Chapter 1
A BRIEF INTRODUCTION TO ACID HYDRAZONES AND THEIR STRUCTURE AND BONDING NATURE TO TRANSITION METALS

1.1. Introduction

Intensive research on the physicochemical properties and molecular structure of complexes with organic derivatives of hydrazine has provided essential new results, which need to be surveyed and compared with previous knowledge. One of the reasons for close attention paid to the formation of complexes of these ligands with transition metals is their biological activity. They are very promising as ligands in the formation of different coordination compounds, because there is a wide range of possible substituents, R1, R2, and R3 (Fig. 1). The existence of tautomeric equilibrium in acid hydrazone make it possible to obtain coordination compounds containing either neutral keto form or deprotonated enol form [1].

![Fig. 1. General formula of acid hydrazone](image)

The chemical properties of hydrazones have been intensively investigated in several research fields because of their chelating capability and their pharmacological applications [2]. The antibacterial and antifungal properties of bis(acylhydrazone) and their complexes with some first transition series metal ions was studied and
reported by Carcelli et al. The evaluation of in vitro antimicrobial properties showed some compounds to exhibit good activity against Gram positive bacteria [3]. Copper(II) complex of salicylaldehyde benzoylhydrazone was shown to be a potent inhibitor of DNA synthesis and cell growth. This hydrazone also has mild bacteriostatic activity and a range of analogues has been investigated as potential oral iron chelating drugs for genetic disorders such as thalassemia [4-5]. This type of diprotic ligand typically acts as tridentate planar chelating agents coordinating through the phenolic and amide oxygen and imine nitrogen. The actual ionization state is depending upon the condition and metal employed [6]. In base solution both phenolic and amide protons ionized, whereas in neutral and acidic solutions the ligands are monoanionic. However in strongly acidic condition the ligands will be bonded to the metal ion in a neutral state. Synthesis and structural studies of nickel(II) complexes of salicyloylhydrazone of picolinaldehyde and its chelating properties were reported by Domiano et al. [7].

The biological activities and chemical and industrial versatility of metal hydrazone complexes continue to attract considerable attention. For example, Schiff base hydrazones of pyridoxal phosphate and its analogues have been studied in an attempt to better understand the mechanism of action for vitamin B₆ containing enzymes [8-9]. Copper (II) complexes of antitumor ligand, salicylaldehyde benzoyl hydrazone have been studied by Ainscough et al. [6]. More recently the related systems have been investigated for their ability to act as iron-chelating drugs. The only agent currently in clinical use for the removal of excess iron from humans for disorder such as thalassemia is desferrioxamine. This is a microbial derived hexadentate trihydroxamic acid, which is given by parental injection or trip [10], but the above type ligands had been evaluated as chelating agents for oral administration.

Binary and ternary complexes copper(II) complexes of the ligand salicylaldehyde acetyl hydrazone and its antitumor activities have been reported by Chan et al. [11]. The studies showed that the monomeric units being bridged through the phenoxyl oxygen. The copper atom has a square pyramidal geometry, with the
basal donor atoms coming from the tridentate ligand (ONO) and symmetry related to phenolate.

Acyl hydrazones of salicylaldehyde subsequently attracted attention. It displays radioprotective properties [12] and a range of acyl hydrazones have been shown to be cytotoxic, the copper(II) complexes again showing enhanced activity.

1.2. Structure, bonding and stereochemistry of the acid hydrazones

Hydrazones have the general formula given below. The functional grouping causes these compound to behave as bidentate ligands for metal ion and owing the availability of -NH-C=O group, acid hydrazones show keto- enol tautomerism (Fig. 2). In solid state they exist in keto form but in solution they exist as an equilibrium mixture keto and enol forms.

