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Synthesis, characterization and biological evaluation of novel binary and mixed ligand complexes of phosphonformic acid

P. Saritha*, C. Gyanakumari and Suchi Tiwari

Department of Chemistry, Osmania University, Hyderabad, India

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ABSTRACT

A new series of binary and mixed ligand complexesof Ni (II), Cu (II), and Zn (II) with phosphonoformic acid (PFA) a base ligand are studied. The structure of these complexes was identified and confirmed by elemental analysis, molar conductivity, FT-IR and magnetic susceptibility. PFA and their derivatives play an important role in biological processes. It is potential chelating agent and a very active in controlling HIV diseases. Complexes of transition metals ions Ni, Cu, and Zn with primary ligand PFA and secondary ligands (alanine, phenyl-alanine, ethylenediamine, 1, 10-penanthroline, oxalic acid and malonic acid) were carried out and tested for biological activity on Eleucine Coracana (ragi), anti-microbial activity on Staphylococcus-aureus, Bacillus subtilis, Escherichia coli and klebsiella pneumoniae.

Key words: synthesis of complexes, characterization and biological activity.

INTRODUCTION

Literature survey showed that the compound PFA [1] which is in the form of trisodiumsalt is known as fascarnet, is an antiviral agent against herpervirus groups (HSV-I, HSV-2VZV, EBV & Cytomegalovirus (CMV)) and AIDS [2-12]. PFA also inhibits virus multiplication both invivo and invitro [13-16] and shows higher affinity to inhibit the hepatitis-B-virus DNA polymerase [17]. Phosphonoformate was also able to block the replication in cell culture of marcks disease herpesviruse [18-20]. Effect of phosphonoformic acid is also studied in mouse kidney [21].

Complexes with phosphonoformic (PFA) have shown of pharmacological activities such as antiviral, antibacterial and anticancer [22-26]. As it is gaining importance in biological activity, and considered as potential chelating agent in recent years, and even the coordination metal ions have been understood to be an essential factor in the discharge of several vital functions in living organism [27-28] and the metal ligand interaction may cause considerable change in the reactivity of ligand, and are known to diminish the toxicity in certain cases. Studies of metal complexes involving PFA moiety can achieve wider attention owing to its applications in various fields like medicine, analytical chemistry and biological systems [29-35].

In this article we report a synthesis, characterization and antimicrobial activity of new binary and mixed ligand complexes with primary ligand (PFA), and secondary ligands like(N-Odonors-α-alanine(ala),phenyl-alanine(phe),(N-Ndonors-ethylenediamine(en), 1,10-penanthroline(phen), and (O^--O^-donors – oxalic(ox) and Malonic acid(mal).

EXPERIMENTAL SECTION

Materials
The ligands Phosphonoformicacid(PFA), ethylenediaminedihydrochloride(en), α-alanine(ala), phenyl-alanine(phe), 1, 10-phenanthroline(phen), oxalic acid(ox), malonic acid(mal) and Metals nitrates(nickel nitrate, copper nitrate, & zinc nitrate) were obtained from sigma-Aldrich chemical company. Double Distilled water was used throughout the investigation. The P^H of the double distilled water is about 6.8 - 6.9.

Physical measurements
The molar conductivity of solution of the synthesized complexes was determined using digital conductivity meter model-DI9009. Elemental analyses were carried out using Perkin Elmer model 2380. FTIR were obtained in the region 4000-400cm^{-1} by the KBr pellet technique using a Perkin- Elmer Model 621 spectrophotometer. The magnetic susceptibility was measured at room temperature by using the Gouy balance model 7750, using mercury tetrathiocyanatocobaltate (II), $\{Hg[Co(NCS)_4]\}$ as calibrant. The data were corrected for diamagnetism using Pascal's constants. Melting points recorded for uncorrected. The molecular weight of the samples was determined by Rast method.

Synthesis of binary complexes of Ni(II), Cu(II), Zn(II) with primary ligand PFA (1:1 ratio)
0.01M of metal salt solutions(MX_2, where M=Ni(II), Cu(II), Zn(II); X= NO_3^-, Cl^-) were mixed with 0.01M concentration of PFA in 1:1 ratio in 10ml double distilled water and stirred for 30mins at room temperature, and then 0.01M of NaOH is added slowly to maintain pH 9, with constant stirring, the colored solid binary complex were precipitated out, are filtered washed with ethanol and dried at 110^{o}C for 1hr.

