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Original Research Article

Analytical chemistry for better drug analysis

Synthesis Of Barbiturate-CU (II) Complexes Using Reflux Method And Evaluation Of Their Antibacterial Activity

Shreekant Ravindra Naik*, Dr. Gayatri Barabde and Dr. Sushama Ambadekar

Department of Analytical Chemistry, The Institute of Science, Mumbai- 400032, Maharashtra, India

Abstract: Actions of metal ions in biology have gain a huge importance of metal-based therapeutics. The present research work deals with the synthesis of copper (II) complexes synthesized from 5-Arylidene Barbituric acid derivatives; chemical analysis was carried out to identify derivatives (ligand) - metal ratio of these complexes in solution. For the determination of complex nature of the material of these complexes, Mole ratio method has been used. The stability constant of the formed complexes was calculated by molar conductance measurement using Modified Job's method, which is also known as method of Continuous Variations. Further, synthesized complexes were characterized by UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction, mass spectroscopy as well as NMR physicochemical techniques. Antibacterial effectiveness of the ligand and its metal complexes has been investigated *invitro* on the growth of Gram-positive *Staphylococcus aureus, Bacillus anthracis, Escherichia coli* and *Klebsiella Pneumonia* bacterial strains. The attained electronic spectral bands are characteristic and reliable with the plausible composition of the ligands as well as its metal complexes. It also provides a further example of the coordination of 5-Arylidene Barbituric acid ligands. Absorption peak values of FTIR are characteristic of the ligand as well as coordinated by two oxygen atoms of two ligands molecules and exhibit their metal coordination. NMR 1H,13C signal variations also correlate with the coordination mediated chemical shifts. Both the metal complexes showed significant antibacterial activity. However, enhanced antimicrobial activity of the metal complexes than Ligands against Gram positive was observed.

Keywords: Barbituric acid, Mole ratio, Job's method, metal complexes, IR, NMR, Mass spectra

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***Corresponding Author**

Shreekant Ravindra Naik , Department of Analytical Chemistry, The Institute of Science, Mumbai- 400032, Maharashtra, India

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1. INTRODUCTION

Biologically transition metal-Ligand complexes have gained huge attention in recent years; they have a special place in pharmaceutical chemistry. Barbituric acid also known as Pyrimidine-2, 4, 6(1H, 3H, 5H)-trione, is the parent compound of barbiturate drugs and it is pharmacologically inactive in nature. It does not give sedative and hypnotic effects but the exchanged derivatives with alkyl or aryl group at fifth position delivers them[1]. The derivatives of barbituric acid have a special place in pharmaceutical chemistry. There are various methods that have been reported for the synthesis of 5-arylidene barbituric acid derivatives; viz. by catalyzing NH_2SO_3H with Grinding technique[2], by using infra-red in absence of solvent,[3] by reaction of microwave irradiation[4], with the help of catalytic Knoevenagel condensation by Ni–SiO2[5], Knoevenagel Condensation Catalyzed by KF–Al_2O_3[6],Natural phosphate [(NP)/KF or NP/$NaNO_3$][7], by using synthetic phosphate ($Na_2CaP_2O_7$)[8], Knoevenagel condensation catalyzed by $K_2NiP_2O_7$[9], by using acidic clay catalysts with dry condensation[10], by using Ni nanoparticles,[11] and by using microwave irradiation[12]. We referred many literatures, most of them have been demonstrated the barbituric acid metal complexes[13] but in our research, first we tried to synthesize the Barbituric acid derivatives by reacting with Aromatic aldehyde with the help of simple grinding technique as mentioned in the literature[14] . The synthesized derivative, which was derived, was in solid form. The derivative was washed, and recrystallized using methanol. Sodium acetate was used as a catalyst to speed-up the reaction. Further, the reaction between these derivatives with transition metal salts was done to prepare the complex derivatives. We executed Job's and Mole ratio methods to understand the relation between Ligand and metal salt using precise proportion to receive the optimum 5-Arylidene Barbituric acid and metal. The amendment of the Job's continuous variation method[15] accomplished by Vesburge and Cooper[16] was applied to find the stoichiometry and formation constant of the complex, this coordination can be calculated by using the following formula

$$mM + nL = MmLn$$

In Mole ratio method, a series of solutions were prepared, in which the total concentration of the metal is kept constant and the concentration of the ligand is varied under similar conditions. A plot (Graph 1) plotted for absorbance as a function of the ratio of moles of ligand to moles of the metal. Molar ratios or conversion factors helps in identifying the number of moles of each reactant needed to form a particular number of moles of each product.. it is really important to know the mole ratio to calculate the reaction yield and to monitor the reaction kinetics.[17].

