for synthesizing model complexes for the active sites of metalloenzymes with mixed N/S donor centers such as nitrile hydratase since they also are having an N$_3$S$_2$ donor set [53]. The active sites of carbon monoxide hydrogenase, acetyl coenzyme synthase A etc also have been recent area of interest.

1.7.4 Radiolabelling and image sensing

There is a wide interest in designing novel imaging probes for biological targets, which can be employed in vivo with a range of molecular imaging techniques to attain research and clinical objectives. Non-invasive techniques such as PET (positron emission tomography) and SPECT (single photon emission computerised tomography), can be used to follow the in vivo distribution of radiolabelled metal complexes of interest in terms of therapeutic and imaging applications. Fluorescence microscopy has been recently used to follow the uptake of such molecules in living cells. The uptake of zinc bis(thiosemicarbazone) complexes in human cancer cells has been studied by fluorescence microscopy and the cellular distribution established, including the degree of uptake in the nucleus [54]. Bis(thiosemicarbonato)copper complexes [55] being fluorescent are found to be useful in radiolabelling. They are hence useful for diagnostic imaging of Alzheimer’s disease by binding to amyloid-β-plaques, the compounds supposed to be associated with the disease [56]. Cu-ATSM has been found to be particularly selective in hypoxia and multidrug resistance. The hypoxic selectivity of Cu(II) bis(thiosemicarbazones) have been found to be dependent on the redox potential of complexes which in turn depends on the back bone substituents of the thiosemicarbazone skeleton [57].

1.7.5 Construction of novel materials and devices

Recently bis(thiosemicarbazones) have been found to be suitable for the construction of discrete multimetallohelicates since they have two long arms
containing two soft sulfur donor atoms [58]. The controlled self assembly of the building blocks resulted in double-stranded dinuclear zinc(II) and tetranuclear Cu(I) helicates. Helicates are used for the construction of novel materials, devices and machines with programmed properties and functions such as luminescence, DNA binding or anion binding.

1.8 Scope and objectives of the present work

As a continuation of the foregoing discussion bis(thiosemicarbazones) has been found to be proligands which are having very versatile donor possibilities to produce different geometries of complexes. Designing of ligand with redox tunable properties can be attained by using highly delocalized systems. It can be inferred that many areas of ring incorporated heterocyclic bis(thiosemicarbazones) are still to be explored. Similarly the behaviour of ring incorporated monothiosemicarbazone and its complexes seem to be an interesting area. Hence it has been decided to select 2,6-diacetylpyridine as the diketone and two tailored ring incorporated thiosemicarbazides containing a heterocyclic unit like morpholine or pyrrolidine as the starting materials for the ligands. Since the compound contains two thiosemicarbazone moieties it can be neutral, dianionic or monoanionic in complex formation. Along with, a free unsubstituted bis(thiosemicarbazone) and a monothiosemicarbazone also are synthesized. Some first row divalent transition metals like Mn(II), Ni(II), Cu(II) and Zn(II) are the selected as metal centers. However a trivalent Fe(III) and a second row divalent Cd(II) also are included in the list.

The objectives of the present work are

a) To design and synthesize some ring incorporated thiosemicarbazones by taking 2,6-diacetylpyridine and the thiosemicarbazide in appropriate ratios.

b) Characterization of the thiosemicarbazones using IR, UV, NMR, CV etc.
c) Study the coordination behavior of these ligands.

d) To synthesize transition metal complexes of transition metals.

e) To study the composition and spectral properties using IR, UV, EPR, NMR, CV etc.

f) To study the structure of the complexes by single crystal X-ray diffraction methods.

g) To analyse any application oriented properties of these complexes.

1.9 Characterization techniques

In order to achieve the above objectives the characterization techniques used are enlisted as follows.

1.9.1 Estimation of carbon, hydrogen, nitrogen and sulfur

Elemental analyses of C, H, N and S present in all the compounds were done on a Vario EL III CHNS elemental analyzer at the SAIF, Cochin University of Science and Technology, Kochi-22, Kerala, India. Based on the elemental composition possible structures were drawn using the ACD/Chemsketch Freeware software.

1.9.2 Conductivity measurements

The molar conductivities of the complexes in DMF solutions (10^{-3} M) at room temperature were measured using a direct reading conductivity meter at the Department of Applied Chemistry, CUSAT, Kochi, India.

1.9.3 Magnetic susceptibility measurements

Magnetic susceptibility measurements of the complexes were carried out on a Vibrating Sample Magnetometer using Hg[Co(SCN)_{4}] as a calibrant at the SAIF, Indian Institute of Technology, Madras and Gouy Balance at the Department of Applied Chemistry, CUSAT, Kochi, India.
1.9.4 IR spectral studies

Infrared spectra of some of the complexes were recorded on a JASCO FT-IR-5300 Spectrometer in the range 4000-400 cm\(^{-1}\) using KBr pellets at the Department of Applied Chemistry, CUSAT, Kochi, India. IR spectra were also recorded on a Thermo Nicolet AVATAR 370 DTGS model FT-IR Spectrophotometer with KBr pellets at the SAIF, Kochi, India.

