



SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF COPPER(II) CHELATES OF 3-NITRO-1,5-DIARYLFORMAZANS

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ABSTRACT

Copper(II) chelates of 3-nitro-1,5-diarylformazans have been synthesized by reacting aqueous solution of copper(II) acetate tetrahydrate with methanolic solution of the ligand. The so synthesized copper(II) chelates of 3-nitro-1,5-diarylformazans were purified, crystallized and characterized on the basis of elemental analysis, IR, Electronic and EPR spectral studies, and magnetic susceptibility measurements. The studies reveal that these copper(II) chelates of 3-nitro-1,5-diarylformazans characterized as bis-chelates having 1:2 stoichiometry, are paramagnetic in character and most probably have a symmetrical six membered chelate ring structure. The anti-bacterial activity of these bis(3-nitro-1,5-diarylformazanato)copper(II) chelates has been evaluated against *Escherichia coli* and *Bacillus subtilis* by disc diffusion method and compared with standard Amoxycillin and they are found to possess potent anti-bacterial activity against selected bacterial strains.

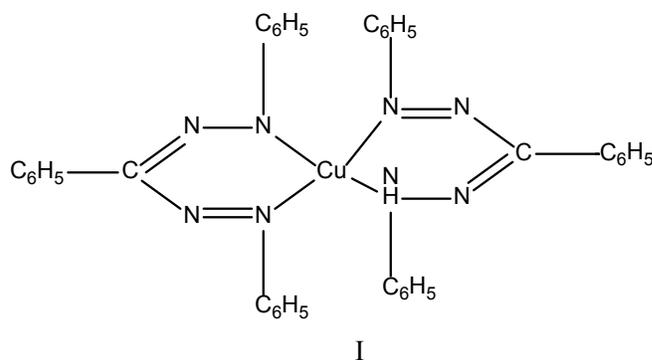
Key Words: 3-nitro-1,5-diarylformazan, Copper(II), Chelates, Anti-microbial activity

INTRODUCTION

3-nitro-1,5-diarylformazan ligands react with copper(II) acetate tetrahydrate in 2:1 molar ratio in methanol to yield bis(3-nitro-1,5-diarylformazan) copper(II) chelates. These compounds have been characterised by elemental analysis, IR, EPR, electronic spectral data and magnetic measurements. The studies suggest that the copper(II) chelates are paramagnetic in character and probably contain planar symmetric six membered chelate rings. The copper(II) chelates of formazans are generally prepared by reacting copper(II) acetate[1-8] or copper(II) chloride[9] with hot alcoholic solution of formazan[2-9]. There are reports in literature that certain 3-acetyl-1,5-diarylformazans[10], 3-benzyl-1,5-diarylformazans[11] and 3-ethoxycarbonyl-1,5-diarylformazans[10] react with copper(II) acetate in ethanol to give copper(II) formazan complex having 1:1 stoichiometry.

The copper(I) complex of 1,3,5-triphenylformazan has structure I, and thus the first stage in this reaction is the oxidation of formazan to tetrazolium salt by cupric ion. Subsequently, 1,3,5-triphenylformazan reacts with cuprous ion to give a

copper(I) complex I, having 2:1 stoichiometry with a constitution comprising one molecule of 1,3,5-triphenylformazan and one molecule of deprotonated 1,3,5-triphenylformazan, i.e. a formazylianion and one copper(I) ion.



The behavior of 1,5-diarylformazans having acetyl-, benzyl- or an ethoxycarbonyl group in the 3-position, with cupric ions contrasts with that of 1,3,5-triphenylformazans that the former are reported to give copper(II) complex II, having a 2:1 stoichiometry under comparable conditions.

