Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences 56 (4): 1–8 (2019)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Formation Constants of Hydrazine Derivative with Co, Ni, Zn, Cd and its Antimicrobial Evaluation

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Abstract: Formation constants of hydrazine derivative with Co, Ni, Zn and Cd were determined by Job's methods of continuous variation using UV/Visible spectrophotometer at 350nm, 392nm and 420nm. The highest values of formation constants were found as 8.771 for Co, Ni and Zn; whereas the lowest formation constant was found as 6.678 for Cd. The evaluation of antimicrobial activities revealed intermediate results against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*

Keywords: Formation constant, Job's method, Antimicrobial activities

1. INTRODUCTION

Azine containing compounds are important because of the wide spectrum of biological activities. Researchers checked symmetrical and asymmetrical azines as potential antitumor and antimicrobial agents [1-2]. Veena screened symmetrical and asymmetrical azines encompassing of naphtha [2,1-b] furan for antibacterial activity [3].

Formation/Stability constants are helpful for chelating ligands that are useful in detoxification of poisonous metals [4]. Formation constants of organic compounds with salts of transition metals were reported by employing UV/visible spectrophotometer using the Job's continuous variation method and stability orders were established [5–8]. Polarographic, potentiometric, pH, spectrophotometric and conductometric techniques were utilized to determine the stability constants [9–13]. Stability constants calculations were also studied by the ligand displacement method which showed the role of stability constants [14].

2. MATERIALS AND METHODS

This research work was carried out in the Chemistry and Zoology Laboratories of respective Departments of Division of Science and Technology, University of Education, Township Lahore, Pakistan. Hydrazine derivative was provided from the organic faculty of University of Education. All other chemicals made of BDH/Merck were used without further purification.

2.1 Determination of Formation Constants

Stock solutions of 1-(2,4-dimethoxybenzylidene)-2-(3,4,5-trimethoxybenzylidene)hydrazine [1x10-3M] and transition metal salts of Co, Ni, Zn and Cd [1x10-3M] were prepared in the mixture of methanol and dimethyl sulphoxide (4:1). Job's method of continuous variation was employed for the calculations of formation constants using UV/visible spectrophotometer at wavelengths 350nm, 392nm and 420nm. A job plot determined the stoichiometry of a binding event and formation constants were calculated by the relation:

Received: March 2019; Accepted: December 2019
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$$K_s = \frac{\left(\frac{A}{Aex}\right)C'}{\left[Cm-\left(\frac{A}{Aex}\right)C'\right]\left[Cx-\left(\frac{A}{Aex}\right)C'\right]}$$

Where,

A= Absorbance peak, A_{ex} = Extrapolated absorbance, C_m = Concentration of metal, C' = Concentration of Complex, C_x = Concentration of Ligand

2.2 Antimicrobial Evaluation

The microbicidal potential of 1-(2,4-dimethoxybenzylidene)-2-(3,4,5 trimethoxybenzylidene) hydrazine was assayed against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* by well diffusion protocols [15, 16]. Ligand was utilized *in vitro* to assess for antimicrobial capacity against three pathogens such as *E. coli, S. aureus* and *C. albicans* by applying agar well diffusion protocols. Wells were punched with sterile metal borer of size 6mm. Two concentrations such as 20mg/100mL and 40mg/100mL of hydrazine derivative were prepared in DMSO:Methanol (1:1) solvent. The specified concentrations of ligand were loaded in wells in agar plates in which selected microbes were seeded by spread plate method. Inoculated plates were incubated for 24 hours at 37°C to check growth inhibition zones which were measured by scale and results were tabulated in Table 2. Biological activities of synthetic compound were taken as standard [17].

