



Original Research Article

Synthesis and Characterization of the Complexes of Isovaleric Acid Hydrazide with M (II) Chlorides (M= Co, Ni, Cu)

A. S. Ojo^{*}, J. N. Nwabueze

Department of chemistry, University of Abuja, P.M.B. 117, Abuja, Nigeria

***Corresponding Author:** A. S. Ojo, Department of chemistry, University of Abuja, P.M.B. 117, Abuja, Nigeria

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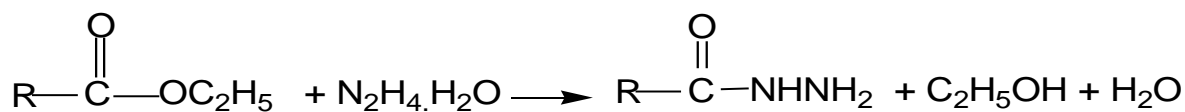
ABSTRACT

Complexes of isovaleric acid hydrazide (IVH) with M (II) chlorides have been synthesized (M = Co, Ni and Cu). The ligand was characterized by melting point and IR studies, while the complexes were characterized by melting point, conductance, IR and electronic Spectral studies. The ligand, complexes and metal salts were screened for antimicrobial activity. The electronic data indicates octahedral coordination for Co²⁺, Ni²⁺ and Cu²⁺ complexes. Infrared spectra data are diagnostic of bidentate coordination for the ligand. They coordinate via the carbonyl oxygen atom and the primary amino nitrogen atom. The Cl⁻ anions are in the outer coordination sphere. Minimum activity was shown by the complexes with some microbes while with others are insignificant, as compared with the metal salts except for [Co(IVH)₂]Cl₂.2H₂O and that showed significant activity against *Salmonella spp.*, *Staph. Aureus*, *P. mirabilis* and *E. coli*. Conductivity studies indicate a non-electrolytic nature for the complexes while the high melting/decomposition temperature indicates a polymeric nature of the complexes.

Keyword: Isovaleric; hydrazide; chloride; antimicrobial; bidentate; ligand; polymeric

INTRODUCTION

The preparation of acid hydrazide from carboxylic acid esters is shown below



Scheme 1: Route to hydrazide preparation [1]

Much attention is given to acylhydrazides and some other hydrazine derivatives because of their biological and physiological activities. They generally exhibit very strong anti-microbial activity. This activity is enhanced on complexation to some metal ion [2]. They are also used to produce high polymer substances and in the determination of metal by complexation [1].

Generally, the acylhydrazides are found to be potentially tridentate ligands. Their metal complexes especially copper (II) have been known to show anti-tumor activity while some others are used in polymer coating and pigments [2].

The remarkable biological activity of acid hydrazides R-CO-NH-NH₂, a class of shift base, their corresponding acylhydrazones, and the dependence of their mode of chelating with transition metal ions present in living system have been of significant interest in the past. The co-ordination compounds of acylhydrazide have been reported to act as enzymes inhibitors and are useful due to their pharmacological application [3].

Isovaleric acid also known as 3-methylbutanoic acid is a natural fatty acid found in a wide variety of plant liquid that is sparingly soluble in water, but extremely soluble in most organic solvent. Isovaleric acid has a strong pungent, cheesy and sweet smell, but its volatile esters have pleasing scents and are used widely by perfumery. Other synonyms of isovaleric acid include delphinic acid, 3-methylbutanoic acid and isopentanoic acid [4].

The coordination chemistry of transition metals with ligands from the hydrazide family based on -CONHNH₂ and their derivatives has been of interest. This is due to the different bonding modes shown by these ligands with metals of varied electropositivity [5]. Hydrazides possessing an azomethine -NHN=CH- constitute an important class of compounds for new drug development. This class of compounds has

been studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal properties and find applications in biomimetic catalytic reactions. The hydrazides based on -CONHNH₂ and their derivatives contain trigonal N- and O- donors making them potential chelating ligands. Hydrazide derivatives and complexes have supra molecular interactions because they include the hydrogen-bonding donors (amino group) and acceptors (carbonyl), and as a result hydrogen-bonding plays an important role in extending and stabilizing the structures of the resultant complexes [6]. The mode of coordination of benzoic acid hydrazide to Li⁺, Zn²⁺, Cd²⁺, Hg²⁺ and Mg²⁺ were reported.