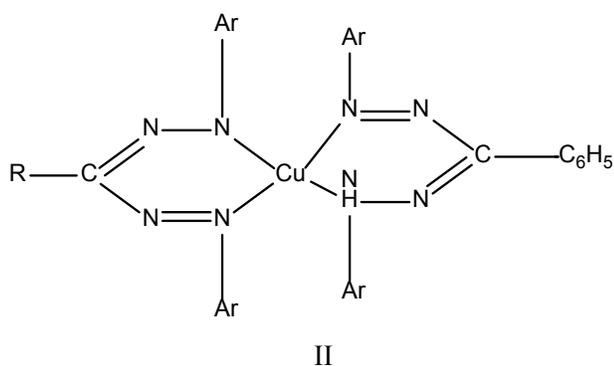
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It is a well known fact that very often the reaction between copper(I) ions and 3-arylformazan results in the formation of a mixture of copper(II) and copper(I) formazan chelates, it was thought worthwhile to investigate whether 3-nitro-1,5-diarylformazans also behave in a similar manner or not. It was also aimed to identify the products of the reaction of copper(II) with 3-nitro-1,5-diarylformazans on the basis of elemental analysis, IR, EPR, electronic spectral data and magnetic measurements. Gilroy (2008)[12] prepared complexes of (Fe^{3+} , Co^{3+} , Ni^{2+} and Pd^{2+}) metals and used 3-substituted diphenyl formazans (3-cyano and 3-nitro formazan) as ligands. Nitika et al [13] prepared 3-nitroformazans by reacting diazotized aromatic amine with nitromethane in cold in an alkaline medium and reported them to be intramolecularly hydrogen bonded having strongly chelated and a symmetrical structure (III). Balt et al[14] reported kinetic study of complex formation between copper(II) and 1-(2-hydroxyphenyl)-3,5-diphenylformazan in an ammoniacal ethanol-water mixture has brought evidence for a step-wise coordination of the tridentate ligand to the metal ion.

Search for new metallic species with enhanced biological applications is ongoing. Among the metal ions, copper, nickel, cobalt and zinc complexes have proved to be excellent candidates. Copper complexes have shown remarkable efficiency in antioxidant[15], DNA-binding and anti-cancer studies[16-17].

In the present work an attempt has been made to synthesize copper(II) chelates of 3-nitro-1,5-diarylformazans. The so obtained chelates were characterized on the basis of elemental analysis, IR, electronic and EPR spectral studies and magnetic measurement studies. The anti-microbial activities of these copper(II) chelates have been evaluated by studying the zone of inhibition against test organisms *E. coli* and *B. subtilis* by Agar Well Diffusion method and compared with standard Amoxycillin.

MATERIAL AND METHODS

Synthesis of copper(II) complexes of 3-nitro-1,5-diarylformazans:

The method of preparation of one of the representative copper(II) complex of 3-nitro-1,5-diarylformazans is given below:

Copper(II)acetate tetrahydrate (200 mg) was dissolved in minimum quantity of distilled water. Solution of the ligand, 3-nitro-1,5-di-p-bromo-phenylformazan(700 mg in 50 ml of methanol) was added to this. The mixture was kept undisturbed for 3-4 hours and then refluxed on water bath for 2 hours. After that the solution was concentrated and cooled by adding a few ml of distilled water. The reddish brown precipitates formed were filtered, washed with distilled water and dried. The crude complex was then column chromatographed using benzene as eluent. The brown fraction which eluted out first was collected, concentrated and re-crystallized from chloroform-hexane. The gummy impurities remained adhered to the column and could not be moved by using methanol as eluent. It was found difficult to crystallize these gummy products but these showed a negative test for copper.

Nitrogen in these copper(II)chelates was estimated by Kjeldahl's method[18] and copper was estimated iodometrically[19].The results are given in Table 1.

The IR spectra of these copper(II) chelates were recorded and the results are as given in Table2. EPR spectra of powdered samples of the complexes were recorded on JES-FE 3XG EPR spectrometer operating at X-band frequency (9.44 GHz), at room temperature. The magnetic susceptibility measurements for the bis(3-nitro-1,5-diarylformazanato)copper(II) complexes were made on finely powdered samples using magnetic susceptibility equipment based on Evans method and manufactured by Johnson Matthey Catalytic systems division standard procedure. μ_{eff} values calculated are reported in Table 2.

EVALUATION OF ANTI-MICROBIAL ACTIVITY OF COPPER(II)CHELATES

Anti-microbial activity was studied by disc diffusion method against *Escherichia coli* and *Bacillus subtilis*, and was compared with the standard Amoxycillin.