Synthesis of mixed ligand complexes of Ni(II), Cu(II), Zn(II) with primary ligand PFA, and secondary ligands (ala, phe, en, phen, ox, mal) in 1:1:1 ratio
0.01M of metal salt solutions (MX_2, where M=Ni(II), Cu(II), Zn(II); X= NO_3^-, Cl^-) were mixed with 0.01M concentration of PFA in 10ml double distilled water with stirring to this 0.01M of secondary ligands (ala, phe, en, phen, ox, mal) and 0.01M of NaOH are slowly mixed with constant stirring at room temperature, to maintain pH-9, the colored solid complexes were precipitated out, are filtered washed with ethanol and dried at 110^{o}C for 1hr

Biological methods
The biological activity of the complexes was performed on the Eleucine Coracana(ragi) seedlings, the accumulation of antioxidants like Glutathione, Ascorbate and Tocopherol were studied by analyzing samples for 24hrs with control seedlings using reversed phase HPLC spectrophotometer C18 column, model Schimadzus.

Antimicrobial test
Antimicrobial activity of the ligand and synthesized complexes were tested against *Staphylococcus-aureus, Bacillus subtilis, Escherichia coli and klebsiella pneumoniae* by using *Kirby Bauer Method*, a Disc diffusion method. PFA, metal salt solutions and DMF are used as controls. 100µgm antimicrobial agents (prepared complexes) were loaded on the discs by soaking in test solution and discs were placed on the surface of the sterile nutrient agar medium with aid of sterile forceps and the plates were incubated at 37^{o}C for 24hrs. Afterwards, the inhibition zones of microbial growth were examined and the diameters measured and recorded in millimeters (mm).

RESULTS AND DISCUSSSION

Physical properties
All Binary and mixed ligand complexes are stable at room temperature and are non-hygroscopic. On heating, they decompose at high temperatures without melting. The complexes are insoluble in water and common organic solvents but are soluble in DMF. The analytical data and physical properties of the ligand and complexes are summarized in Table 1, 2&3.

Elemental analysis
The analytical data for metals (Ni,Cu,Zn), and elements like C,H,N,O,P of the complexes and their compositions are assigned and formulated as presented in Table-1. The theoretical values of the elements in these complexes are calculated on the assumption that the metal and ligands are in the ratio 1:1 in binary complexes and 1:1:1 ratio in mixed ligand complexes and each complex is also been associated with two water molecules. It is clear from the data that the experimental values shown for each of the compound are in good agreement with the theoretical values calculated for both binary and mixed ligand complexes.

Molar Conductance

The Molar Conductance of metal complexes were measured using 10^{-3}M DMF solvent, the obtained values are in the range of 0.124-0125Ω^{-1} cm^2 mol^{-1} suggest the presence of a non-electrolytic nature[38,39] and that no anions are present outside the coordination sphere.

Magnetic susceptibility

Magnetic data was obtained at RT by Gouy technique using Hg [Co $(NCS)_4$] as calibrant. The data were corrected for diamagnetism using Pascal's constants. The magnetic moment values for Ni(II) and Cu(II) binary and mixed ligands were found to be higher than the calculated values for unpaired electrons indicating their paramagnetic nature.

The configurations possible for the metal complexes [Ni(II), Cu(II), & Zn(II)] were evaluated using the semi empirical and density functional theory calculations respectively. PM3[40-42] a semi empirical self-consistent field method was employed to obtain the 3D-Geometry and relative energies of the possible isomers of Ni^{2+},Cu^{2+},Zn^{2+} complexes and molecular modeling studies shows the octahedral geometry for all the synthesized complexes.

Table-1: Physical properties and analytical data of PFA, binary and mixed ligand complexes of Ni(II), Cu(II) ,Zn(II)