The luminescence properties of Zn(II) and Cd(II) complexes of N'-(2-hydroxybenzoyl) isonicotino hydrazide and N'-(2-hydroxybenzoyl) nicotino hydrazide had been investigated. Also, the mode of coordination and the biological activity of some Zn(II) complexes with acetic acid (3-chloro-4-hydroxyl benzylidene)-hydrazide and acetic acid(2-nitro-benzylidene) hydrazide have been found out to have interesting biological applications [6]. Hence, our interest in the syntheses, physicochemical properties, mode of coordination and the stability of some metal (II) complexes of isovaleric acid hydrazide.

MATERIALS AND METHODS

Reagents and Instruments

Isovaleric acid was obtained from Sigma-Aldrich Chemical Company Ltd. All other chemicals, the metal chloride and nitrate were of analytical grade and were obtained from BDH Chemicals Co Ltd, England. All chemicals were used without further purification.

Preparation of Ethyl Isovalerate

30 mL (27.81g, 0.27mole) of isovaleric acid was added to 13.15 mL (12.42g, 0.27mole) of ethanol in a flask. 1ml (1.84g) of conc. Sulphuric

acid was added drop wise with swirling, boiling chips was added to avoid bumping and the solution was refluxed for 2 hours. After refluxing the solution was allowed to cool to room temperature, the reaction mixture was washed with sodium bicarbonate until effervescent stops; the mixture was transferred into a separating funnel and allowed to separate. The organic layer collected was dried using anhydrous magnesium sulphate [7].

Preparation of Isovaleric Acid Hydrazide

5.12 mL (5.3g, 0.106 mole) of hydrazine hydrate was added to 15 mL ethyl isovalerate with 50 mL of ethanol, pieces of antipumping granules were added to prevent bumping. The mixture was refluxed for six hours in a 500 mL round bottom flask and concentrated by heating in a water bath, and then it was left for three weeks to crystallize. The resulting crystal was dried over silica gel in a vacuum desiccator and weighed [8].

Preparation of the Complexes

The complexes were prepared by the reaction between aqueous solutions of the metal salts and the ethanolic solutions of the ligands in a 2:1 molar ratio [9].

Preparation of Cobalt Chloride IVH Complex

0.8g (0.007 mole) of IVH was dissolved in 6 mL of absolute ethanol. 0.83 g (0.0035 mole) cobalt chloride was dissolved in 10 mL of ethanol. The ligand solution was gently added with constant stirring to the solution of the metal salt. A purple coloured precipitate was immediately formed. The precipitate was removed by filtration and dried over silica gel desiccator and weighed. (Yield 3.41g, 41.09%).

Preparation of Nickel Chloride IVH Complex

0.8g (0.007 mole) of IVH was dissolved in 6 mL of absolute ethanol. 0.83 g (0.0035 mole) nickel chloride was dissolved in 10 mL of ethanol. The ligand solution was gently added with constant

stirring to the solution of the metal salt. A sky blue coloured precipitate was immediately formed. The precipitate was removed by filtration and dried over silica gel desiccator and weighed. (Yield 3.41g 58.49%).

Preparation of Copper Chloride IVH Complex

0.8g (0.007 mole) of IVH was dissolved in 6 mL of absolute ethanol. 0.83 g (0.0035 mole) copper chloride was dissolved in 10 mL of ethanol. The ligand solution was gently added with constant stirring to the solution of the metal salt. A green coloured precipitate was immediately formed. The precipitate was removed by filtration and dried over silica gel desiccator and weighed. (Yield 3.41g, 54.59 %).