![Fig. 2. Tautomerism in acid hydrazone](image)

These types of compounds expected to exist in a trans form, but such situation the compound may act as a unidentate ligand, by bonding through enolate oxygen. It is well evident that the stereochemistry of the ligand is much decided by the steric effects of the various substituents in the hydrazone moiety. It is found that most of the compound is in cis form, while coordinating to the metal ions. This phenomenon is assumed to be due to chelate effect- increased stability due to better electron delocalisation in chelated ring system- resulting from the coordination with the metal atoms.

Dept. of Applied Chemistry

February 2004
The composition and structure of the complexes are determined by the electronic structure of complex forming metal as well as preparation condition [13-14]. They form coordination compounds through the oxygen atom of either carbonyl [15-16] or the enol group and through the imine nitrogen atom, a five membered ring being produced. (Fig. 3)

![Fig. 3. General mode of coordination of acid hydrazones](image)

Neutral metal complexes can be formed. This type of hydrazones may behave as a terdentate chelating agent, provided R1 residue provides a donor atom in a adequate position, as it occurs in salicyloylhydrazide derivatives, although in these cases, the compounds acts commonly as ON donor ligands if R2 and R3 do not show chelating tendency [17-19] or in the pyridylhydrazide derivatives (NON) ligands. Terdentate ligands may also be obtained if R2 and/or R3 residues are groups such as o-hydroxyphenyl (ONO) ligands [19-21]. Chelating ligand with higher number of coordination position may be prepared by convenient selection of the condensation products: aroylhydrazide (R1-CONHNH2) and di or tri ketones [22-30]. The oxidation state of the metal ion determines the softness and hardness and hence plays an important role in predicting the stability of the complexes of acid hydrazones. The stability of the complexes also increases with the in crease in pi electron delocalisation, size of the molecule and also the nature and size of the rings formed [31].

Hydrazones obtained by condensing substituted acid hydrazide with aromatic 2-hydroxyaldehyde, β-diketones, and keto-acids of the pyruvic type are most effective in complex formation. The systems of this type, which have been studied
most, are benzoyl hydrazone 2-hydroxy aldehyde, which with transition metals ions can give at least four type of coordination (Fig. 4-7).

![Fig. 4.](image)

![Fig. 5.](image)

![Fig. 6.](image)

![Fig. 7.](image)

Different modes of coordination of substituted acid hydrazones with metal

The reactions of VO(IV), Mn(II), and Cu(II) with these type of ligands in neutral or weakly alkaline solution gives mainly complexes containing enol forms [32-36]. The IR spectra of these complexes contain no bands due to -NH, -OH, or
-C=O groups and bands due to stretches of the -C=N-N=C- hydrazone grouping is shifted to lower wavenumbers compared to the initial aroyl hydrazones, being found in the ~1590 cm\(^{-1}\) [1].

1.3. Objectives and scope of the work

- Preparation of different acid hydrazones and their structural studies and studies on their antimicrobial activity.
- Synthesis and spectral characterization of the different complexes of copper(II), oxovanadium(IV), manganese(II), nickel(II), zinc(II), etc.
- Effect of incorporation of heterocyclic bases to the coordination sphere.
- Change in the biological activity of ligand upon coordination.
- Development of X-ray quality single crystals and its X-ray diffraction studies
- Studies on the redox behavior of the coordinated metal ions.
- Correlation between the stereochemistry and biological activities.

1.4. Structural characterization techniques

Various techniques are used to elucidate the bonding, structure and stereochemistry of the ligands and the complexes prepared. While the ligands are characterized by usual methods such as elemental analysis, IR, UV-visible and NMR spectral techniques, it differs for complexes depending on the nature of the ligands and the metal ions involved. Ligands on complexation with some metal ions to form complexes having paired or unpaired electrons give diamagnetic or paramagnetic respectively. Some of the common physicochemical methods adopted by inorganic chemists are discussed below.
1.4.1. Magnetic measurements