1.9.5 Electronic spectral studies

Electronic spectra in the range 200-500 nm were recorded on a Cary 5000 version 1.09 UV-VIS-NIR Spectrophotometer using solutions in acetonitrile/DMF at the SAIF, Kochi, India. The spectra in the range 200-900 nm were recorded on a UV-vis Double Beam UVD-3500 spectrometer at the Department of Applied Chemistry, CUSAT, Kochi, India.

1.9.6 NMR spectral studies

The \(^1\)H, \(^13\)C NMR spectra, D\(_2\)O exchange and DEPT experiments were recorded using Bruker AMX 400 Spectrometer, with CDCl\(_3\) as solvent and TMS as standard at the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore, India and using Bruker 400 Spectrometer with DMSO as solvent at the SAIF, Kochi.

1.9.7 EPR spectroscopy

EPR spectra were recorded in a Varian E-112 X-band EPR Spectrometer using TCNE as a standard at SAIF, IIT, Bombay, India. The \(g\) factors were quoted relative to the standard marker TCNE (\(g = 2.00277\)).

1.9.8 X-ray crystallography

Crystallography is the experimental science of determining the arrangement of atoms in crystals. For an object to be visible, its size needs to be at least half the wavelength of the light being used to see it. Since visible
light has a wavelength much longer than the distance between atoms, molecules are not seen in it. In order to see molecules it is necessary to use a form of electromagnetic radiation with a wavelength of the order of bond lengths, such as X-rays. When X-rays are beamed at the crystal, electrons diffract the X-rays, which cause a diffraction pattern. Using Fourier transformation, these patterns can be converted into electron density maps. These maps show contour lines of electron density. Since electrons more or less surround atoms uniformly, it is possible to determine where atoms are located. Unfortunately since hydrogen has only one electron, it is difficult to map hydrogen. A three dimensional picture is obtained by rotating the crystal at different angles. A computerized detector produces two dimensional electron density maps for each angle of rotation. The third dimension comes from comparing the rotation of the crystal with the series of images. Computer programs use this method to come up with three dimensional spatial coordinates.

Single crystal X-ray crystallographic analysis of one of the zinc compound was carried out using Siemens SMART CCD area–detector diffractometer at the Analytical Science Division, Bhavnagar, Gujarat, India. The structures were solved by direct methods with the program SHELXS-97 and refined by least-square on $F_0^2$ using the SHELXL software package [59].

X-ray diffraction measurements of other complexes were carried out on a CrysAlis CCD diffractometer with graphite-monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation at National Single Crystal X-ray Facility, IIT Bombay, Mumbai, India and University of Hyderabad, Hyderabad, India. The program CrysAlis RED was used for data reduction and cell refinement [60]. The structures were solved by direct methods using SHELXS and refined by full-matrix least-squares refinement on $F^2$ using SHELXL. The graphical tools used were Diamond version 3.1f [61] and Mercury [62].
1.9.9 Cyclic voltammetry

Cyclic voltammetric measurements were done on a PC interfaced electrochemical analyzer (BAS Epsilon Bioanalytical system USA) with a three electrode compartment system consisting of a glassy carbon working electrode, platinum wire counter electrode and Ag/Ag⁺ reference electrode, at the Department of Applied Chemistry, CUSAT, Kochi, India. The solutions of complexes in DMSO (10⁻³M) after degassing (N₂ bubbling for 15 mts) containing 0.1M TBAC (tetrabutylammonium chloride) as the supporting electrolyte have been used to study the electrochemical properties. The voltammogram is run between the potentials of −200 and +200 mV at a scan speed of 100 mV/s.

1.9.10 Fluorescence spectrophotometry

Fluorescence spectroscopy is an important investigational tool in many areas of analytical science, due to its extremely high sensitivity and selectivity which is used across a broad range of chemical, biochemical and medical research. It is an essential investigational technique allowing detailed, real-time observation of the structure and dynamics of intact biological systems with extremely high resolution. In the pharmaceutical industry it has almost completely replaced radiochemical labeling.

Fluorescence studies are conducted with a Cary Eclipse fluorescence spectrophotometer with scan software 1.1(132) at the International School of Photonics, Cochin University of Science and Technology, Kerala.

1.10 Conclusion

This chapter deals with a brief historical outline on the studies of thiosemicarbazones, bonding and geometrical aspects, applications, scope and various characterization techniques. The ligands decided to be synthesized are thiosemicarbazones of 2,6-diacetylpyridine.
References


Thiosemicarbazones – A Conceptual Framework