Biological Activities of Synthetic Compounds

Diameter of Zone of Inhibition (mm)	
Resistant	10 or less
Intermediate	11 – 15
Susceptible	16 or more

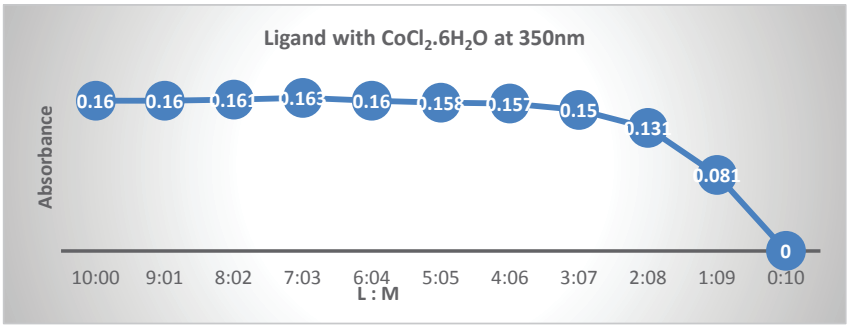
3. RESULTS AND DISCUSSION

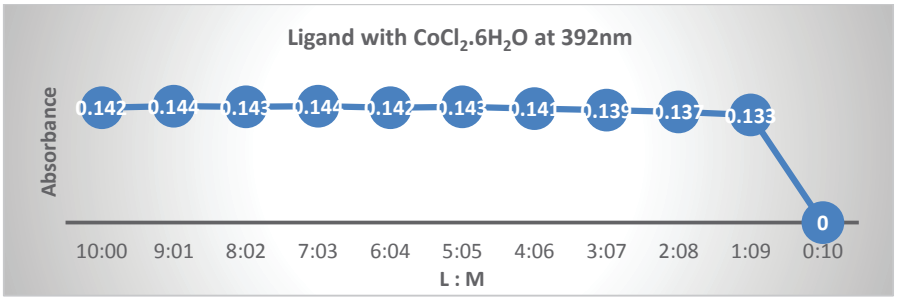
Dark brown crystals of 1- (2 , 4 - dimethoxybenz -ylidene)-2-(3,4,5-trimethoxybenzylidene) hydrazine were dissolved in CH_3OH:DMSO (4:1) to record the λmax as 392nm. Job's Method of Continuous Variation was utilized for the determination of formation constants of the ligand with transition metal salts such as $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $ZnCl_2$ and $CdCl_2$ $6H_2O$ by UV/visible spectrophotometer at appropriate wavelengths such as 350nm, 392nm and 420nm (Table 1, Figures 1-2). Log values of formation constants were calculated and stoichiometric ratios from the plotted graphs were obtained. The decreasing order of formation constants (log values) at 350nm, 392nm and 420nm was established as mentioned below:

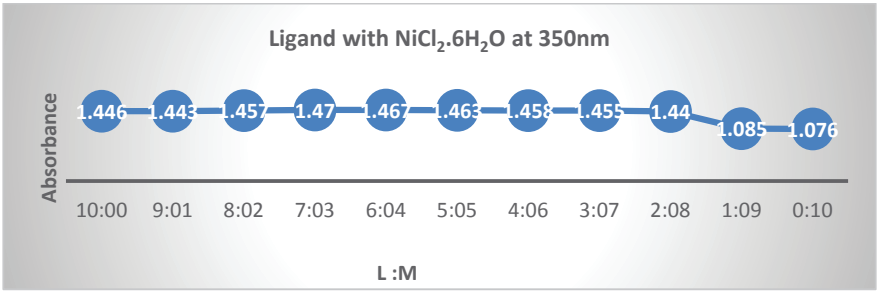
Ni > Zn > Co > Cd	At 350nm
Zn > Co > Ni > Cd	At 392nm
Co > Ni > Zn > Cd	At 420nm

