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## Formation and Stability of Binary and Ternary Metal Complexes Containing Chelating Ligands and Their Biological Activity

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### Abstract

Antioxidant activity of Ni(II) and Cd(II) binary and their mixed ligand complexes. Binary and mixed complexes were synthesised and were subjected to elemental analysis, conductivity studies, IR spectral studies, magnetic properties were analysed. Their biological activity in plants showed that the Ni(II) mixed ligand complexes with ethylenediamine and alanine showed more response than all other mixed ligand complexes of Ni(II) and Cd(II).

**Keywords:** antioxidant activity; Ni(II) and Cd(II) complexes; mixed ligand complexes; IR spectra; biological activity.

### 1. Introduction

The interconversion of Serine to Phosphoserine is of major significance in regulation of biochemical process. The phosphorylation of serine and dephosphorylation of DL-Phosphoserine are catalysed by the enzyme phosphoserine phosphohydrolase which requires the presence metal ions [1,2]. This may operate by formation of a metal bridged mixed ligand complex. The scientific reviews indicate that Dr. Tolonen's phosphoserine extracted from soyabeans is a pure natural supplement and has no side effects nor does it react adversely with any medication. It helps to improve concentration and short term memory, increase intelligence, mood alleviate depression in the elderly Survey of literature shows that synthesis, stability and structures of the binary and mixed ligand complexes contains OrthoPospho-DL-Serine which is not been investigated [3].

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Mixed ligand complexes are of great importance[4-8] in enzyme metal concentration substrate system [8,9]. Studies have shown the importance of Ni(II) in biological systems[10-19]. In the present study synthesis of binary and mixed ligand complexes containing OrthoPospho-DL-Serine were carried out and these complexes were supported by their geometries. The complexes were identified on the basis of their chemical analysis. The levels of antioxidants induced due to strain caused metal and metal complexes in ragi seedlings were estimated. The activity of antioxidants enzymes were also studied in ragi seedlings.

## **2. Experimental Methods and Materials**

### **2.1. Materials**

All chemicals used were Analor grade (BDH)-The ligands OrthoPospho-DL-Serine, Ethylene diamine dihydrochloride,  $\alpha$ -alanine are obtained from Aldrich chemicals, USA. The ligand oxalic acid, metallic salts like Nickel nitrate, sodium hydroxide and Dimethyl formamide (DMF) are of analor grade. Double distilled water is used throughout the research work.

### **2.2. Methods**

The mixed ligand complexes were prepared by mixing equal amount 0.01M salt solution of the ligand OrthoPospho-DL-Serine and 0.01M solution of the metallic(Ni(II)) so in the ratio of 1:1 in 10ml double distilled water and refluxed for half an hour. To this mixture 0.01M solution of secondary ligand (A) (ethylene diamine,  $\alpha$ -alanine, oxalic acid) were added and 0.01M solution of NaOH is added slowly to maintain the pH and stirred for half an hour and solid mixed ligand complex separates out. The solid product obtained is filtered, washed with ethanol, distilled water and then with acetone and dried at 110<sup>0</sup>C for an hour.

The synthesis of binary complexes of metallic salts with primary ligand and also with secondary ligand has been carried out in the ratio 1:1 even though it has been already reported by the chemists to maintain the same temperature and for accurate comparative study.

The synthesized binary and mixed ligand complexes are subjected to elemental analysis using Perkin Elmer elemental analyser. The percentage of metal contents was determined by atomic absorption spectroscopy on Perkin Elmer model 2380. The molar conductivity of a solution of these synthesized complexes was determined using digital conductivity meter model D 19009. The magnetic data of the metal complexes were obtained at room temp. by the Guoy temperature technique using Hg[Co(NCS)] as calibrant. The molecular weight of the samples was determined by using Rast method. The melting points of the complexes were recorded.

### **2.3. Biological activity of the Metal complexes in plants:**

Four day old Eleusine Coracena(ragi) seedlings were treated with Ni(II), Cd(II) precursors and their binary and mixed ligand complexes separately at 50 concentration(solvent used is ethanol). The effect of heavy metals and their complexes on the accumulation of antioxidants like Glutathione, Ascorbate, Tocopherol were studied by analysing the samples for 24 hours exposure with control seedlings using reverse phased

HPLC.(Methanol:water in 1:1 ratio) and the analyte volume-25 $\mu$ l, spectrophotometer C<sub>18</sub> column, model used Shimadzu.