2. MATERIALS AND METHODS

2.1 Synthesis of 5-Arylidene Barbituric Acid

A mixture of barbituric acid (10 mmol), aromatic aldehyde (10 mmol) and sodium acetate (10 mmol) which acts as a catalyst were grounded at room temperature; after grinding the mixture for half an hour the solid mixture was washed and recrystallized with the help of methanol. The following products we synthesized during our research:

1. 5-Benzylidene pyrimidine-2,4,6(1H,3H,5H)-trione- Derivative I
2. 5-(4-Bromo benzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione- Derivative II
3. 5-(4-Fluoro benzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione- Derivative III
4. 5-(4-Methylbenzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione- Derivative IV

Above synthesized products were then analyzed with the help of IR and NMR. Additionally, to make complex derivatives we used copper salt; Cupric nitrate trihydrate [Cu $(NO_3)_2.3H_2O$].To make complex derivatives it is always important to know the relation between ligand and metal salt. The exact proportion, which converts the derivative to its most complex form, should be noted by using; Job's method and Mole ratio method.

2.2 Job's method (continuous variation method) [17]

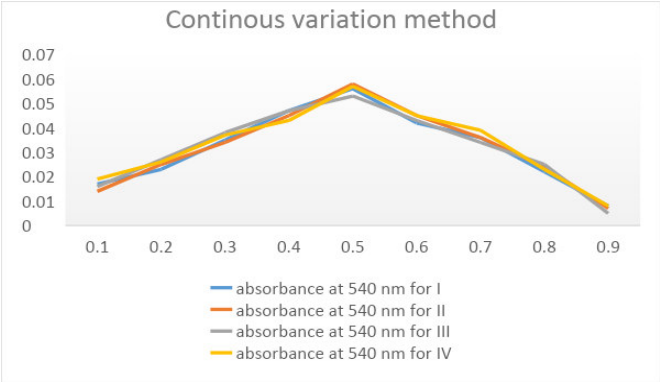
A series of extractions were carried out in which the mole fraction of Cu(II) and 5-Arylidene Barbituric acid were varied from 0.2M to 0.8M as shown in Table I. Absorbance of the organic extracts were measured against DMSO as a blank in lcm cells after diluting them to 25 ml with the same solvent, these dilutions were measured at absorbance 540nm; and same has plotted in Graph I

2.3 Mole ratio method [18]

8ml of pH 8 buffer was added to a series of Cu (II) and variable volumes of ligand in DMSO was extracted. The total volume was filled 10ml with the DMSO. After shaking the mixture thoroughly it was allowed to standstill until the mixture was separated. The organic mixture was completed to 25 ml with DMSO.

Table 1. Job's plot for derivatives

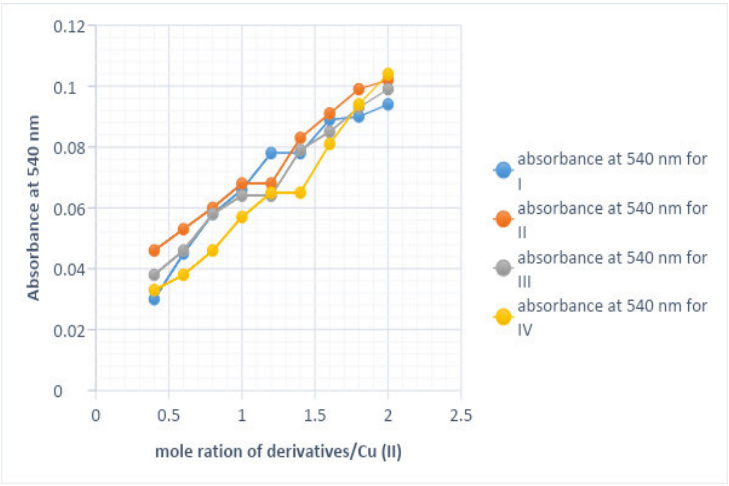
Concentration of Cu(II) in ml	Volume of derivative I/II/III/IV	Mole Fraction	absorbance at 540 nm for I	absorbance at 540 nm for II	absorbance at 540 nm for III	absorbance at 540 nm for IV
1	9	0.1	0.017	0.014	0.016	0.019
2	8	0.2	0.023	0.025	0.027	0.026
3	7	0.3	0.035	0.034	0.038	0.037
4	6	0.4	0.047	0.045	0.047	0.043
5	5	0.5	0.056	0.058	0.053	0.057
6	4	0.6	0.042	0.045	0.043	0.045
7	3	0.7	0.036	0.036	0.034	0.039
8	2	0.8	0.022	0.024	0.025	0.023
9	1	0.9	0.008	0.007	0.005	0.008