Determination of Co²⁺ in Cobalt (II) Complexes

3.722g of EDTA was weighed and dissolved into 1000 mL of deionized water in a volumetric flask. The EDTA solution was standardized using 0.1M ZnSO₄ solution. 25 mL of ZnSO₄ pipetted out into a 250 mL conical flask, 5 mL of pH 10 buffer was added and diluted to 100 mL with deionized water. 30mg of solid EBT + KNO₃ indicator mixture was added, then titrated against EDTA solution with continuous swirling until the colour of the solution changes from wine red to blue. The titration was repeated to get three concurrent results.

This made a standard solution of 0.008M EDTA. It was then poured into a burette.

0.20g of each of the Cobalt (II) complexes was digested and made up to 100 mL with deionized water; 25 mL of each was pipetted into two conical flasks and diluted further with 50 mL of water. 10 mL of the ammonium buffer, pH 10 and 5 drops of the Eriochrome Black T indicator were added. The 0.008M EDTA prepared was titrated against it. The colour change was from pink to violet. The process was repeated twice for each of the complexes and the average volume of EDTA used was noted. The Cobalt (II) present in each complex was calculated [8].

Determination of Ni²⁺ in Nickel (II) Complexes

0.20g of each of the Nickel (II) complexes was digested and made up to 100 mL with deionized water; 25 mL of each was pipetted into two conical flasks and diluted further with 50 mL of water. 5 mL of buffer pH8 of aqueous NH₃/NH₄Cl was added and the murexide indicator 5 drops was also added. The 0.008M EDTA prepared was titrated against it. The colour change was from yellow to violet. The process was repeated twice for each of the complexes and the average volume of EDTA used was noted. The Nickel (II) present in each complex was calculated.

Determination of Cu²⁺ in Copper (II) Complexes

Then 0.20g of each of the four Copper (II) complexes of was weighed into two different Conical flask. Digestion was done and then made up 100 mL with deionized water in two different conical flasks. 5 drops each of indicator (fast suiphon black F) was added together with 5 mL each of concentrated ammonium solution. The 0.008M EDTA was then titrated against the solution and the colour changes from purple to dark green. The process was repeated two times for each of the complexes and the average titre value (of EDTA used) noted. The Copper (II) present in each complex was calculated.

Instrumental Measurements

The ultra-violet electronic absorption spectral of the complexes were carried out with methanol as solvent using a UV-2500PC UV-Visible spectrophotometer and IR studies were carried out on a 8400S FTIR machine using KBr pellets.

The conductivity measurements were made using WTW LF 340 meters, while Gallenkamp apparatus was used to determine the decomposition temperature/melting point of both the ligands and the complexes. The conductivity of the complexes was measured in methanol.

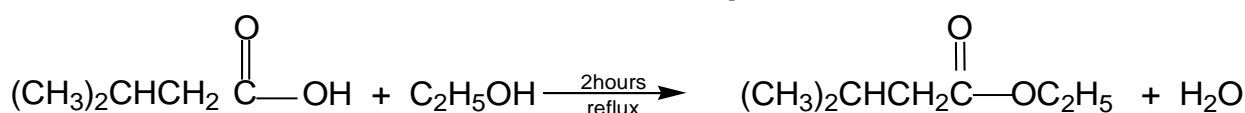
Antibacterial Screening

For the microbial sensitivity tests, the isolates (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudo moasaeruginosa* and *Proteus mirabilis*) were obtained from the micro biology laboratory of UNIVERSITY OF ABUJA TEACHING HOSPITAL, Gwagwalada, Abuja. They were cultured on a nutrient agar media. Solution of the test compounds was prepared by dissolving 0.1g of each compound in 2 mL of methanol. The petridishes to be used were impregnated with disks containing the solutions of the tests compounds and dried in an open air for 4 hours. 0.2 mL of the compounds under test were then incubated on the plate by striking uniformly with sterile wire loop after which the disk at 2 cm spacing was applied to the surface of the plate with sterile fine forceps and pressed gently to ensure full contact with the medium and moistening of the disk. They were transferred into the incubator and incubated for 24 hours at 37 °C. Antibacterial activity was measured by measuring the zone diameter of inhibition around the disc [10].