#### 2.4. Antimicrobial activity of metal complexes:

Synthesised complexes were plated for antimicrobial activity overnight broth of test culture E. Coli using Agar-well diffusion method. The standard protocol was followed as given by Kurby-Bayer method. The basal medium useful was nutrient agar medium. Test samples were loaded with respect to each test sample at concentration of 25 $\mu$ l into the wells. The nutrient agar plates were incubated in presence of above test compounds at 37<sup>o</sup>C for 24hrs.

### 3. Results and discussion

All the complexes are insoluble in common organic solvents but dissolve in DMF and DMSO respectively. The electrolytic conductance in DMSO suggests the non-electrolytic nature of the complexes.

Table 1. Physical Characteristics and elemental analysis of PS, its Binary and Mixed Ligand Ni(II) Complexes.

Sl.no	Ligand complex/formula	Colour	Mol.wt	Magnetic moment	M.pt/ decomposition	Ni(II)	C	H	N	P	O
1	PS C <sub>3</sub> H <sub>8</sub> NO <sub>6</sub> P	White	185.07	--			19.45	4.3	7.6	6.7	52
2	PS+Ni(II) C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	294.69	3.072	>330	19.92	12.22	3.732	4.750	10.52	48.86
3	PS+ Ni +Ala C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	364.69	3.902	>330	16.09	19.8	4.113	7.677	8.5	43.87
4	PS+Ni +PheAla C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	440.69	3.764	>320	13.32	32.7	4.31	6.35	7.03	36.31
5	PS+ Ni +His C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	431.69	3.843	>340	13.59	25.02	4.17	12.97	7.18	37.06
6	PS+ Ni +Asp C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	422.69	3.832	>320	13.88	22.71	4.02	6.624	7.333	45.42
7	PS+ Ni +En C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	336.69	3.896	>360	17.43	17.82	5.05	12.47	9.21	38.02
8	PS+ Ni +phen C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	460.69	3.842	>360	12.74	39.07	3.690	9.116	6.73	27.784
9	PS+ Ni +Ox C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	364.69	3.912	>320	16.09	16.45	2.47	3.84	8.50	52.64
10	PS+ Ni +Mal C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PNi	Green	378.69	3.944	>320	15.50	19.01	2.905	3.70	8.19	50.70

The magnetic moment values of complexes of Cd(II) shows that they are diamagnetic and while the Ni(II) complexes are paramagnetic. The elemental analysis data of the complexes (Tables 1&2) shows the formation 1:1:1 ratio of complexes. It is found that the theoretical values are in good agreement with the obtained values.

Table 2. Physical Characteristics and elemental analysis of PS, its Binary and Mixed Ligand Cd(II) Complexes

Sl.no	Ligand complex/formula	Colour	Mol.wt	Magnetic moment	M.pt/ decomposition	Cd(II)	C	H	N	P	O
1	PS C <sub>3</sub> H <sub>11</sub> NO <sub>6</sub> P	White	185.07				19.45	4.3	7.6	6.7	52
2	PS+Cd(II) C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	348.4	DM	>340	32.26	10.33	3.16	4.02	8.91	41.33
3	PS+Cd+Ala C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	418.411	DM	>330	26.866	17.20	3.584	6.692	7.41	38.24
4	PS+Cd+PheAla C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	494-411	DM	>330	22.73	29.12	3.842	5.663	6.270	32.36
5	PS+Cd+His C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	486.411	DM	>320	23.157	22.24	3.71	11.54	6.3861	32.96
6	PS+Cd+Asp C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	476.411	DM	>320	23.59	20.15	3.57	5.88	6.507	40.30
7	PS+Cd+En C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	390.41	DM	>360	28.79	15.37	4.354	10.76	7.94	32.78
8	PS+Cd+phen C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	510.41	DM	>360	22.02	35.27	3.331	8.23	6.073	25.08
9	PS+Cd+Ox C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	418.411	DM	>320	26.87	14.34	2.151	3.346	7.409	45.89
10	PS+Cd+Mal C <sub>3</sub> H <sub>11</sub> O <sub>9</sub> N PCd	White	432.411	DM	>320	26.00	16.65	2.54	3.2376	7.17	44.40