In a magnetic field, the paramagnetic compounds will be attracted while the diamagnetic compounds repelled. In paramagnetic complexes, often the magnetic moment gives the spin only value corresponding to the number of unpaired electron. The variation from the spin only value is attributed to the orbital contribution and it varies with the nature of coordination and consequent delocalisation. In some cases two magnetic centers may be coupled together and may result in extraordinary increase or decrease in the magnetic moment of the complex. For example, a mononuclear complex of copper of the formula \([\text{CuLX}]\) where \(\text{Cu}\) is in the +2 oxidation state, the complex is expected to have magnetic moment of 1.73 BM - corresponding to \(d^9\) configuration, but in case of \([\text{Cu(OAc)}_2]\) the value is lower than 1.73 BM. This is explained on the basis of the assumption that the individual magnetic moments are aligned in opposite directions so that they cancel each other to some extent or can be ascribed as due antiferromagnetic coupling. Thus the value of magnetic moment of a complex would give valuable insights into its constitution and structure. In some cases the variation in the magnetic moment can be explained on the assumption that the compound may be an equilibrium mixture of tetrahedral and square planar geometries- the number of unpaired electrons differ in either geometries and hence the magnetic moment. The magnetic susceptibility measurements thus help to predict the oxidation state of the metal ion to a limited extent and to establish the possible geometry of the compound.

The most widely adopted method for determining the magnetic moment of a complex is by Gouy’s Method in which the weight difference experienced by a given amount of a substance in the presence and absence is measured. This is compared with that of a standard substance and magnetic moment is determined with the help of suitable equations. Faraday Balance and Vibration Susceptibility Magnetometer are the other instruments used for the magnetic susceptibility measurements [37].
1.4.2. Electronic spectroscopy

Electronic spectroscopy is an important and valuable tool for most chemists to draw important information about the structural aspects of the complexes. The ligands, which are mainly organic compounds, have absorption in the ultraviolet region - hence do have bands in the region of the 200 to 350 nm of the electromagnetic spectrum - and in some cases these bands extends over to higher wavelength region due to conjugation. But upon complexation with transition metal ions, due to interaction with the metal ion there will be an interesting change in the electronic properties of the system. New features or bands in the visible region due to d-d absorption and charge transfer spectra from metal to ligand (M→L) or ligand to metal (L→M) can be observed and this data can be processed to obtain information regarding the structure and geometry of the compounds [38].

This technique along with other spectral techniques viz., EPR serves to find out the structural features and to calculate the bonding parameters such \((\alpha^2, \beta^2, \gamma^2, K_\alpha, K_\beta, \text{etc.})\) [39-42] and Racah Parameters (B and C) [43].

The electronic spectroscopy is also widely used to explore the change in the structural features with change in the pH of the medium. The electronic and structural features of the complexes are widely utilized to investigate the kinetics and mechanisms of the reactions involving transition metal complexes [44-45].

The kinetics of 4-nitrophenylacetate cleavages, by oxime in the presence of \(\text{Zn}^{2+}\) ions was investigated [46-47].

1.4.3. Infrared Spectroscopy

The IR spectroscopy is the widely used as a characterization technique for metal complexes. The basic theory involved is that the stretching modes of the ligands changes upon complexation due to weakening/strengthening of the bonds.
involved in the bond formation resulting in subsequent change in the position of the bands appearing in the IR spectrum. The changes in the structural features of the ligands are observed as changes in bands observed, mainly in the fingerprint region i.e., in the 1500 - 750 cm\(^{-1}\). Nakamoto discusses at length the characterization of metal complexes with the help of IR spectroscopy [48]. The bands due to the metal ligand bonds are mainly observed in the far IR region i.e., 50 - 500 cm\(^{-1}\).

