

**STUDIES ON TRANSITION METAL COMPLEXES OF HERBICIDAL COMPOUNDS.
II: TRANSITION METAL COMPLEXES OF DERIVATIZED 2-CHLORO-4-
ETHYLAMINO-6-ISOPROPYLAMINO-S-TRIAZINE (ATRAZINE)**

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ABSTRACT. 2(2-Hydroxyethyl)hydrazino-4-ethylamino-6-isopropylamino-*s*-triazine (HEATZ), a derivative of 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, atrazine (ATZ) – a well known herbicide has been studied for complex formation with cobalt(II), nickel(II), and copper(II). Analytical, conductance, thermal, and spectral studies suggest that HEATZ is a monobasic NNO donor and it forms binuclear octahedral metal complexes, possessing alkoxide bridging. Cobalt(II) complex exhibits thermochromism. Antimicrobial studies on *E. coli* and *S. aureus* show the absence of growth inhibitory activities for HEATZ and its metal complexes which is attributed to the combined effect of derivatization of ATZ and metal complexation.

KEY WORDS: Metal complexes, Atrazine derivatives, Hydrazinoethanol substitution, Monobasic tridentate NNO donor, Diminished (reduced or minimized) antimicrobial activities

INTRODUCTION

Symmetrical triazines are a class of six-membered heterocyclics containing three azomethine functions which exhibit potential binding characteristics [1-3]. Major research efforts and field tests have established unique herbicidal properties and growth promoting influences due to symmetrical (*-s-*) triazines. Several compounds of this class like atrazine, simazine, prometryn, aziprotryn, etc. have gained worldwide recognition for their outstanding herbicidal properties [4].

However, the wide utilization of these herbicides in the crop management has created a challenging problem in terms of residual herbicides. These are remnant herbicides in the agricultural products which are causing serious symptoms of illness in animal and human consumers. Two approaches are currently validated in the detoxification of remnant herbicides. One of them is derivatization of the herbicides into less harmful compounds after crop development while the other is the metal ion associated degradation or deactivation of the herbicides [5-8]. Studies have shown that metal ions can show remarkable effect in catalyzing the decomposition of herbicides [9-10].

These two approaches are combinedly considered in the present investigation to achieve deactivation of a popular *s*-triazine herbicide, atrazine (ATZ), (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine). Literature survey reveals that polarographic, voltammetric, and chromatographic studies [11-12] on metal complexes of atrazine have been reported. They also indicate that Fe(III) ion can promote photodegradation of atrazine in moderately acidic solution [12].

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Reports also show that derivatization of atrazine by the substitution of chloro group in position-2 by -OH, -SH, -NH₂, -NHNH₂, -CN, -N₃, etc. functions are possible [5-8, 13]. In this report, the derivatization of atrazine to 2-(2-hydroxyethyl) hydrazino-4-ethylamino-6-isopropylamino-*s*-triazine (HEATZ) and synthesis of Co(II), Ni(II), and Cu(II) complexes with this derivative are presented.

The results of preliminary screening of ATZ, HEATZ, the metal complexes and related compounds for antimicrobial properties against *E. coli* and *S. aureus* are also presented.

EXPERIMENTAL

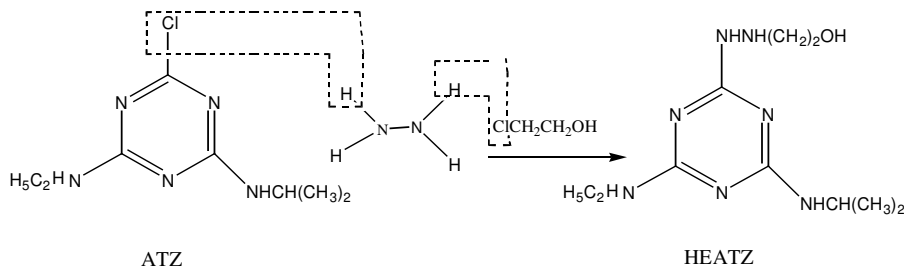
All of the chemicals used were of Analar grade. The solvents such as methanol, ethanol, petroleum ether, chloroform, *iso*-propanol, *n*-butanol, 1,4-dioxane, and 2-chloroethanol were purified by known procedures [14]. A pure sample of atrazine was obtained from Promochem (Wesel, Germany). Hydrazine hydrate (100%) from BDH Chemicals, and Analar grade metal chlorides of Co(II), Ni(II), and Cu(II) from Aldrich Chemicals were used.