IR spectral data of Cd (II) and Ni (II) complexes (Tables 3, 4) reveal the broad bands in the range 800-900cm<sup>-1</sup> corresponds to coordinated water molecules. The absorption in the range of 720cm<sup>-1</sup> to 760 cm<sup>-1</sup> corresponds to M-N bond. The absorption at 980cm<sup>-1</sup> corresponds to P-O bond. The magnetic moment value of Ni(II) complexes shows that they are paramagnetic and that of Cd (II) complexes shows that they are diamagnetic in nature.

Table 3: IR Spectral bands of Primary ligand PS, its binary and Mixed ligand complexes of Cd(II).

Sl.no	Compound	$\nu$ (C=O) $\text{cm}^{-1}$	$\nu$ (OH) $\text{cm}^{-1}$	$\nu$ (N-H) $\text{cm}^{-1}$	$\nu$ (P=O) $\text{cm}^{-1}$	$\nu$ (POC) $\text{cm}^{-1}$	$\nu$ (N-N) $\text{cm}^{-1}$	$\nu$ (M-O) $\text{cm}^{-1}$	Co- ordinated water $\text{cm}^{-1}$	N-H deformation $\text{cm}^{-1}$	Chelate ring formation $\text{cm}^{-1}$
1	PS	1618	3500	3174	1260	1047	-	-	813.4 743.4	1474	-
2	PS+Cd	1602	3367	-	-	1063	806	536	800	1411	-
3	PS+Cd+Ala	1586	3653	3321	1357	1087	760	543	826	1417	-
4	PS+Cd+PheAla	1574	3563	3268	1350	1077	760	549, 400- 500	-	1416	2352
5	PS+Cd+His	1555	3600	3217	1355	1079	761	546	829	1416	-
6	PS+Cd+Asp	1593	3580	3316	-	1068	759	549	827	1420	-
7	PS+Cd+En	1586	3605	3321	1358	1076	761	549	824	1416	-
8	PS+Cd+phen	1500- 1600	3586	3417	1348	1110	724	418	857	1426	-
9	PS+Cd+Ox	1611	3849	3449	1310	1083	774	516	890	1416	-
10	PS+Cd+Mal	1557	3500	3270	1359	1085	760	549	-	1416	-

Table 4. IR Spectral bands of Primary ligand PS, its binary and Mixed ligand complexes of Ni(II).

Sl.no	Compound	$\nu$ (C=O) $\text{cm}^{-1}$	$\nu$ (OH) $\text{cm}^{-1}$	$\nu$ (N-H) $\text{cm}^{-1}$	$\nu$ (P=O) $\text{cm}^{-1}$	$\nu$ (POC) $\text{cm}^{-1}$	$\nu$ (M-N) $\text{cm}^{-1}$	$\nu$ (M-O) $\text{cm}^{-1}$	Co- ordinated water $\text{cm}^{-1}$	N-H deformation $\text{cm}^{-1}$	Chelate ring formation $\text{cm}^{-1}$
1	PS	1618	3500	3174	1260	1047	-	-	813.4	1474	-
2	PS+Ni	1635	-	3445	1261	1038	633	400- 500	-	1404	-
3	PS+ Ni +Ala	1654	3641	3448	1384	1083	-	400- 500	917	1504	2280
4	PS+ Ni +PheAla	1589	3644	3368	1348	1069	754	557	-	1496	2922

5	PS+ Ni +His	1592	3640	3421	1384	1077	720	525	-	-	2900
6	PS+ Ni +Asp	1578	3588	3431	1384	1086	774	517	-	1508	2280
7	PS+ Ni +En	1596	3644	3412	1384	1087	600	458	-	-	2922
8	PS+ Ni +phen	1654	3649	3412	1384	1060	700	471	-	1508	2922
9	PS+ Ni +Ox	1597	3643	3448	1384	1079	-	537	-	1500	2345
10	PS+ Ni +Mal	1637	3569	3430	1384	1085	-	459	-	-	2920