Graph 1. Continuous variation method

Table 2. Mole ratio for derivative/Cu (II)

Derivative volume in ml(I/II/III/IV)	Mole ratio	Absorbance at 540 nm for I	Absorbance at 540 nm for II	Absorbance at 540 nm for III	Absorbance at 540 nm for IV
2	0.4	0.03	0.046	0.038	0.033
3	0.6	0.045	0.053	0.046	0.038
4	0.8	0.058	0.06	0.058	0.046
5	1	0.066	0.068	0.064	0.057
6	1.2	0.078	0.068	0.064	0.065
7	1.4	0.078	0.083	0.079	0.065
8	1.6	0.089	0.091	0.085	0.081
9	1.8	0.09	0.099	0.093	0.094
10	2	0.094	0.102	0.099	0.104



Graph 2. Mole ratio for derivative and Cu (II)

With the help of found absorbances in Table 2, the absorbances were plotted against the mole ratio of derivative/Cu (II), Graph 2 shows that the point of intersection is at 1.2, 1.0, 1.0 and 1.2-mole ratio for D1, D2, D3 and D4 respectively. This proves that the complex is a 1:2 complex.

2.4 Synthesis of complex derivatives of copper (II)

Each derivative (1.5gm) was dissolved in ethanol (40 ml) with copper metal salt (1.5 gm) in ethanol (15 ml). Continuous stirring for duration of 30 minutes using a magnetic stirrer dissolved the mixture. The pH was maintained by adding concentrated ammonia (6ml). The whole mixture was refluxed for a duration of 60 minutes at approximately 90°C in a water bath. The derived brown colored precipitate of the reaction was filtered and washed with ethanol. The extracted precipitate was kept for about 15 minutes to dry in an oven at 70°C.

2.5 Antibacterial Activity

All the test solutions (including Derivatives and Metal complexes) were prepared with known amount of complex derivative in DMSO. Then test solutions were diluted to a concentration of 50, 100, 150, 200 and 500mcg/ml further by using Whatman no.1 sterile filter discs of diameter with 6mm were soaked and allowed to dry at room temperature for 24 hours; to grow the bacterial strains Mueller Hinton Agar obtained from Himedia, 24 hours old culture of bacterial strains mixed with saline water and Mueller Hinton agar and suspended in sterile water. Further Petri plates were prepared with 10 ml of Mueller Hinton agar and allowed to solidify; Prepared discs were then introduced to plate and incubated at 37°C for 24hours; after all this process Inhibition zone(mm) was measured as diameter; the results were compared using Ciprofloxacin suspension as a standard antibacterial medicine.

3. RESULTS AND DISCUSSION

3.1 Elemental analysis and physical properties

The elemental analysis of C, H and N of the Cu(II) 5-arylidene barbituric acid complexes are listed in table 3. The results of C, H and N percentage are in confirmation with the composition suggested for the complexes. The data clearly aligned with the suggested molecular formula of data further suggest that all these complexes are bis binuclear and ratio of derivative to metal is 1:2. In table 4 basic physical parameters molecular formula, weight and colour of complexes have been mentioned

Table 3. Elemental analysis

Metal complex	Element	mass-percent
D1Cu	C	53.26
	H	3.25
	N	11.3
D2Cu	C	40.28
	H	2.46
	N	8.55
D3Cu	C	49.47
	H	3.02
	N	10.5
D4Cu	C	54.78
	H	4.22
	N	10.65

Table 4. General properties of derivative-metal complexes

DM complex	Mol.formula	M.P.	Mol.Wt.	Color
D1Cu	$C_{22}H_{18}N_4O_6Cu$	317	497.95	White
D2Cu	$C_{22}H_{16}N_4O_6Br_2Cu$	356	655.46	White
D3Cu	$C_{22}H_{16}N_4O_6F_2Cu$	391	533.65	Dark brown
D4Cu	$C_{24}H_{22}N_4O_6Cu$	374	525.7	Dark yellow