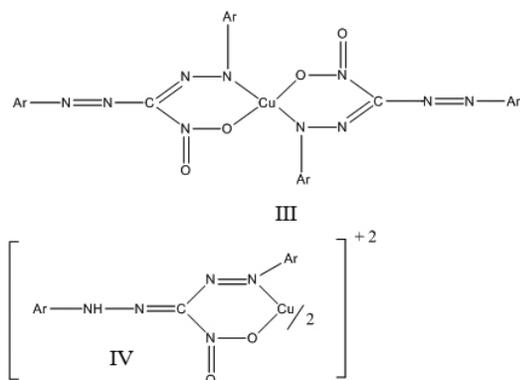
RESULTS AND DISCUSSION

Elemental Analysis and Spectral Studies

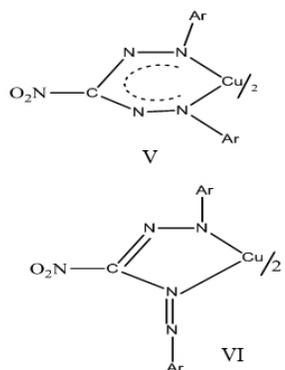
The elemental analysis of copper(II) chelates of 3-nitroformazans shows that they have 1:2 stoichiometry. The lack of NH absorption band ($2900\text{-}3200\text{ cm}^{-1}$) in their IR spectra as compared to those of ligands reveal that these complexes are formed by the replacement of imine hydrogen atom of formazan ring by copper(II). The bis(3-nitro-1,5-diarylformazan)copper(II) complexes are neutral and are soluble in organic solvents. Therefore, they are not Copper(I) chelates of 3-nitro-1,5-diarylformazans. Thus, it appears that in case of 3-nitro-1,5-diarylformazans only copper(II) chelates

and not a mixture of copper(II) and copper(I) chelates are formed.

Having established the composition of reaction between copper(II) and 3-nitro-1,5-diarylformazans, effort was made to establish the structure of these chelates. The $\nu\text{NO}_2(\text{asym})$ and $\nu\text{NO}_2(\text{sym})$ appear approximately in the same range in the IR of these copper(II) chelates as compared to $\nu\text{NO}_2(\text{asym})$ and $\nu\text{NO}_2(\text{sym})$ of the corresponding ligands. Had the -NO_2 group been involved in coordination with copper(II), it would lead to the linkage of the type, III or IV.



In which event, the $\nu\text{NO}_2(\text{asym})$ and $\nu\text{NO}_2(\text{sym})$ which appear in the IR of the ligands in the range $1570\text{-}1520\text{ cm}^{-1}$ and $1290\text{-}1250\text{ cm}^{-1}$ respectively would disappear and two new absorption bands in the range $1390\text{-}1360\text{ cm}^{-1}$ and $1110\text{-}1050\text{ cm}^{-1}$ characteristic of metal-nitrito type of linkage would have appeared, as very strong bands. No such change is observed in the IR of bis(3-nitro-1,5-diarylformazanato)copper(II) chelates. There is only a slight decrease in $\nu\text{N}=\text{N}(\text{sym})$, $\nu\text{N}=\text{N}-\text{C}-\text{N}(\text{sym})$ and $\nu\text{N}-\text{N}=\text{Ar}$ of the free ligands as compared to the complexes as expected because the coordination of copper(II) with formazyl group is bound to decrease these frequencies slightly. On the basis of above IR data, the following two structures V and VI are likely.



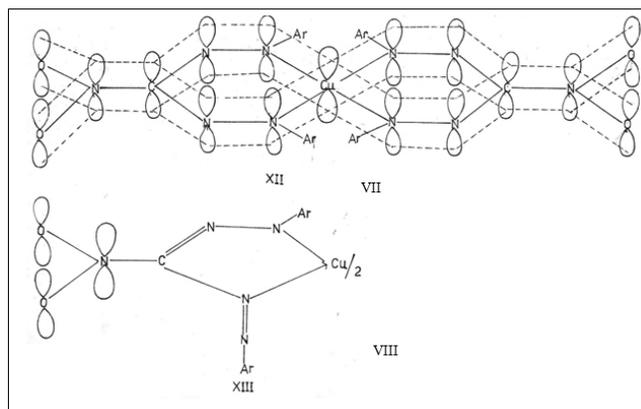
The electronic spectra of copper(II) chelates are similar to the electronic spectra of corresponding ligands, except for a low energy band between $550\text{-}600\text{ nm}$. The $360\text{-}380\text{ nm}$ broad band and 460 nm of ligands slightly shifts in position and intensity in their copper(II) complexes. Therefore, these