Vibrational (IR) spectra were recorded on a Pye Unicam SP 2000 spectrophotometer in the range 200–4000 cm⁻¹ with a KBr disc as reference material. Electronic spectra of the complex solution in methanol were measured in the range 200–900 nm at room temperature with a Beckman DU-65 spectrophotometer. Conductance measurements of the complexes were made in freshly prepared aqueous solutions (10⁻³ M) using a Philip Harris conductivity meter. Melting points (decomposition temperatures) of the ligands and the metal complexes were determined on a Bock-Monoscop apparatus and are uncorrected. The thermochromism (colour changes with temperature) of the metal complexes was also monitored on the Bock-Monoscop apparatus. The purity of the ligand and the complexes was tested by thin layer chromatography (TLC). A Philips PW 9409 digital pH/mV meter was used for the measurements of pH.

The metal complexes were analyzed for metal and chlorine. Cobalt(II) was estimated complexometrically using EDTA. Nickel(II) and copper(II) were estimated gravimetrically using dimethylglyoxime as Ni(DMG)₂ and Cu(benzoin- α -oxime), respectively. Chloride contents were analyzed as AgCl [15].

Synthesis of hydrazinoethanol derivative of atrazine (HEATZ)

In this synthesis 2-chloroethanol serves the purpose of a solvent and also of a reagent. Formation of hydrazinoethanol from hydrazine and chloroethanol is a known reaction, which in turn replaces chlorine from atrazine (Scheme 1) [3].



Scheme 1. Synthesis of HEATZ.

To 0.5 g (2.32 mmol) of atrazine, 2-chloroethanol (10 mL) and hydrazine hydrate (0.14 mL, 2.32 mmol) were added and the mixture was refluxed on steam bath with continuous stirring for eight hours. The off white solid separated during this time was filtered and washed with 2-chloroethanol. The product was dried under vacuo. It was recrystallized from methanol. Yield: 77.6%. Melting point/decomposition temperature: 160-162 °C.

Synthesis of metal complexes

The following general method was employed for the synthesis of metal complexes. To the methanolic solution (10 mL) of divalent metal chloride (0.19 mmol), 46.6 mg, cobalt(II); 46.4 mg, nickel(II); 33.4 mg, copper(II); 0.1 g (0.39 mmol) of the methanolic suspension (10 mL) of HEATZ was added in small portion to obtain a clear solution. The pH of the reaction mixture was adjusted to 6 by adding 5% (v/v) methanolic ammonia solution and then the mixture was refluxed for 6 h. Finally the reaction mixture was concentrated. The coloured product was filtered in hot condition and was washed successively with small amounts of methanol and petroleum ether. It was dried in vacuum. The purity of each complex was tested by TLC using different solvents. The yields of the compounds are given in Table 1.

Table 1. Analytical and conductance data of the complexes.

Complex with formula	Colour	M.p./decomp. temperature (°C)	Yield (%)	Calculated (found) (%)		Λ_m ($\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$)
				Chlorine	Metal	
Co(II) complex [Co(II)(HEATZ)(H ₂ O) ₂]Cl	Pink	Changes its colour from pink to blue at 200 and to green at 220.	36.7	(9.20) 9.21	(15.22) 15.28	71
Ni(II) complex [Ni(II)(HEATZ)(H ₂ O) ₂]Cl	Light green	210 - 212	42.3	(9.18) 9.21	(15.27) 15.23	87
Cu(II) complex [Cu(II)(HEATZ)(H ₂ O) ₂]Cl	Green	199 - 202	39.3	(9.60) 9.10	(16.74) 16.78	110

Anti microbial studies

This was done by the disc-diffusion method in a nutrient agar medium against the three bacteria (*Escherichia coli* and *Staphylococcus aureus*).

The bacteria were incubated on nutrient agar in separate test tubes for 48 h. The exponentially growing cultures (0.5 mL) of each bacterium were then diluted further with sterile nutrient broth. These were seeded on nutrient agar/broth media by the top and bottom technique.