							Elemental Analysis, %found(%calculated)					
Ligand/complexes	**color**	**Molecular formula**	**M.Wt (g)**	**Yield %**	**M.P (ºC)**	**Magnetic susceptibility (B.M)**	**C**	**H**	**N**	**O**	**P**	**Ni,Cu,Zn**
PFA	white	CH_3O_5P	300	99	90	-	4.0	0.6	-	26.66	10.33	-
Ni-PFA	Light green	CH_8O_9PNi	253.69	85	290	2.9	4.73 (4.64)	3.15 (3.19)	-	56.80 (56.93)	12.24 (12.32)	22.91 (22.98)
Ni-PFA-ala	Dark green	$C_4H_{10}NO_9PNi$	305.69	80	>320	3.12	15.72 (15.82)	3.26 (3.23)	4.57 (4.62)	47.24 (47.20)	10.15 (10.11)	19.02 (19.23)
Ni-PFA-phe	Dark green	$C_{10}H_{14}NO_9PNi$	381.69	87	>360	3.14	31.48 (31.50)	3.66 (3.72)	3.68 (3.72)	37.80 (37.91)	8.12 (8.16)	15.21 (15.22)
Ni-PFA-en	green	$C_3H_{10}N_2O_7PNi$	273.69	82	>340	3.0	13.17 (13.69)	2.93 (2.89)	10.24 (10.35)	41.04 (41.06)	11.34 (11.39)	21.23 (21.26)
Ni-PFA-phen	green	$C_{13}H_{12}N_2O_7PNi$	397.69	80	>340	3.2	39.20 (39.22)	3.28 (3.01)	7.04 (7.01)	28.11 (28.16)	7.71 (7.79)	14.72 (14.80)
Ni-PFA-ox	green	$C_3H_4O_{11}PNi$	305.69	82	>360	3.3	11.81 (11.85)	1.30 (1.36)	-	57.27 (57.32)	10.14 (10.16)	19.02 (19.05)
Ni-PFA-mal	green	$C_4H_6O_{11}PNi$	319.69	79	>360	3.32	15.02 (15.06)	1.86 (1.89)	-	55.19 (55.15)	9.71 (9.75)	18.17 (18.21)
Cu-PFA	blue	CH_8O_9P Cu	258.5	89	260	2.05	4.63 (4.68)	3.11 (3.09)	-	55.72 (55.70)	11.97 (11.99)	24.39 (24.37)
Cu -PFA-ala	Bluish green	$C_4H_{10}NO_9P$ Cu	310.5	85	>360	2.15	15.42 (15.48)	3.21 (3.25)	4.51 (4.55)	46.38 (46.37)	9.97 (9.95)	20.44 (20.46)
Cu -PFA-phe	Bluish green	$C_{10}H_{14}NO_9P$ Cu	386.5	80	>360	2.17	31.02 (31.09)	3.63 (3.62)	3.61 (3.68)	37.27 (37.25)	8.01 (8.06)	16.42 (16.40)
Cu -PFA-en	Bluish green	$C_3H_{10}N_2O_7P$ Cu	278.5	78	>340	2.10	12.92 (12.89)	2.88 (2.85)	10.06 (10.03)	40.18 (40.20)	11.14 (11.10)	22.78 (22.80)
Cu -PFA-phen	Bluish green	$C_{13}H_{12}N_2O_7PCu$	402.5	77	>360	2.17	38.79 (38.72)	2.95 (2.92)	6.90 (6.95)	27.79 (27.82)	7.68 (7.71)	15.71 (15.75)
Cu -PFA-ox	Light blue	$C_3H_4O_{11}P$ Cu	310.5	75	>360	1.96	11.61 (11.65)	1.27 (1.23)	-	56.69 (56.65)	9.97 (9.95)	20.43 (20.45)
Cu -PFA-mal	Light blue	$C_4H_6O_{11}P$ Cu	324.5	75	>360	2.18	14.77 (14.72)	1.83 (1.87)	-	54.21 (54.25)	9.56 (9.59)	19.55 (19.56)
Zn-PFA	white	CH_8O_9P Zn	260.39	80	>300	DM	4.59 (4.63)	3.06 (3.02)	-	55.29 (55.31)	11.91 (11.87)	25.10 (25.16)
Zn -PFA-ala	white	$C_4H_{10}NO_9P$ Zn	312.39	82	>350	DM	15.35 (15.38)	3.21 (3.26)	4.47 (4.49)	46.10 (46.08)	9.91 (9.96)	20.92 (20.95)
Zn -PFA-phe	white	$C_{10}H_{14}NO_9PZn$	388.39	80	>330	DM	30.88 (30.86)	3.61 (3.64)	3.60 (3.66)	37.08 (37.04)	7.79 (7.75)	16.82 (16.84)
Zn -PFA-en	white	$C_3H_{10}N_2O_7PZn$	280.39	85	>360	DM	12.84 (12.89)	2.84 (2.85)	9.99 (9.95)	39.10 (39.08)	11.02 (11.05)	23.36 (23.30)
Zn -PFA-phen	white	$C_{13}H_{12}N_2O_7PZn$	404.39	85	>360	DM	38.55 (38.57)	2.92 (2.96)	6.90 (6.94)	27.68 (27.65)	7.63 (7.66)	16.15 (16.11)
Zn -PFA-ox	white	$C_3H_4O_{11}P$ Zn	312.39	77	>360	DM	11.54 (11.52)	1.27 (1.26)	-	56.35 (56.33)	9.91 (9.95)	20.92 (20.85)
Zn -PFA-mal	white	$C_4H_6O_{11}P$ Zn	326.39	75	>360	DM	14.69 (14.74)	1.82 (1.87)	-	53.94 (53.98)	9.48 (9.50)	20.01 (20.06)