RESULTS AND DISCUSSION

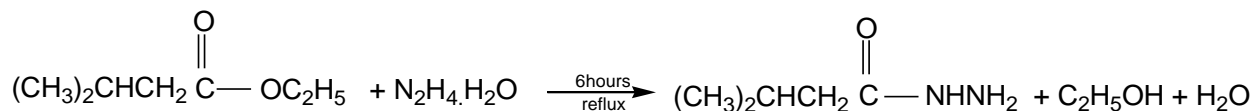
The Ligand

Ethyl isovalerate was first synthesized by refluxing isovaleric acid with ethanol



Scheme 2: Route to Ethyl isovalerate preparation

While the ligand was synthesized by refluxing the ethyl isovalerate with hydrazine hydrate

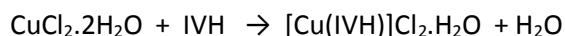
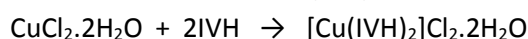
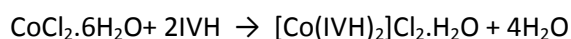


Scheme 3: Route to IVH preparation

The Complexes

The complexes were formed from the ligand with Co^{2+} , Ni^{2+} and Cu^{2+} chlorides. It was discovered that the filtrate of cobalt and copper chloride complexes when left over the night formed another complex of different colour and texture; this could be attributed to small stability difference between the octahedral and tetrahedral cobalt (II) and copper (II) complexes [10]. The different chloride complexes of cobalt and copper were analyzed differently.

The complexes were synthesized as shown in the equations below.



The physical properties of these complexes and their analytical data are given in Table 1, which includes solubility, conductivity, and melting point/ decomposition temperature.

The solubility test was carried out in different solvent and the result showed the complexes are insoluble in most organic solvent except methanol. This may be due to their polymeric nature [5].

The complexes produced have a low conductance values in methanol, indicating their low ionic character. Thus they are non-electrolytes. The value of $0.88 \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ highest value is weak when compared with a strong conductivity value of $1.5 - 2.0 \text{ ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ [12].

Melting Point/ Decomposition Temperature

The complexes melted at high temperature, above 200°C while the ligand melted at 72°C as shown in Table 1 [5].

The solubility test was carried out in different solvent and the result showed the complexes are insoluble in most organic solvent except methanol as shown in table 2. This may be due to their polymeric nature [13].

Table 1: Some Analytical Data and Physical Constants for the Ligand and Complexes

Compound	Formula	Formula Weight	Colour	Melting/ Dec. Temp. $^\circ\text{C}$	%Yield	% M (Calc.)	Conductivity $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$
IVH	$\text{C}_5\text{H}_{12}\text{N}_2\text{O}$	116.16	White	72	37.00		0.05
$[\text{Co}(\text{IVH})_2]\text{Cl}_2\cdot\text{H}_2\text{O}$	$\text{C}_{10}\text{H}_{26}\text{N}_4\text{O}_3\text{Cl}_2\text{Co}$	380.25	Purple	243	41.09	15.50(15.65)	0.88
$[\text{Co}(\text{IVH})]\text{Cl}_2\cdot 3\text{H}_2\text{O}$	$\text{C}_5\text{H}_{18}\text{N}_2\text{O}_4\text{Cl}_2\text{Co}$	299.93	Light pink	203	6.67	19.59(19.26)	0.08
$[\text{Ni}(\text{IVH})_2]\text{Cl}_2\cdot\text{H}_2\text{O}$	$\text{C}_{10}\text{H}_{26}\text{N}_4\text{O}_3\text{Cl}_2\text{Ni}$	380.01	Sky Blue	277	58.49	15.44(15.34)	0.47
$[\text{Cu}(\text{IVH})_2]\text{Cl}_2\cdot 2\text{H}_2\text{O}$	$\text{C}_{10}\text{H}_{28}\text{N}_4\text{O}_4\text{Cl}_2\text{Cu}$	402.87	Green	267	54.59	15.77(15.87)	0.68
$\text{Cu}(\text{IVH})\text{Cl}_2\cdot\text{H}_2\text{O}$	$\text{C}_5\text{H}_{14}\text{N}_2\text{O}_2\text{Cl}_2\text{Cu}$	269.16	Green	270	21.28	23.52(23.78)	0.11