The metal complexes and controls were dissolved in distilled water to make 25 mg/mL concentration. Two different concentrations of each sample were studied. 10 μL (250 mg) and 20 μL (500 mg) of each sample, controls and solvent were absorbed on paper discs (antibiotic - assay disc, diam. 0.25 inch) using eppendorf. The soaked paper disks were then placed on the inoculated agar plates at relatively regular intervals and incubated in the inverted fashion at 37 °C for 24 h. Any inhibition zones were looked for and their diameters measured using a ruler.

RESULTS AND DISCUSSION

General characteristics of the compounds

HEATZ is soluble in water and most of the common polar solvents. Solubility in water is a characteristic feature. This is probably due to the hydrogen bonding caused by ethanol function. Increasing solubility associated with increasing electron-donating capacity of the substituents at C-2 (Figure 1) has been observed in some earlier studies [3]. The derivative gives qualitative tests for alcoholic function. Co(II), Ni(II), and Cu(II) complexes are brightly coloured crystalline compounds and are stable to atmospheric condition. They do not melt but decompose. The decomposition temperatures are distinctly different from that of the free ligand. The complexes dissolve in water and most of the common polar organic solvents.

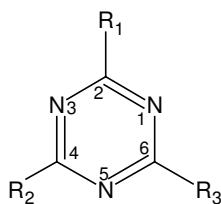


Figure 1. Structure of *s*-triazine. R₁ – Cl; R₂ – NHC₂H₅; and R₃ – NHCH(CH₃)₂ (atrazine).

Elemental analysis of the complexes

The analytical data of all the complexes is given in Table 1. Metal and chloride analyses show M:L:Cl ratio as 1:1:1 in all the complexes. Analyses also suggest the presence of two molecules of water per metal ion. Thus, the general composition of the complexes is given as ML(H₂O)₂Cl, where M = Co(II), Ni(II), and Cu(II).

Conductivity study

Conductance recorded in 10⁻³ M freshly prepared aqueous solutions (Table 1) shows that all the complexes are 1:1 electrolytes and the chloride ion is present in the ionization sphere. The data also suggests that the ligand could be present as mononegative ion in these complexes, probably due to the deprotonation of alcoholic group during complex formation. Hence, the complexes may be formulated as [ML(H₂O)₂]Cl.

IR spectral study of HEATZ complexes

The IR spectrum of the ligand shows a very broad and strong band in the region 3600–2500 cm⁻¹ assignable to four types of ν_(NH) vibrations and one ν_(OH). All these functions are likely to involve inter – as well as intramolecular hydrogen bonding interactions, due to which the broad band has resulted. Another multiple bands in the region 1650–1590 cm⁻¹ are due to ν_(C=N) also with δ_(OH, NH) vibrations. Other characteristics are the strong band at 1100 cm⁻¹ and medium intensity band at 1000 cm⁻¹ assignable to ν_(C-O) (alcoholic) and ν_(N-N) of hydrazine moiety [16, 17]. The spectra of metal complexes show better resolutions, probably due to diminished hydrogen bonding and stereochemical changes accompanying metal-ligand interaction. Characteristics IR frequencies of the complexes are given in Table 2.

Table 2. Characteristic infrared frequencies (cm^{-1}) of the ligand and complexes.

Compound	$\nu_{\text{(NH)}} + \nu_{\text{(OH)}}$	$\nu_{\text{(C=N)}}$	$\nu_{\text{(C-O)}}$	$\nu_{\text{(N-N)}}$	New bands
Ligand	3600–2500	1650–1590	1100	1000	---
Co(II) complex	3500–3300	1620–1610	1140–1145	1010–1015	875, 920, 525, 485
Ni(II) complex	3500–2900	1620	1140	1025	900, 970, 550, 490
Cu(II) complex	3500–3000	1610	1110–1140	1020	950, 920, 555, 495

Notable features in the spectra of complexes in comparison with the free ligand spectrum are:

(i) Modification of the broad band ($3600\text{--}2500\text{ cm}^{-1}$) into a relatively narrow band with components in the region $3500\text{--}2900\text{ cm}^{-1}$. The absence of band structure in the region $2500\text{--}2900\text{ cm}^{-1}$ can be attributed to deprotonation of alcoholic group and its subsequent involvement in metal bonding. This derives further support from the absence of $\delta_{\text{(OH)}}$ and shifting $\nu_{\text{(C-O)}}$ alcoholic bands.