Infrared spectra of PFA, Binary and Mixed ligand Complexes

The significant infrared spectra bands of PFA, binary and mixed ligand complexes are presented in table-2 and spectra obtained in the region 4000-400cm^{-1}. The infrared spectra of pure ligand shows a sharp band at 1694cm-1 corresponds to υ(C=O) stretching frequency [36-38]. The absorption bands 1435cm^{-1}&1313cm^{-1}is assigned to be υ(P-C) and υ(P=O) and symmetry stretching frequency 1281cm^{-1} and 919cm^{-1} are due to presence υ(P-O). The absorption bands at 2944cm^{-1} show presence of υ(P-O-H) stretching vibrations and at 3800-3500cm^{-1}is attributed to the existence of stretching υ(OH) band [39-42].

The infrared data of prepared binary and mixed ligand complexes shows a shifting of various absorption bands to lower frequency and appearance of some new bands in the spectra when compared with free ligand. The broad band in the region of 4000 - 3854cm^{-1} is attributed to existence of coordinated water molecules in the complex [43-54]. The bands at 3644 and 3465cm^{-1} are due to υ(OH) stretching frequency indicating that it is involved in the complexation with a coordinate bond to metal ion[55-57]. The band at 1694cm^{-1} corresponds to υ(C=O) absorption for carboxylate group in free ligand is shifted to lower frequency in the complexes 1500-1649cm^{-1}, υ(P─C) and υ(P─O) band absorption at 1435-1313 cm^{-1} in free ligand is shifted to a lower frequency in the complexes to 1426-1259cm^{-1}. The absorption bands at 1033cm^{-1} and 918cm^{-1} corresponds to υ(P─O─C) and υ(P─O). All complexes shows new absorption bands due to υ(M-N), υ(M-O) vibrational modes in the region 417-600cm^{-1}[58-61]. A characteristic peaks corresponds υ(O─O) & υ(M─O─O) stretching frequency are absorbed in the region 745-845cm^{-1} shows that there is a complexation, which is further confirmed by absorption peak at 2638cm^{-1}which is expected for a chelate ring.

Table-2 Infrared spectra of PFA, Binary& Mixed ligand Complexes

Ligand/complexes	**υ(C=O)**	**υ(P-C)**	**υ(P=O)**	**υ(P-O)**	**υ(OH)**	**υ(N-H)**	**υ(M-N)**	**υ(M-O)**	**υ(O-O)**
PFA	1694	1435	1313	1281	-	-	-	-	-
Ni-PFA	1694	1426	1259	-	3644(BB)	-	-	588	846
Ni-PFA-ala	1578	1384	-	1037	3642	3447	800-700	518	-
Ni-PFA-phe	1617	1384	-	-	3642	3422	750	526	-
Ni-PFA-en	1649	1364	-	-	3854	3434	656	-	-
Ni-PFA-phen	1649	1427	-	-	3845(BB)	3447,1516	725	-	845
Ni-PFA-ox	1636	1384	-	-	3646	-	-	519	-
Ni-PFA-mal	1585	1384	-	1021	3647	-	-	520,447	-
Cu-PFA	1579	1362	1280	918	3468	-	-	527,424	-
Cu -PFA-ala	1617	1384	-	-	3568	3422	698	463	-
Cu -PFA-phe	1620	1324	-	-	3500	3332	755	557	-
Cu -PFA-en	1578	1436	1360	-	3600	3466	600	422	-
Cu -PFA-phen	1585	1432	-	-	3854	3412	721	458	-
Cu -PFA-ox	1678	1414	1278	-	3535	-	-	498	810
Cu -PFA-mal	1578	1363	1279	937	3643	-	-	492	745
Zn-PFA	1654	1384	-	-	3854(BB)	-	-	432	-
Zn -PFA-ala	1596	1384	-	-	3854	3421	650	428	-
Zn -PFA-phe	1621	1386	-	-	3650	3334	722	470,417	837
Zn -PFA-en	1550	1383	-	-	3800	3448	-	435	-
Zn -PFA-phen	1600	1384	-	-	-	3374	724	484	-
Zn -PFA-ox	1578	1384	-	-	3751	-	-	436	-
Zn -PFA-mal	1586	1384	-	-	3433	-	-	428	-