two bands in copper(II) complexes are mainly ligand based bands. But another band of lower energy and of comparatively low intensity could be of ligand field origin if the geometry of the chelate is assumed to be tetrahedral. The PMR of all the bis(3-nitro-1,5-diarylformazanato)copper(II) chelates, recorded in CDCl_3 were broad and highly contact shifted, thereby indicating the absence of a strong copper-copper interaction. This is also supported by magnetic studies on these chelates. The magnetic moments of bis(3-nitro-1,5-diarylformazanato)copper(II) were calculated by Evans method and reported in Table 3.

The magnetic moments of the other bis(3-nitro-1,5-diarylformazanato)copper(II) chelates, calculated by the same procedure are given in Table 3.

From the magnetic moment data it is difficult to say anything about the geometry and the size of the ring. The magnetic moment of these bis(3-nitro-1,5-diarylformazanato)copper(II) chelates was found to have μ_{eff} (1.8-2.20 BM) which is normal for copper(II) complexes containing one unpaired electron. Thus, the magnetic studies again confirm that the complexes under study are not copper(I) but definitely copper(II) chelates. The magnetic susceptibility of the copper(II) complexes does not permit a distinction between (i) a square planar configuration [20-21] as in bis(acetylacetonato)copper(II) (μ_{eff} 1.90 BM) [22] in bis(salicylaldehydato)copper(II) diagram (μ_{eff} 1.90 BM) [23], (ii) bridged structure [24] as in bis(8-hydroxyquinolato)Copper(II) (μ_{eff} 1.89 BM) [25] and (iii) a tetrahedral one as in $[\text{Ph}_3\text{MeAs}]_2[\text{CuBr}_4]$ (μ_{eff} 1.96 BM) [26] (iv) distorted tetrahedral as in (2-2-bis(salicylaldehyde-imine)6,6'-dimethyldiphenyl)Copper(II) (μ_{eff} 1.95 BM) [27]. All these geometries have μ_{eff} in the range 1.8-2.20 BM. The two factors which are likely to determine the size of formazan ring could be:

- Steric hindrance of the Ar groups of formazan ring around Copper(II).
- π -bonding capacity of πP_z orbital of the metal with the πP orbitals of the atoms of formazan ring which will highly delocalise the π -electrons and stabilize the system.



It is obvious from above structures VII and VIII that the stabilisation through π -electron delocalisation will be more predominant in a planar symmetric six membered ring structure, VII as compared to unsymmetric five membered ring structure VIII. This extensive π -electron delocalization in a symmetric six membered structure VII tends to favour aplanar configuration as in case of bis(1,3,5-triphenylformazan)Copper(II), bis(1,3-diphenyl-5-p-tolylformazan)Copper(II), bis(1,3-diphenyl-5-p-chlorophenylformazan)Copper(II) and bis-(1,3-diphenyl-5-o-carboxyphenylformazan)Copper(II)[28]. Also, the ring strain in five membered structure VIII, is more than in the six membered structure VII. This fact also favours a six membered structure VII in these bis(3-nitro-1,5-diarylformazan)Copper(II).

Consideration of both of these factors as mentioned above led Ermakova et al[29] to believe that bis(formazan)Copper(II) complexes have a planar symmetric sixmembered structure II, (where $Ar^1=p$ -tolyl, $R=Ar=C_6H_5$; $Ar^2=p$ -chlorophenyl, $R=Ar=C_6H_5$; $Ar^3=o$ -carboxyphenyl, $R=Ar=C_6H_5$). This was later on confirmed by X-ray studies[30].