1.4.4. EPR spectroscopy

Electronic structure is important concept in many area of chemistry such as inorganic, physical, material and biological. Among the main techniques for describing electronic structure, electron paramagnetic resonance spectroscopy is unique in its ability to selectively probe paramagnetic molecules. In fact EPR spectroscopy can provide a wealth of information about the electronic structure of paramagnetic molecules including those involving metal ions. For complexes those are paramagnetic, in addition to the elemental analysis, IR, and electronic spectroscopic techniques, Electron Paramagnetic Resonance (EPR) spectroscopy acts as an effective and valuable tool to explore the structural features and bonding characteristics of metal complexes. The advances in the ESR spectroscopy have benefited the inorganic chemists with the help of high field and high-resolution spectrometers that helps to resolve the \( g_\parallel \) and \( g_\perp \) features of the paramagnetic species. The information obtainable from a low temperature spectrum of diamagnetically diluted paramagnetic species provides important clues to structural traits and bonding properties of the complexes [49].

The single crystal EPR spectrum measurements are also widely employed to derive more information about the geometry of the paramagnetic species formed [50]. Hathaway had extensively surveyed the studies on complexes by using EPR spectroscopy [51]. Various simulation packages are extensively used to simulate the experimental spectrum and hence help to establish the absolute geometry and accurate bonding and structural characteristics of the complexes [52-55]. However,
for diamagnetic complexes, NMR spectroscopy still remains as a valuable tool for establishing the structural characterizations.

1.4.5. X-Ray crystallography

The diffraction/scattering of X-ray radiations by array of atoms in a single crystal of a compound is exploited to establish the structure and geometry of the complexes. At present this versatile techniques is valued as the final word by many chemists for establishing the accurate structure of the complex compounds.

1.4.6. NMR techniques

It is a technique used to characterize ligands and diamagnetic complexes. It consists of four techniques, $^1$H NMR, $^{13}$C NMR, COSY ($^1$H - $^1$H correlation) and HMQC ($^1$H - $^{13}$C correlation).

The proton NMR ($^1$H NMR) spectrum was recorded at 500 MHz. The assignment is done on the basis of chemical shifts, multiplicities and coupling constants.

$^{13}$C NMR technique is a relatively new one. There are considerable differences between $^1$H NMR and $^{13}$C NMR spectra, both in modes of recording as well as appearance. $^{13}$C has a spin quantum number equal to $\frac{1}{2}$ and its nuclear magnetic resonance can be observed in the magnetic field of 23,000 guass at 25.2 MHz. Whereas with the same magnetic field $^1$H NMR at 100 MHz. The insufficiency of this method is clear from the fact that only one line can be observed at a given point in time and hence to excite the whole band of frequency simultaneously. It is done by using strong pulse of radio frequency covering a large band of frequencies, which is capable to excite the whole, all resonance of interest at once. Each $^{13}$C in organic molecule is spin coupled not only to the directly attached proton, but also to the protons, which are two to four bonds away. Because of this long range coupling
\(^{13}\text{C}\) spectra appear as broad peaks. By using \textit{proton noise decoupling} technique simplified \(^{13}\text{C}\) NMR spectra can be obtained.

Assignments of protonated carbons were made by two dimensional heteronuclear-correlated experiment using delay values, which corresponds to \(^1J(\text{C,H})\). The HMQC experiment provides correlation between protons and their attached heteronuclei through the heteronuclear scalar coupling. Thus we can determine the connectivity of each proton to the carbon. This sequence is very sensitive, compare to the older HETCOR, as it is based on proton detection, instead of the detection of the least sensitive low gamma heteronuclei.