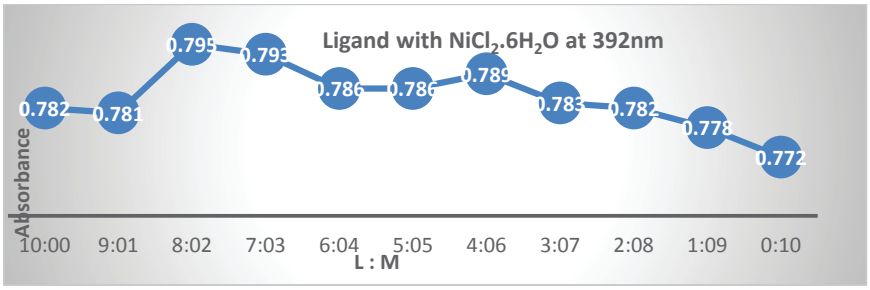
The highest values of formation constants were found as 8.771 (L2:M1) at 420nm for Co(II) and Ni(II); (L4:M1) at 350nm for Ni(II); and (L3:M2) at 350nm for Zn(II). The lowest values of formation constants of Cd(II) were found as 6.779 (L3:M2) at 420nm and 6.678 (L4:M1) at 350nm.

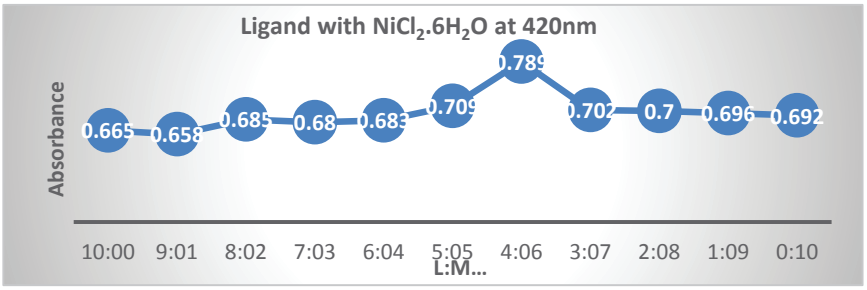
The results regarding antimicrobial evaluation (Table 2, Figure-3) showed that 1-(2,4-dimethoxybenzylidene)-2-(3,4,5-trimethoxybenzylidene) hydrazine inhibited the growth of both gram positive and gram negative bacteria but ineffective against fungus such as *C. albicans* even at high concentration. From the above results, it is noted that increased chemical concentration has positive relation with growth inhibition of bacteria.

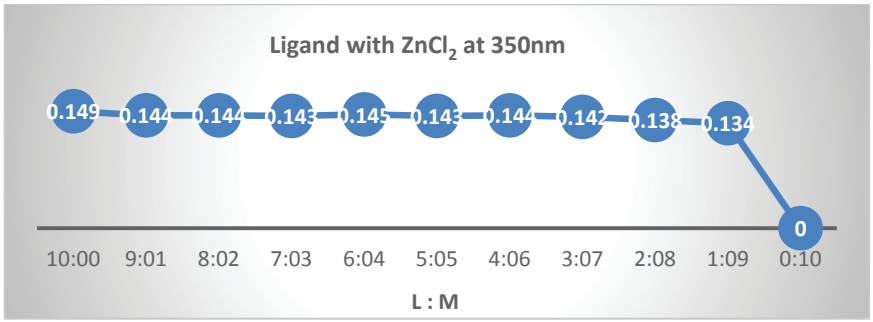


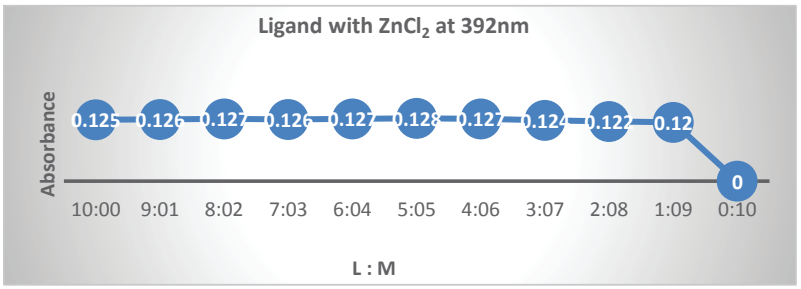

Ligand with $CoCl_2.6H_2O$ at 392nm
Absorbance
0.142
0.144
0.143
0.144
0.142
0.143
0.141
0.139
0.137
0.133
0
10:00 9:01 8:02 7:03 6:04 5:05 4:06 3:07 2:08 1:09 0:10
L : M