3.2 Infrared spectral analysis

IR spectroscopy helps in identification of functional groups. Therefore, IR spectroscopy measured as important basis for getting binding mode of the 5-Arylidene Barbituric acid derivative to metal in the complexes formation. Some primary bands are of interest of the 5-Arylidene Barbituric acid derivatives and their metal complexes, which provide the information about the coordination pattern of derivative to metals and the complexes 1–4 show the broadband at 3247.75–3566.09 cm−1 is due to νH2O. The characteristic band of aldehyde carbonyl was found to be 1571.88-1719.79 cm−1. These frequencies lower by the unit 20–40 cm−1 in the complexes, indicating that the aldehydes carbonyls donate the pair of electrons toward the metal and forming metal–oxygen bond[19]. The νM–O frequencies are obtained in the complexes at the 525.62 – 671.17 cm−1 and depend on the metal complexes[20]. In the case of the mixed ligand complexes 1–4 the νM–N frequencies are obtained at 702.65 –853.48 cm−1. The derivatives donate the electron from the C = N atom toward the metal in the case of mixed ligand complexes are listed in table 5, it lower down significantly and it clearly indicates an establishment bond between ligand and metal in complexes[21].

Table 5. IR spectra analysis

Compound	√(OH)as	√(OH)s	√(NH)	√(C=O)	√(C=N)	√(CH2)bend	√(C-N+C-O)	√(M-N)	√(M-O)
D1	3840.18		3566.13	1681.48	1594.12	1368.68			
D1Cu		3247.75	2360.04	1571.88	1531.03	1108.18 1231.03	823.18	796.24 823.18	668.25
D2	3829.76	3806.60	3778.48	1694.60	1577.59				
D2Cu			3484.93	1668.21	1575.87	1233.35 1375.31	1008.22 1107.73	775.10 853.48	671.17
D3	3579.15		2996.16	1643.94	1482.50	1408.50	1230.52 1129.25		
D3Cu	3840.18		3566.13	1681.48	1594.48	1338.69	1065.38	810.35	525.62
D4	3575.15		2589.53	1719.79	1641.62	1080.24	1019.25		
D4Cu	3566.09		2450.34	1681.80	1574.60 1472.90	1081.54	789.26	702.65	612.68

3.3 UV-Visible spectral analysis of copper complex and their magnetic moments

The UV-Visible spectroscopy was carried out for the complexes, which were scanned from 200 to 800 nm in the UV–Vis spectrophotometer. The concentration of complexes was in pure DMSO (10−3 M). The ultraviolet absorption spectrum of the D1Cu(Figure 1) shows three bands at positions of 275 nm, 361 and 441 nm, D2Cu(Figure 2) shows bands at 282nm, 348nm and 493 nm, D3Cu(Figure 3) Shows

bands at 286nm, 358nm and 624nm, D4Cu(Figure 4) shows four bands at 289nm, 397nm, 479nm and 589nm which are assigned to π → π*, π → π* and n → π*respectively.