1.4.7. \textit{Cyclic Voltammetry}

Cyclic voltammetry is widely used to study the redox behavior of the coordinated complexes. It gives an insight into the stability of the compound under investigation against electrolytic oxidation and reduction in the solution. In this technique, the potential of a small stationary working electrode is changed linearly with the time starting from a potential where no electrode reaction occurs and moving to potentials where reduction or oxidation of a solute (material being studied) occurs. After traversing the potential region in which one or more electrode reaction takes place, the direction of the linear sweep is reversed and the electrode reactions of the intermediates and products formed during the forward scan, often can be detected. The technique can be carried out using a suitable reference, working and counter electrodes, the selection of which can be made depending on the nature of the compound and the solvent used, in the presence of a supporting electrolyte \cite{56}. The supporting electrolyte is usually added to repress the migration of charged reactants and products. For our systems measurements were made on degassed (\(\text{N}_2\) bubbling for 10 minutes) solution in DMF (10\(^3\text{M}\)) containing 0.1 M tetrabutyl ammonium tetrafluoroborates as supporting electrolyte. The three electrode system consists of a glassy carbon (working) platinum wire (counter or auxiliary) and Ag/AgCl (reference) electrodes. Having knowledge of the species involved and an idea about
the redox properties, one can select the range of voltages, and the variation in voltammogram can be recorded at different sweep rates. The peaks in the forward and reverse sweeps can be interpreted to assess the stability of the species. Depending on the nature of the voltammogram obtained they may be termed as reversible ($i_{pa}=i_{pc}$), quasi-reversible ($i_{pa}>i_{pc}$) and irreversible process. If some chemical reaction occurs the return peak of the cyclic voltammogram will be reduced in magnitude, and it will be completely absent if the reaction half-life is much less than the scan duration [57]. The cyclic voltammetric techniques can also be utilized, coupled with electronic spectroscopy, to obtain information about the presence of a new species formed during the oxidation-reduction process and the related stereo chemical and structural changes [58].

1.4.8. Biological studies

The biological activities or the therapeutical ability of any compound depends on the minimum amount by which the chemical or substance is required to inhibit the growth or to kill the micro organism that causes the disease, along with a minimum cytotoxicity - a potential to act as a toxin that may generate undesirable symptoms that are harmful to health of living organism and hence decides a drug value of the same

The synthesized chemical ligands and complexes were tested for their antimicrobial activity. Antimicrobial activity is the ability of a compound to inhibit the growth of a given microorganism. The antimicrobial agent may be either bacteriostatic or bactericidal.

The effectiveness of an antimicrobial agent in sensitivity testing is based on the size of the zones of inhibition. When the test substances are introduced on to a lawn of bacterial culture by disc diffusion method, if the bacteria are sensitive, there develops a zone of no growth around the disc, which is referred to as the zone of inhibition. The diameter of the zone is measured to the nearest millimeter. Test
substances which produce zone of inhibition of diameters, 9 mm or more are regarded as positive, i.e. having antimicrobial activity; while those cases where the diameter is below 9 mm, the bacteria are resistant to the sample tested and the sample is said to have no antimicrobial activity.

Test organisms

The microorganisms used as test organisms were bacteria isolated from clinical samples.

Two Gram positive bacteria and three Gram negative bacteria were used as test organisms.

A) Gram Positive
1. Staphylococcus aureus
2. Bacillus sp

B) Gram Negative
3. Escherichia coli
4. Salmonella paratyphi
5. Vibrio cholerae O1

The disc diffusion method was used for screening for the antimicrobial property of the test samples. The MIC (minimum inhibitory concentration) of the complexes showing a positive antimicrobial property was done using the same method.

1. Preparation of discs

   Discs of 4 mm diameter were cut out of Whatman No.1 filter paper and autoclaved at 15 psi for 15 minutes. 5 µL of each of the test chemical samples were dispensed on to the discs under aseptic conditions. The discs were dried at 30°C and stored in sterile vials until further use.

2. Media

   Unless otherwise specified, the medium used for growing the cultures was nutrient agar.

Dept. of Applied Chemistry

February 2004
Disc diffusion method

A loopful of an overnight slant culture of the test organism was inoculated to 5 ml of sterile physiological saline to make a uniform suspension. This suspension culture was surface spread on nutrient agar plate by swabbing with a sterile cotton swab so as to get a uniform lawn culture.

The discs with the test complexes prepared as mentioned above, were placed on the swabbed surfaces of the plates (4 discs per plate) using sterile forceps.

The plates were incubated at 37°C for 24 hours and then checked for zones of inhibition around the discs.

References


Dept. of Applied Chemistry

February 2004


Dept. of Applied Chemistry February 2004