Similar very extensive π -electron delocalisation exists in case of bis(3-nitro-1, 5-diarylformazan)Copper(II). The μ_{eff} in case of bis(3-nitro-1,5-diarylformazan)Copper(II) complexes also fall in the range 1.8-2.23 BM as in case of bis(3-arylformazan)Copper(II) complexes (TABLE 3). Hathaway[28] has reported that in practice moments of magnetically dilute compounds of Copper(II) are in the range 1.8-2.23 BM with compounds whose geometry approaches octahedral having moment at the lower end and those with geometries approaching tetrahedral having moments at the higher end, but their measurements cannot be used diagnostically with safety unless supported by other evidences. The ' g_{av} ' values calculated for bis(3-nitro-1,5-diarylformazanato)Copper(II) complexes reported in Table 2 are consistent with the planar structure of these Copper(II) complexes. Therefore, a planar six membered symmetric ring structure V is indicated to be the structure of bis(3-nitro-1,5-diarylformazanato)copper(II) complexes. However, this is merely a conjecture, unless X-ray studies are carried out.

Results of study of antimicrobial activity of copper(II) chelates of 3-nitro-1,5-diarylformazans

Different compounds were studied for zone of inhibition against the test organism *E.coli* and *Bacillus subtilis* using Well diffusion method. The maximum activity was observed at 100% concentration of different compounds and standard Amoxycillin. The zone of inhibition of compounds is Table 4.

The study reveals that bis(3-nitro-1,5-diarylformazan) copper(II) chelates possess promising anti-microbial activity against *E. coli* and *B. subtilis*.

CONCLUSIONS

From the above results it is concluded that

An attempt has been made to synthesize bis(3-nitro-1,5-diarylformazanato)copper(II) complexes by reacting methanolic solution of 3-nitro-1,5-diarylformazan with aqueous solution of copper(II)acetate tetrahydrate. These chelates were found to have stoichiometry 1:2 and paramagnetic in character. These bis(3-nitro-1,5-diarylformazanato)copper(II) chelates have μ_{eff} between 1.8-2.23 BM. All the bis(3-nitro-1,5-diarylformazanato)copper(II) complexes reported here probably have a planar six membered symmetric ring structure, as supported by their μ_{eff} and ' g_{av} ' values. The antimicrobial activity of these copper chelates was evaluated against *E. coli* and *Bacillus subtilis* by Agar well diffusion method. The observations show that the zones of inhibition for bis-(3-nitro-1,5-di-p-chlorophenylformazanato)copper(II) against *E. coli* and *Bacillus subtilis* are good and are quite comparable to standard Amoxycillin.

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Table 1: Characterization data of some bis(3-nitro-1,5-diarylformazanato)copper(II)

S. No.	Compound	Molecular Formula	Melting point* (°C)	Nitrogen found (Calculated) (%)	Copper found (Calculated)
1	Bis(3-nitro-1,5-diphenylformazanato) Cu(II)	C ₂₆ H ₂₀ O ₄ N ₁₀ Cu	130	23.01(23.35)	10.83(10.59)
2	Bis(3-nitro-1,5-di-p-tolylformazanato) Cu(II)	C ₂₆ H ₂₈ O ₄ N ₁₀ Cu	120	21.12(21.35)	9.87(9.68)
3	Bis(3-nitro-1,5-di-p-anisylformazanato) Cu(II)	C ₃₀ H ₂₈ O ₈ N ₁₀ Cu	A	19.57(19.45)	8.97(8.82)
4	Bis(3-nitro-1,5-di-p-nitrophenylformazanato) Cu(II)	C ₂₆ H ₂₀ O ₁₂ N ₁₄ Cu	A	24.87(25.14)	8.32(8.14)
5	Bis(3-nitro-1,5-di-p-bromophenylformazanato) Cu(II)	C ₂₆ H ₁₆ O ₄ N ₁₀ Br ₂ Cu	180	14.97(15.29)	7.27(6.93)
6	Bis(3-nitro-1,5-di-p-chlorophenylformazanato) Cu(II)	C ₂₆ H ₁₆ O ₄ N ₁₀ Cl ₂ Cu	242	18.63(18.98)	8.47(8.61)

* All the melting points are uncorrected.

a - The compound does not melt upto 250°C.