Table 2: Solubility Data

Compound	Water	Ethanol	Acetone	P. Spirit	Methanol	Benzene	Dichloromethane
IVH	S	S	SS	IS	S	IS	S
[Co(IVH) ₂]Cl ₂ .H ₂ O	S	S	SS	S	S	IS	S
[Co(IVH)]Cl ₂ .3H ₂ O	IS	IS	S	IS	S	IS	S
[Ni(IVH) ₂]Cl ₂ . H ₂ O	SS	SS	IS	IS	S	IS	S
[Cu(IVH) ₂]Cl ₂ .2H ₂ O	IS	S	SS	IS	S	IS	S
Cu(IVH)]Cl ₂ . H ₂ O	IS	SS	S	IS	S	IS	S

S=Soluble, IS =Insoluble and SS = Sparely Soluble

Infrared Spectra

The infrared spectra of the complexes taken in the region 350cm^{-1} to 4500cm^{-1} were compared with that of the ligand. There are some significant changes between the complexes and the free ligand for chelation as expected. The main stretching frequency of the IR spectra of the ligand and the complexes are tabulated in Table .3. An exhaustive comparison of the IR spectra of the ligand and complexes gave information about the mode of bonding of the ligand in the metal complexes. The IR spectra of the complexes show that the ligand acts as neutral bidentate donors through the primary amino nitrogen and carbonyl oxygen. The assignment of bands above 3000cm^{-1} are only tentative since bands due to the asymmetric vibration of OH and NH group appear in this region as unresolved bands [14]. However, the bands in the region above 3400cm^{-1} in the hydrated complexes have been assigned to $\nu(\text{O-H})$ of the associated water molecules that may be water of hydration or coordinated molecule, while bands in the region around 3200cm^{-1} was assigned to $\nu(\text{N-H})$ stretching vibration[15]. The N-H bending vibration (scissoring) appears as a medium to strong intensity band at around 1560cm^{-1} [16]. The amino nitrogen bending vibration band $\nu(\text{N-H})$ which was located at 1537.32cm^{-1} in the spectra of the ligand, is lowered between $20.26 - 88.73\text{cm}^{-1}$ in the complexes, indicating weakening of the bond and coordination via primary amino nitrogen [17].

The band appearing in the spectra of the ligand at 1640.51cm^{-1} is assigned to $\nu(\text{C=O})$ vibration, which is lowered by 4.82 , 28.93 and 42.43cm^{-1} in complex $[\text{Ni}(\text{IVH})_2]\text{Cl}_2.\text{H}_2\text{O}$, $[\text{Co}(\text{IVH})_2](\text{NO}_3)_2.\text{H}_2\text{O}$ and $[\text{Co}(\text{IVH})]\text{Cl}_2.3\text{H}_2\text{O}$ respectively, suggesting weakening of the bond and participation of the carbonyl oxygen in coordination, except for that of $[\text{Co}(\text{IVH})_2]\text{Cl}_2.2\text{H}_2\text{O}$ which shows 1.93cm^{-1} shift in $\nu(\text{C=O})$ vibration band, which is insignificant for coordination [17]. Additional evidence of the participation of the two groups $\nu(\text{C=O})$ and $\nu(\text{C-N})$ in coordination is the presence of new band around 500cm^{-1} for $\nu(\text{M-O})$ and 400cm^{-1} for $\nu(\text{M-N})$ respectively[19-21].

The chloride complexes gave a white precipitate with silver nitrate, indicating that the chloride is outside the coordination sphere as a counter ion [22].