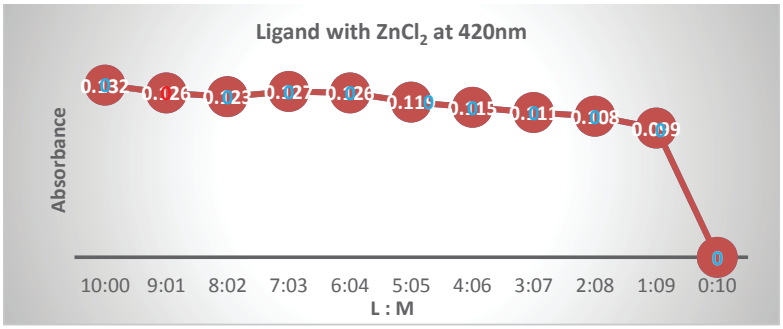

Ligand with $NiCl_2.6H_2O$ at 350nm
Absorbance
1.446
1.443
1.457
1.47
1.467
1.463
1.458
1.455
1.44
1.085
1.076
10:00 9:01 8:02 7:03 6:04 5:05 4:06 3:07 2:08 1:09 0:10
L :M

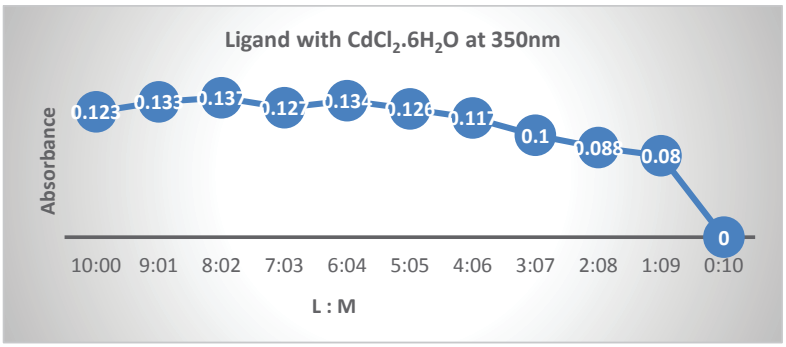

Ligand with $NiCl_2.6H_2O$ at 392nm
Absorbance
0.782
0.781
0.795
0.793
0.786
0.786
0.789
0.783
0.782
0.778
0.772
10:00 9:01 8:02 7:03 6:04 5:05 4:06 3:07 2:08 1:09 0:10
L : M

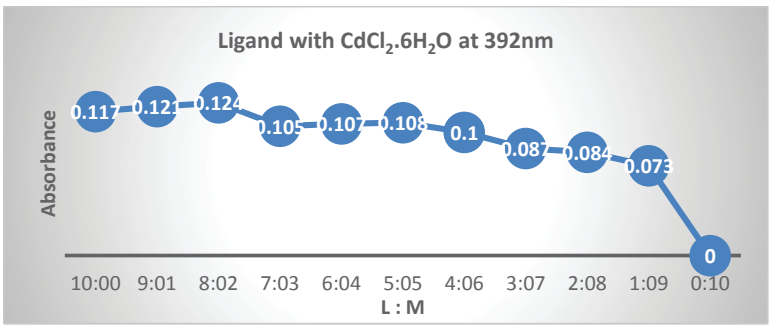

Ligand with $NiCl_2.6H_2O$ at 420nm
Absorbance
0.665
0.658
0.685
0.68
0.683
0.709
0.789
0.702
0.7
0.696
0.692
10:00 9:01 8:02 7:03 6:04 5:05 4:06 3:07 2:08 1:09 0:10
L:M...