Table 2: Electronic spectral data of some bis(3-nitro-1,5-diarylformazanato)copper(II)

S. No.	Compound	Molecular Formula
1	bis(3-nitro-1,5-diphenylformazanato)copper(II)	540 nm (1,900), 430nm Sh (6,600), 380 nm (9,000)
2	bis(3-nitro-1,5-di-p-tolylformazanato)copper(II)	550 nm (1,300), 410nm(4,078), 360 nm (6,250)
3	bis(3-nitro-1,5-di-p-anisylformazanato)copper(II)	540 nm Sh (25,400), 460nm (4,000), 370 nm (3,1200)
4	bis(3-nitro-1,5-di-p-nitrophenylformazanato)copper(II)	550 nm (625), 420nm (17,200), 380 nm (11,000)
5	bis(3-nitro-1,5-di-p-bromophenylformazanato)copper(II)	550 nm (1,400), 400nm Sh (3,700), 360 nm (5,700)
6	bis(3-nitro-1,5-di-p-chlorophenylformazanatos)copper(II)	270 nm (16,600), 460nm Sh (15,400), 600 nm (4,700)

*Electronic spectra were recorded in Benzene.

Table 3: Some important IR frequencies (cm⁻¹), μ_{eff} and g_{av} of bis(3-nitro-1,5-diarylformazanato)copper(II)

S. No.	Compound	ν_{NO_2} (asym)	$\nu_{\text{N}=\text{C}-\text{N}}$ (asym)	$\nu_{\text{N}=\text{N}}$ (asym)	$\nu_{\text{N}=\text{C}-\text{N}}$ (sym)	ν_{NO_2} (sym)	$\nu_{\text{N}-\text{N}-\text{Ar}}$	$\psi-\text{N}$	$\nu_{\text{C}-\text{N}}$ of $\text{C}-\text{NO}_2$ (asym)	μ_{eff} (B.M.)	g_{av}
1	Bis(3-nitro-1,5-diphenylformazanato) copper(II)	1570	a	1405	1340	1280	1200	1070	850	1.93	2.08
2	Bis(3-nitro-1,5-di-p-tolylformazanato) copper(II)	1570	14909	1405	1320	1275	1150	1100	824	1.95	2.10
3	Bis(3-nitro-1,5-di-p-bromophenylformazanato)copper(II)	1570	1480	1405	1380	1280	1180	1060	820	1.94	2.13
4	Bis(3-nitro-1,5-di-p-chlorophenylformazanato)copper(II)	1560	a	1400	1333	1278	1180	1071	858	2.18	2.10
5	Bis(3-nitro-1,5-di-p-chlorophenylformazanato)copper(II)	1510	a	1405	1370	1290	1250	1050	825	2.13	2.10

*All the IR spectra were recorded in Nujol Mull.

a - Hidden under the broad and intense Nujol peak.

Table 4: Results of Antibacterial Studies

Concentration of Sample	Test Organism	Zone of Inhibition (in mm)	
		Bis-(3-nitro-1, 5-di-p-chlorophenyl formazanato) copper(II)	Bis-(3-nitro-1, 5-di-m-chlorophenyl formazanato) copper(II)
25%	<i>E. Coli</i>	10	10
	<i>Bacillus subtilis</i>	10	11
50%	<i>E. Coli</i>	13	14
	<i>Bacillus subtilis</i>	13	14
75%	<i>E. Coli</i>	15	16
	<i>Bacillus subtilis</i>	14	15
100%	<i>E. Coli</i>	16	18
	<i>Bacillus subtilis</i>	15	17
Standard (Amoxicillin)	<i>E. Coli</i>	19	19
	<i>Bacillus subtilis</i>	20	20