Table 3: Diagnostic IR Bands for the Ligand and the Complexes

Compound	V(OH)	V(N-H)	V(C=O)	$\Delta V(C=O)$	V(N-H)	$\Delta V(N-H)$	V(C-N)	V(M-O)	V(M-N)
IVH	-	3234.73	1640.51	-	1537.32	-	-	-	-
[Co(IVH) ₂]Cl ₂ .H ₂ O	3427.63	3255.95	1638.58	-1.93	1448.59	-88.73	1184.33	493.79	405.06
[Co(IVH)]Cl ₂ .3H ₂ O	3503.81	3219.30	1598.08	-42.43	1487.17	-50.15	1193.98	492.83	399.23
[Ni(IVH) ₂]Cl ₂ .H ₂ O	3407.37	3237.63	1635.69	-4.82	1460.16	-77.16	1183.37	515.98	387.70
[Cu(IVH) ₂]Cl ₂ .2H ₂ O	3458.48	3227.02	1616.40	-24.11	1463.06	-74.26	1177.58	585.42	401.21
Cu(IVH)]Cl ₂ .H ₂ O	3466.20	3211.59	1606.76	33.75	1517.06	-20.26	1207.48	502.47	405.06

Electronic Spectra

The electronic spectra in the UV-visible region of the complexes were done in methanol using UV-2500pc series spectrophotometer. The data are shown in Table 4.

The electronic spectra of [Co(IVH)₂]Cl₂.2H₂O complex show band at 512.5nm while the other [Co(IVH)]Cl₂.3H₂O complex show two bands at 467nm and 501nm. The band observed in the spectra of [Co(IVH)₂]Cl₂.2H₂O complex has been assigned to the transitions from ⁴T_{1g}(F) ground term to ⁴A_{2g}(P), while the two bands observed for the other [Co(IVH)]Cl₂.3H₂O complex have been assigned to the transitions from ⁴T_{1g}(F) ground term to ⁴T_{1g}(P) and ⁴A_{2g}(P) respectively. An octahedral geometry is therefore proposed

for the complexes, with some proposed distortion for [Co(IVH)]Cl₂.3H₂O complex [23].

The electronic spectra of the nickel complex show three bands. [Ni(IVH)₂]Cl₂.2H₂O complex show band at 356.5, 594 and 765nm. The band observed in the spectra of the complexes has been assigned to the transitions from ³A_{2g}(F) ground term to ³T_{1g}(P), ³T_{1g}(F), and ³T_{2g}(F) respectively. An octahedral geometry is therefore proposed for the two complexes [23]. The electronic spectra of [Cu(IVH)₂]Cl₂.2H₂O and [Cu(IVH)]Cl₂.H₂O complex show band at 769.5 and 777.5nm respectively. The band observed in the spectra of both complexes has been assigned to be due to d → d transitions. Distorted octahedral geometry is proposed for both complexes [23].

Table 4: Electronic Data for the Complexes

Compound	λ (nm)	Assignment	Geometry
[Co(IVH) ₂]Cl ₂ .H ₂ O	512.5	⁴ T _{1g} (F) → ⁴ A _{2g}	Octahedral
[Co(IVH)]Cl ₂ .3H ₂ O	467	⁴ T _{1g} (F) → ⁴ T _{1g} (P)	
	501	⁴ T _{1g} (F) → ⁴ A _{2g}	
[Ni(IVH) ₂]Cl ₂ .H ₂ O	356.5	³ A _{2g} (F) → ³ T _{1g} (P)	Octahedral
	594	³ A _{2g} (F) → ³ T _{1g} (F)	
	765	³ A _{2g} (F) → ³ T _{2g} (F)	
[Cu(IVH) ₂]Cl ₂ .2H ₂ O	769.5	d → d	Octahedral
Cu(IVH)]Cl ₂ .H ₂ O	777.5	d → d	Octahedral

Microbial Screening

The result of the microbial screening as shown in Table 5 shows that the ligand is inactive against all the microorganisms used, while cobalt salts are active against all the used microorganisms with the zone of inhibition diameter ranging between 17 to 26 mm, when compared with that of the complexes, the zone of inhibition is greatly reduced and totally inactive against some of the organisms except nitrogen or chloride as a result of the bulkiness or polymeric nature of the complexes [24].

for $[\text{Co}(\text{IVH})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ which was very active against *Salmonella spp*, *Staph. Aureus*, *P. mirabilis* and *E. coli* with zone of inhibition diameter between 13 mm and 19 mm, as shown in the table. The minimal activity of the complexes against some of the microbes which ranges between 4 to 8 mm and inactiveness of some others could be attributed to the use of or reduction of the percentage