(ii) Appearance of a relatively sharp band in the region $1610\text{--}1620\text{ cm}^{-1}$ in place of $1590\text{--}1650\text{ cm}^{-1}$ band of the free ligand. If the low frequency component is assumed to be due to $\delta_{\text{(OH)}}$, its absence in the spectra of complexes further supports the deprotonation. Downward shift of band from $1650\text{--}1620\text{--}1610\text{ cm}^{-1}$ can be attributed to the involvement of one ring nitrogen in coordination. N1 or N3 may be involved in this process (Figure 1) [18–20].

(iii) Positive shifts in $\nu_{\text{(C-O)}}$ and $\nu_{\text{(N-H)}}$ are strong indications of the participation of the deprotonated alcoholic oxygen and hydrazino nitrogen in metal binding process. Particularly the shift in $\nu_{\text{(C-O)}}$ ($\Delta\nu_{\text{(C-O)}} = 40\text{--}45\text{ cm}^{-1}$) is very large, which distinctly points towards bridging interaction of the alkoxide oxygen [16, 17].

(iv) Appearance of new/non-ligand bands in the spectra of complexes assignable to rocking and wagging modes of coordinated water and M-N and M-O vibrations confirm the interaction between metal, ligand, and water molecules [21]. From IR data, it can be concluded that the ligand HEATZ behaves as a tridentate towards Co(II), Ni(II), and Cu(II), involving a ONN donor system in bonding process with characteristic alkoxide bridging. Consequently, the complexes could be formulated as binuclear compounds with the general formula $[\text{M}_2\text{L}_2(\text{H}_2\text{O})_4]\text{Cl}_2$.

Electronic spectra

HEATZ is an off white coloured compound with no absorptions in the visible region. The spectra of metal complexes show characteristic bands in visible/near IR region which are assignable to d-d transitions [22, 23]. Based on the data presented in Table 3, along with assignment of transitions, octahedral geometry has been assigned to all the complexes. Of particular interest is Co(II) complex, which shows temperature dependent colour changes. The complex is pink in colour at room temperature and it changes to green through blue colour on heating to $T > 210\text{--}220\text{ }^\circ\text{C}$. These colour changes are reversible on cooling. This provides an interesting example of thermochromism.

When the complex is heated to a constant temperature of $220\text{ }^\circ\text{C}$, the green product obtained shows weight loss equivalent to four water molecules per formula weight of $[\text{Co}_2\text{L}_2(\text{H}_2\text{O})_4]\text{Cl}_2$. It is suggested that the pink octahedral complex of the composition $[\text{Co}_2\text{L}_2(\text{H}_2\text{O})_4]\text{Cl}_2$ may lose water molecules in a two step process. Scheme 2 is proposed for the deaquaation steps occurring on heating.

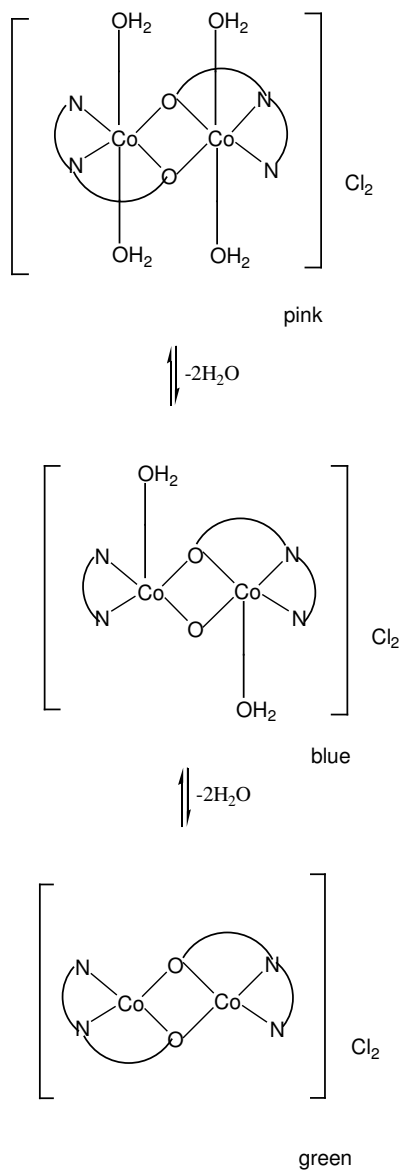
Scheme 2. Deaquation of thermochromic $[\text{CoL}_2(\text{H}_2\text{O})_4]\text{Cl}_2$.