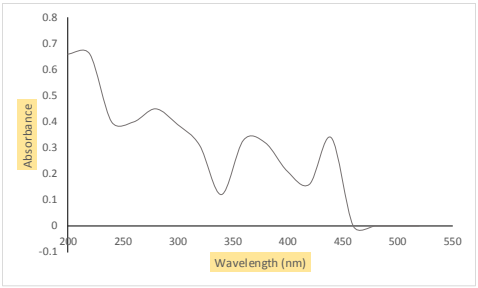


Fig 1. D1Cu

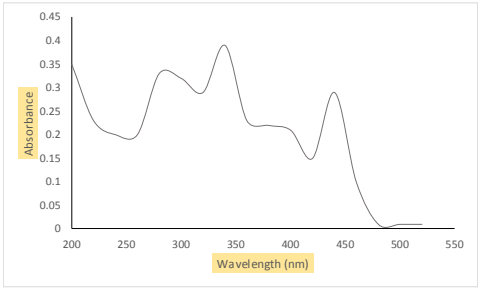


Fig 2. D2Cu

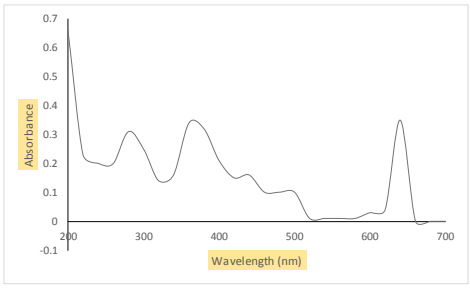


Fig 3. D3Cu

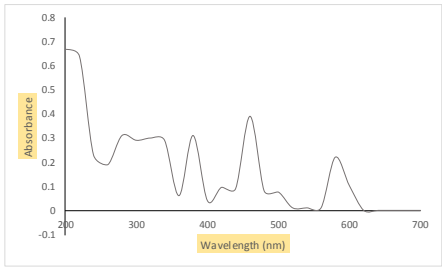


Fig 4. D4Cu

3.4 Nuclear magnetic resonance spectral analysis of complexes

The ^{1}H NMR spectra of synthesized metal complexes were recorded in DMSO-d6. These synthesized complexes appeared the multiples signals were in the region at δ=7.00-8.00 ppm, which assigned to proton of aromatic group[22].

D1Cu-(300MHz,DMSO-d6)δ:3.559(s,2H,CH_2),3.661(s,2H,CH_2),7.555(s,1H,CH),7.682(s,1H,CH), 7.725(s,1H,CH),7.731(s,1H,CH),7.756(s,1H,CH),7.791(s,1H,CH),7.815(s,1H,CH),7.867(s,1H,CH),6.354(s,1H,CH),10.702(m,2H,NH),10.932(m,2H,NH)
D2Cu-(300 MHz, DMSO-d_6) δ: 11.622(m,2H, NH),11.444(m,2H, NH),7.690(m,2H, CH),7.664,(s,1H, CH),7.589(m,2H, CH),7.331(m,3H, CH),3.555(m,4H, CH_2)
D3Cu-(300 MHz, DMSO-d_6) δ: 10.128(m,2H, NH),10.015(m,2H, NH),7.535(m,2H, CH),7.510,(m,2H, CH),7.392(m,2H, CH),7.434(m,4H, CH_2),3.411(m,4H, CH_2)
D4Cu- 300 MHz, DMSO-d_6) δ: 10.980(m,2H, NH),10.302(m,2H, NH),7.465(m,4H, CH),7.298,(m,4H, CH), 3.408(m,4H, CH_2),2.195(m,6H, CH_3)

^{13}C NMR spectra of the complexes were observed the signal due to aryl group moieties at the region 140.00-120.00; respectively.
D1Cu-(300MHz,DMSO-d6)150.101,165.573,189.396,79.119,132.12,131.692,129.103,124.331,19.592
D2Cu-(300MHz,DMSO-d6)120.393,152.139,145.369,190.005,90.639,135.391,133.322,132.99,30.12
D3Cu-(300MHz,DMSO-d6)159.622,150.201,162.51,191.398,91.193,135.549,125.31,110.325,101.296,27.319
D4Cu-(300MHz,DMSO-d6)154.183,165.745,187.565,87.531,138.619,134.502,128.451,28.301,21.193

3.5 Mass spectral analysis of complexes

The mass spectra of all synthesized complexes have been analyzed at 300°;70 eV. All the spectra exhibit parent peaks due to molecular ions and proposed molecular formula of these complexes was confirmed by comparing their molecular formula weights with m/z values. In the spectras of Cu(II) exhibit M^+ peak; the molecular ion peaks at m/z 495, m/z 655, m/z 535 and m/z 527, respectively, supporting the composition of synthesized complexes.

3.6 X-ray diffraction pattern of D1Cu, D2Cu and D3Cu metal complex

The powder XRD pattern figures below of all the homoleptic and heteroleptic complexes were recorded over the 2θ = 20°–80° range. The all Cu metal complexes with derivatives exhibit a crystalline nature with sharp peaks. This XRD study is also one of the portions of indication about development of metal-ligand complexes[23]. The XRD unit cell parameters such as crystal nature, Bravais lattices, diffraction angles, cell length, volume, etc, are illustrated. The XRD peak at the lowest side is extensive in all the complexes to indicate smaller particle size may be in nanometers. Average size of these complexes was calculated by Scherer's formula[24] where λ is wavelength of X-ray radiation, β is the full width at half maximum of diffraction line and θ is the diffraction angle. Table 6 shows the found values and structure of synthesized complexes