Table 5: Microbial Sensitivity Test for the Ligand and Complexes, Zone of Inhibition (mm)

Compound	<i>Salmonella spp</i>	<i>P. aeruginosa</i>	<i>Staph.aureus</i>	<i>P. mirabilis</i>	<i>E. coli</i>
IVH	-	-	-	-	-
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	18	27	20	20	23
$[\text{Co}(\text{IVH})_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$	13	5	15	12	19
$[\text{Co}(\text{IVH})]\text{Cl}_2 \cdot 3\text{H}_2\text{O}$	4	-	-	8	5
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	22	21	23	18	19
$[\text{Ni}(\text{IVH})_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$	8	6	-	-	12
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	17	10	23	22	15
$[\text{Cu}(\text{IVH})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$	8	-	5	4	6
$\text{Cu}(\text{IVH})\text{Cl}_2 \cdot \text{H}_2\text{O}$	-	-	-	-	-

CONCLUSION

The ligand isovaleric acid hydrazide was successfully synthesized and it formed complexes with the titled metal salts. The ligand acted as a neutral bidentate donor via the carbonyl oxygen and the primary amino nitrogen to metal ions, except for copper in which there was no significant coordination via the carbonyl oxygen. The stoichiometry, physiochemical properties and chelating mode of the ligand would suggest a polymeric

structure. The complexes formed are of octahedral geometry. The anions appear to be in the outer coordination sphere. Minimum activity was shown by the complexes with some microbes while with others are insignificant, as compared with the metal salts except for nickel complex that showed significant activity against *Salmonella spp*, *Staph. Aureus*, *P. mirabilis* and *E. coli*. On the basis of their physiochemical studies, the suggested structure of the complexes is given below.

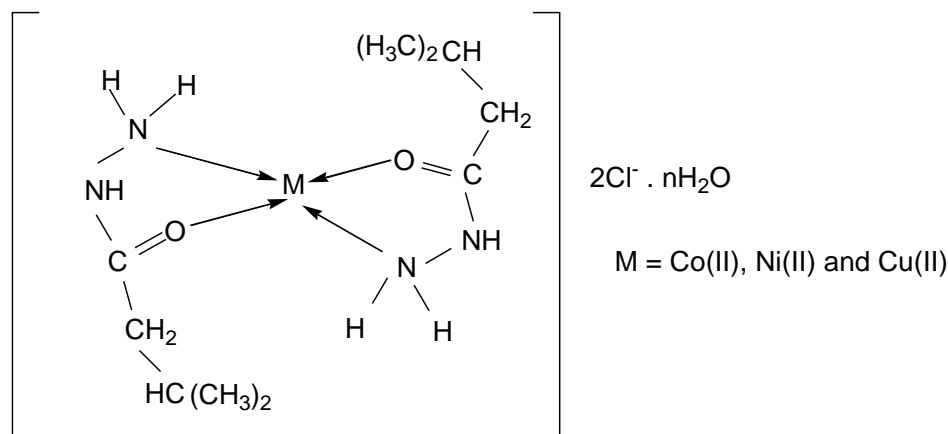


Fig. 1: Suggested structure for M (II) Chloride complexes

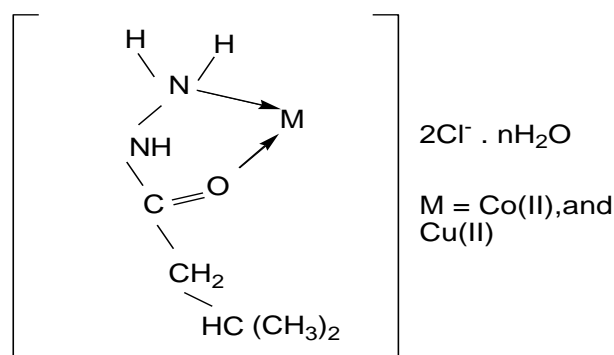


Fig. 2: Suggested structure for other M (II) Chloride complexes (M= Co and Cu)

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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