Ligand with $ZnCl_2$ at 350nm
0.149
0.144
0.144
0.143
0.145
0.143
0.144
0.142
0.138
0.134
0
10:00 9:01 8:02 7:03 6:04 5:05 4:06 3:07 2:08 1:09 0:10
L : M









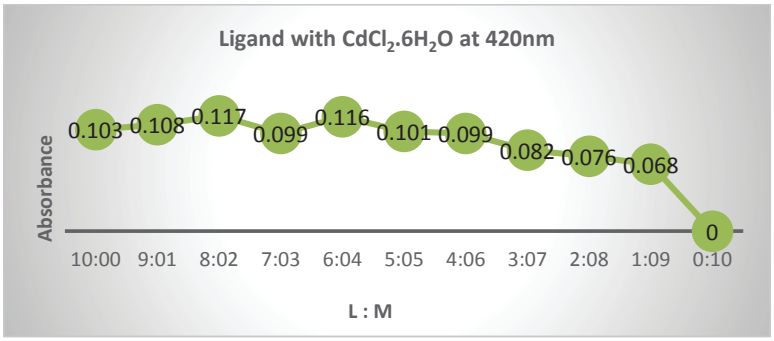


Fig. 1. Determination of Formation Constants by Job's Method

Table 1: Formation constants of 1-(2,4-dimethoxybenzylidene)-2-(3,4,5-trimethoxybenzylidene) hydrazine with metals

Metal	Wavelengths	Formation Constants		L:M
		Ks	log Ks	
	350 nm	2.76×10^{8}	8.4409	2:1
$CoCl_2.6H_2O$	392nm	1.27×10^{8}	8.1028	1:1
		1.27×10^{8}	8.1028	2:1
	420 nm	5.09×10^{8}	8.7708	2:1
		1.27×10^{8}	8.1028	3:2
	350 nm	5.09×10^{8}	8.7708	2:1
	392 nm	2.08×10^{7}	7.3180	2:3
$NiCl_2.6H_2O$		2.77×10^{7}	7.4434	4:1
	420nm	5.09×10^{8}	8.7708	4:1
		2.06×10^{8}	8.3138	1:1
	350nm	5.09×10^{8}	8.7708	3:2
	392nm	3.90×10^{8}	8.5910	4:1
$ZnCl_2$		3.90×10^{8}	8.5910	1:1
	420nm	6.54×10^{7}	7.8155	2:1
	350nm	8.17×10^{6}	6.9122	3:2
		4.76×10^{6}	6.6776	4:1
$CdCl_2.6H_2O$	392nm	1.22×10^{7}	7.0875	1:1
		1.55×10^{7}	7.1900	4:1
	420nm	6.02×10^{7}	6.7795	3:2
		1.37×10^{7}	7.1373	4:1