Table 6. XRD spectra analysis

Complex	a	b	c	α	β	□	Volume Å	Structure
D1Cu	5.59	11.4	15.36	42.1	55.7	79.8	495.69	Triclinic
D2Cu	5.67	7.50	16.92	52.76	63.45	103.27	464.34	Triclinic
D3Cu	6.35	6.43	15.59	90	90	90	692.45	Tetragonal
D4Cu	8.36	8.28	11.39	90	90	90	826.37	Tetragonal

3.7 Antimicrobialactivity[25]

In antimicrobial activity of 5-Arylidene Barbituric acid derivatives and their Copper complexes against gram-positive and gram-negative bacteria was carried out. Only the mixed ligands synthesized complexes were dissolved in a minimum quantity of DMSO and adjusted to make up the volume to get 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL concentrations. The zone of inhibition was observed and measured to compare with ciprofloxacin as standard drug. The result (Table 7) obtained clearly showed that all complexes were effective against the entire microorganisms of *Staphylococcus aureus*, *Bacillus anthracis*, *Escherichia coli* and *Klebsiella Pneumonia*. The zone of inhibition was described in Supplementary material Table below. The Complexes D1Cu to D4Cu showed more activity as compared with Derivatives and plain barbituric acid[26]. Many researchers found that the less active compound became more active upon complexion[27]. This is due to the decrease in polarity by forming a chelate complex[28].

Table 7. Antimicrobial activity (zone of inhibition in mm)

Name of the bacterial strain	Name of the compound examined										
	Barbituric acid	D1	D2	D3	D4	D1-Cu(II)	D2-Cu(II)	D3-Cu(II)	D4-Cu(II)	DMSO	Ciprofloxacin suspension
Staphylococcus aureus	17	21	20	23	18	24	23	27	22	15	24
Bacillus anthracis	17.5	19	21.1	18	20	23	25	25	21	16	23
Escherichia coli	16	23	18	22	23	21	22	25	24	13	21
Klebsiella Pneumonia	18	19.2	21	22	21	23	25	24	27	16	26

Based on various interpretations of elemental analysis, spectroscopic methods (UV-Vis, IR, 1H NMR, 13C NMR, Mass) and XRD measurements, it has been observed that 5-Arylidene Barbituric acid derivatives (ligands) bonded to metal ion through oxygen of carbonyl group. The complexes were produced by having action of derivative with the metal ion in 1:2 molar ratios in ethanol. By nature Cu(II) complexes are paramagnetic. At room temperature the derivatives and its Cu(II) complexes are stable with non-hygroscopic nature. The XRD data shown the D1Cu(II) and D2Cu(II) metal complexes are shown triclinic geometry and D3Cu(II) and D4Cu(II) metal complexes showed tetragonal geometry. All the synthesized compounds showed significant antibacterial actions against used bacterial strains in the experiment and their complexes showed better activity as compared to the parent derivatives under equal experimental circumstances. Between all the compounds, D3Cu(II) was found to be most effective antibacterial agent. The proposed structure of metal complex can be seen below (Figure 5):

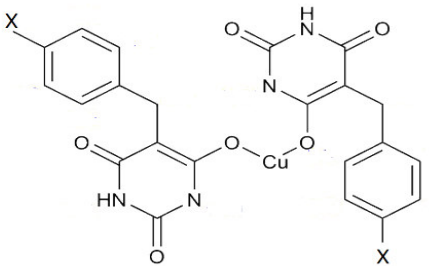


Fig 5. Where X can be changed depending on Aromatic aldehyde

4. CONCLUSION

In conclusion, copper (II) complexes were synthesized from 5-Arylidene Barbituric acid derivatives and its chemical analysis was carried out to identify derivatives (ligand) - metal ratio of these complexes in solution. Further study on this approach could cover a mode for the development of 5-Arylidene Barbituric acid derivatives-metal complex based antibacterial agent.

5. AUTHORS CONTRIBUTION STATEMENT

Shreekant Naik and Dr. Gayatri Barabde contributed to the design and implementation of the research, Dr. Sushama Ambadekar and Dr. Gayatri Barabde to the analysis of the results and helped to the formatting of the manuscript. All authors had discussed the methodology and results and contributed to the final manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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