The antioxidants like ascorbate, glutathione and Tocopherol were analysed in ragi seedlings exposed to metal and metal complexes by reverse phase HPLC Compared to control seedlings (Table 5) Cadmium shown more activity of ascorbate (14.3) and glutathione(4-7) compared to Ni (5-3 and 1.0) whereas Tocopherol level was more in Ni(II) treated plants(0.52)-compared to Cd(II) treated plants(0.41)

A comparison of binary complex levels, ascorbate was found only in Cd(II) treated plants whereas the levels of Tocopherol and glutathione were less(1.7 and 5.8) compared to Ni(II) treated plants(9.12 and 11.9). Among the binary complexes of Ni(II) oxalate as secondary ligand has induced the production of Tocopherol 24.4. Oxalate complexes with Cd(II) and PS had shown ascorbate and glutathione levels of 10.9 and 3.6 respectively. Ethylenediamine hydrochloride as secondary ligands with PS and Ni(II) has shown increased levels of ascorbate (12.02) and tocopherol(21.6), compared to en, PS and Cd(II) levels were 9.7 and 3.4 respectively.

Ternary complexes of Ni(II) with Alanine and PS exhibited increased levels of tocopherol and glutathione(22.2 and 19.4) compared to similar complexes with Cd(II) which has shown 1.7 and 5.2 respectively. However ascorbate was found with levels of 7.9.

Shorter exposure of low concentration of Cd(II) led to the II augmentation of antioxidant activity, both in callus and seedling, while longer exposure and high concentration of Cd(II) led to a concentration dependent decrease in callus super oxide dismutase, ascorbate peroxidase, glutathione reductase and catalytic activities as well as reduced and oxidised glutathione concepts in all samples of leaves, roots and stolons were increased in presence Cd(II) when compared to control plants the concentration of glutathione are strongly correlated with ability to hyper accumulate Ni(II) in various thlaspi hyper accumulators. Aravidopsis plants with low glutathione levels were hypersensitive to Cd due to limited capacity of these plants to make phytochelations. The biological functions of glutathione levels.

From this result we can conclude that the ternary complex of Ni(II) with PS and ethylenediamine hydrochloride or Alanine was inducing more stress in the plants. Therefore biological activity was induced by both the metal ternary complexes of Ni(II) and Cd(II) but when compared to Cd(II), Ni(II) complexes, the plants treated with

Ni(II) ternary complexes with en and alanine have shown more response which can be estimated by the levels of antioxidants and antioxidant enzymes when compared to other complexes.

The diameter of inhibition zone was measured. It was observed that the ternary complex has a larger inhibition zone (Table 6) indicating it to have higher antimicrobial activity. The E.Coli is used along with Staphylococcus aureus as a standard strain to test the antimicrobial activity of unknown compounds. The result of antimicrobial testing reveals an increase of inhibition zone with the increase of the chelates weight placed on the bacterial culture.

Table 5- MDA activity of Binary and Ternary complexes

SAMPLES	ASCORBATE	TOCOPHEROL	GLUTATHIONE
CONTROL	0.73	0.01	0.15
NICKEL	5.3	0.52	1.0
Ni+Ps	--	9.12	11.9
Ni+Ps+Ox	--	24.4	--
Ni+Ps+En	12.02	21.6	--
Ni+Ps+Ala	--	22.2	19.4
CADMIUM	14.3	0.41	4.7
Cd+Ps	10.6	1.7	5.8
Cd+Ps+Ox	10.9	--	3.6
Cd+Ps+En	9.7	1.3	3.4
Cd+Ps+Ala	7.9	1.7	3.2

Table 6- Test compounds for Antimicrobial activity

Test compounds		Diameter of inhibition Zone
Phosphoserine(10mm)		
Cadmium nitrate	Control	20mm
Cadmium+ Phosphoserine	Binary	28mm
Cadmium+ Phosphoserine+1, 10 phenenthroline	Ternary	33mm

#### 4. Conclusion

The ternary complexes of Ni(II) with Ps ,en or ala induce more stress in plants which indicates that the antioxidant activity of these complexes have shown more response. The studied inhibition zones of ternary complexes of Cd(II) with Ps and en showed increased levels which reveal that the antimicrobial activity of these

complexes. We would like to extend the biological activity of the complexes using different metal ions like Co(II), Cu(II) and Mn(II).

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