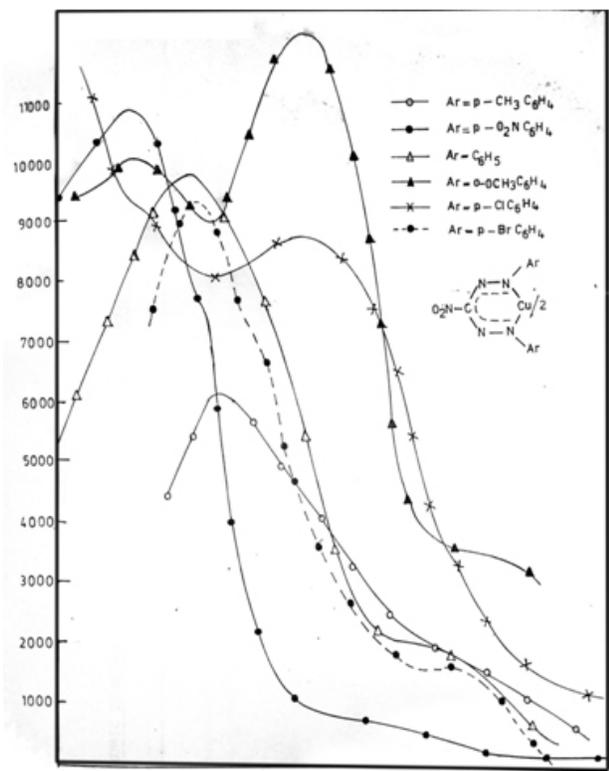


Figure 1: Electronic Spectra of bis(3-nitro-1,5-diaryl-formazanato)copper(II).

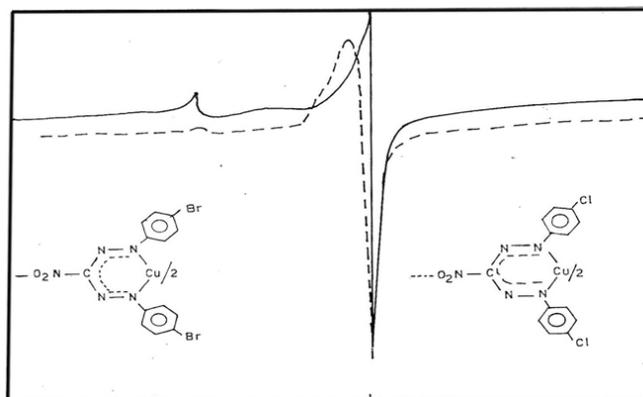
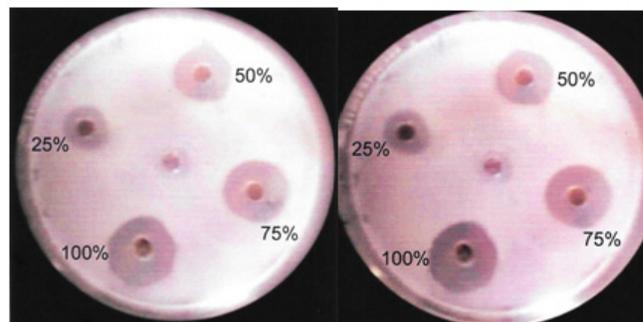


Figure 2: EPR Spectrum of bis(3-nitro-1,5-diaryl-formazanato)copper(II).



E.coli

B. subtilis

Figure 3: Zone of Inhibition of bis(3-nitro-1,5-di-p-chlorophenyl-formazanato)copper(II).

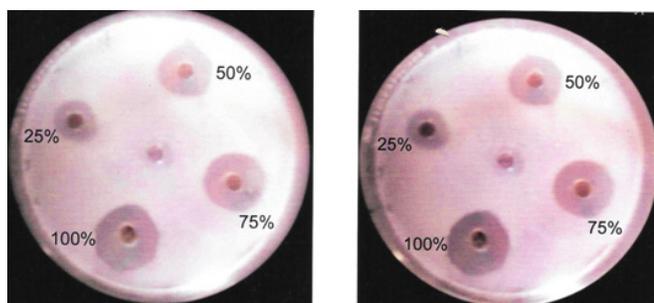
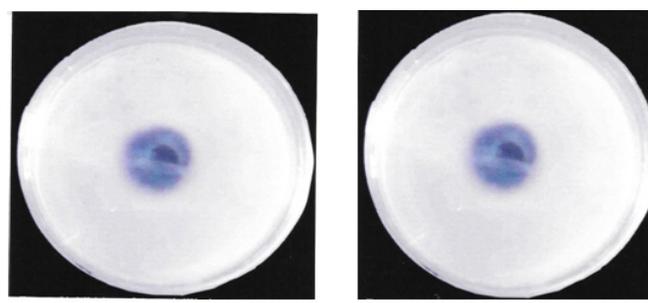


Figure 4: Zone of Inhibition of bis(3-nitro-1,5-di-m-chlorophenylformazanato)copper(II).



E. coli

B. subtilis

Figure 5: Zone of Inhibition of Amoxicillin.