Table 3. Electronic spectral data of the complexes.

Compound	Band position (ν_{\max} cm^{-1})	Assignment of transitions
Co(II) complex	19,092–20,000	$^4T_1(F) \rightarrow ^4T_1(P)$
Ni(II) complex	11625	$^3A_2 \rightarrow ^3T_2$
	14124	$^3A_2 \rightarrow ^3T_1(F)$
	21739	$^3A_2 \rightarrow ^3T_1(P)$
Cu(II) complex	11780	$^3B_1 \rightarrow ^2A_2$
	12468	$^2B_1 \rightarrow ^2B_2$
	13158	$^2B_1 \rightarrow ^2E$

Antimicrobial studies

HEATZ, metal complexes of HEATZ and related compounds have been screened for antimicrobial properties against *Escherichia coli* (a gram negative bacterium) and *Staphylococcus aureus* (a gram positive bacterium), following agar diffusion method. The preliminary screening has been carried out in view of the existing reports of similar studies on *s*-triazine herbicides like prometryn, simazine, and atrazine against microbes like *E. coli* and *Bacillus subtilis* [24].

When physiologically active compounds are bound to metal ions, their properties may be enhanced or reduced depending on the binding sites, the electronic distribution and availability or non-availability of active functional groups which are responsible for the activity.

The observations show that the ligand, HEATZ, and the metal complexes under investigation do not exhibit any growth inhibitory activity against the two microbes, i.e., *E. coli* and *S. aureus*. Thus these studies prove to be good verification of the two proposed approaches leading to the detoxification of herbicides, viz. derivatization and metal complexation. Derivatization of ATZ to HEATZ and metal complex formation with Co(II), Ni(II), and Cu(II) may then be suggested as models to detoxify atrazine. Further studies on several herbicides may lead to accumulation of data which forms a good basis for working out a solution in the direction of residual herbicide toxicity. However, the utility of the models depends on the ease of their application on the crops, as and when required.

CONCLUSION

Based on analytical, conductance, thermal, and spectral studies it is concluded that HEATZ behaves as tridentate NNO donor employing ring nitrogen, hydrazino nitrogen and alkoxide oxygen (bridging) in bonding. Octahedral geometries have been proposed for the complexes (Figure 2). Thermochromism is an interesting feature observed with the Co(II) complex.

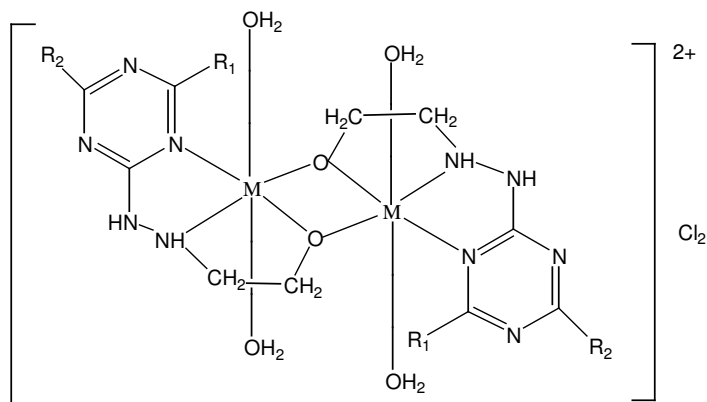


Figure 2. Metal complexes of HEATZ. $R_1 = \text{NHC}_2\text{H}_5$, $R_2 = \text{NHCH}(\text{CH}_3)_2$ and $M = \text{Co}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$.

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