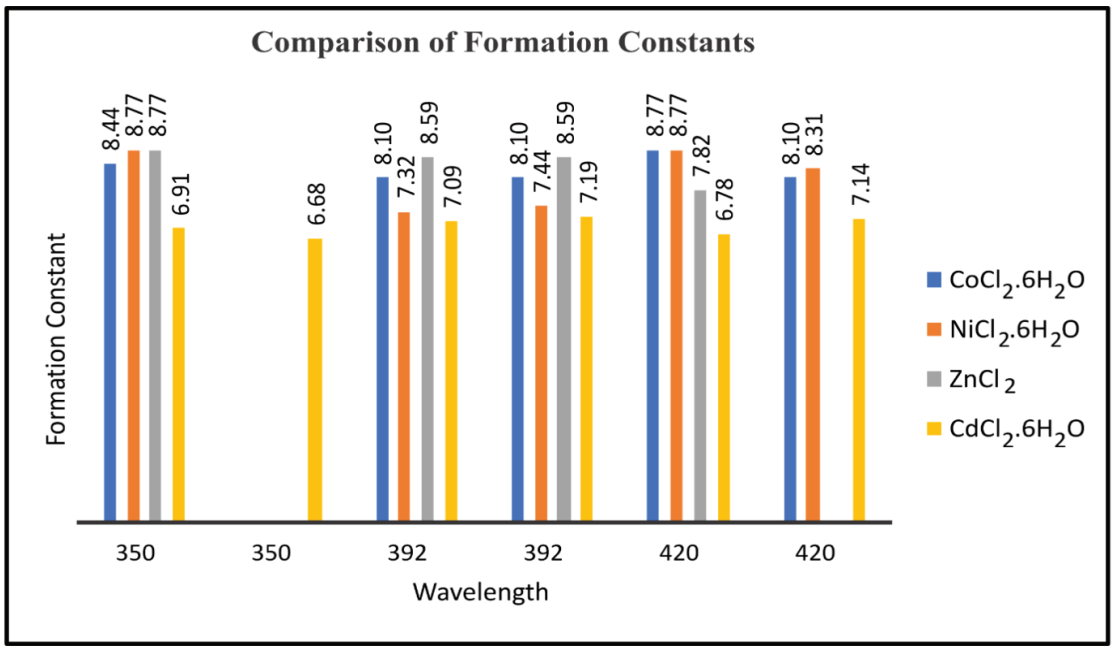


Fig. 2. Comparison of Formation Constants

Table 2: Growth inhibition shown by 1-(2,4-Dimethoxybenzylidene)-2-(3,4,5-trimethoxy-benzylidene) hydrazine

Organism	Zone of Inhibition (mm)	
	20 mg / 100mL	40 mg / 100mL
E. coli	15	20
S. aureus	15	19
C. albicans	5	10

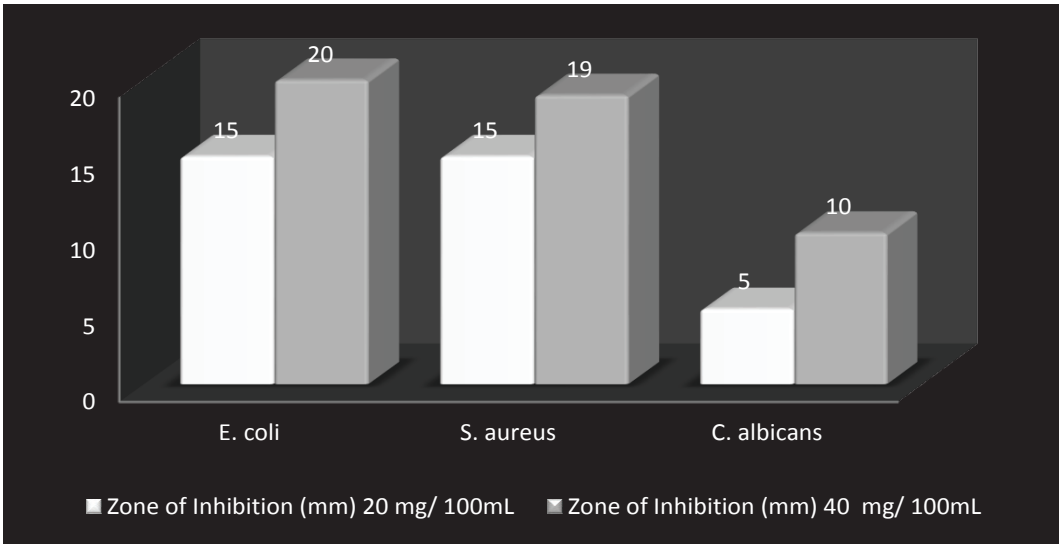


Fig. 3. Antimicrobial Activities of 1-(2,4-dimethoxybenzylidene)-2-(3,4,5-trimethoxybenzylidene) hydrazine

4. CONCLUSIONS

Job's methods of continuous variation was employed spectrophotometrically to determine the formation constants of 1-(2,4-dimethoxybenzylidene)-2-(3,4,5-trimethoxybenzylidene) hydrazine at 350nm, 392nm and 420nm against the metals such as Co(II), Ni(II), Zn(II) and Cd(II). The highest values of formation constants were found as 8.771 (L2:M1) at 420nm for Co and Ni; (L4:M1) at 350nm for Ni; and (L3:M2) at 350nm for Zn and the lowest values of formation constants were found for Cd as 6.779 (L3:M2) at 420nm and 6.678 (L4:M1) at 350nm. Hydrazine derivative was found effective/susceptible against *S. aureus* and *E. coli* but ineffective against *C. albicans*.

Results revealed that increased chemical concentration has positive relation with growth inhibition of bacteria.

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