Biological activity of the binary and mixed ligand complexes on Eleucine Coracana (ragi seedlings

Plants require metals like Nickel, Copper and Zinc etc., in minute quantities for certain metabolic process but at high levels metals can damage membranes, DNA and other cell components. However most plants try to protect themselves from the damaging effects caused by high levels of metals, by increasing the production of antioxidants [62] like glutathione[63-66], Ascorbate and Tocopherol which refer to a broad class of compounds that protects cells from damage when exposure to certain highly reactive compounds.

The result reveals that activity of metal precursors on Control seedlings (ragi seedlings) exhibited very low levels of Ascorbate (0.9µg/ml) Glutathione (0.9µg/ml) and Tocopherol (1.1µg/ml). In seedlings treated with Ni precursor the Ascorbate levels were elevated (24.8µg/ml) compared to those treated with Cu (4.5µg/ml) and Zn (6.4µg/ml). However the antioxidants levels are increases to large extent when treated with binary and ternary complexes for different antioxidants. The binary complexes of these metals showed increased levels of Ascorbate with Ni (24.8µg/ml), Cu (7.4µg/ml) and Zn (37.6µg/ml). Theternary complexes caused an enhancement of Ascorbate levels of Ni, Cu, and Zn in the seedlings to 9.2µg/ml, 13µg/ml and 21µg/ml.

Glutathione, an ubiquitous antioxidant, was found to be present in high levels in seedlings treated with Zn (6.6µg/ml) and Ni (3.6µg/ml). However the levels of Glutathione in plants treated with Cu had the same levels as control (0.9µg/ml). The binary complexes of Cu and Zn had little effect on the accumulation of glutathione (2.9µg/ml and 1.5µg/ml) as compared with that of control and accumulation of glutathione of Ni is 14.6µg/ml. Treatment with Zn mixed ligand complexes showed maximum increase in the level of glutathione (9.1µg/ml) and

Cu ternary showed minimum increase (1.8µg/ml) and Ni an intermediate response (2.4µg/ml). Tocopherol levels are high for zinc ternary (22µg/ml) compare to nickel ternary complex (15µg/ml) and the copper complex same as binary. From present study thus concluded that antioxidants levels for same plants species with different metal complexes shows varying results not only the metals but also the metal complexes also induce biological activity by increasing in the antioxidant levels like Ascorbic acid, Glutathione and Tocopherol in the plant.

Table – 3 Biological activity of Binary and Mixed ligand complexes on Eleucine Coracana (ragi) (µg/ml)

Antioxidants	control	Ni	Ni-Bin	Ni-Ter	Cu	Cu Bin	CuTer	Zn	Zn-Bin	Zn-Ter
Ascorbate	0.9	24.8	24.8	9.2	4.5	7.4	13	6.4	37.6	21
Glutathione	0.9	3.6	14.6	2.4	0.9	2.9	1.8	6.6	1.5	9.1
Tocopherol	1.1	7.8	3.5	15	1.1	1.1	1.1	10.5	0.2	22

Antimicrobial activities

The antimicrobial activity of ligand PFA, metal precursors, synthesized binary and mixed ligand complexes of Ni(II), Cu(II), Zn(II) and the solvent DMF were tested against *Staphylococcu saureus, Bacillus subtilis, Escherichia coli and klebsiella pneumoniae*. All the metal ions used have varying antibacterial influence on bacterial species. The antibacterial activity of the synthesized complexes has greater effect. The Staphylococcus aureus is inhibited by mixed ligand complexes the best, followed by binary complexes. *Bacillus subtilis and klebsiella pneumoniae* inhibition was found to be moderate and *Escherichia coli* least. The antibacterial activity results were given in the table 4. The Staphylococcus aureus, a Gram +ve bacterium is getting inhibited by the complexes shows an encouraging result. The high antibacterial activity is due to chelate formation. The result of antimicrobial activity test reveals copper is the best microbial inhibitor compare to the nickel and zinc and with increase in chelate weight on bacterial culture, increases the inhibition zone.

Table 4 Antimicrobial activity data of PFA, binary and mixed ligand complexes and controls

S.No	*Ligand/complexes/100µg/disc*	Growth inhibition zone in millimeters(mm)			
		Gram positive		Gram negative	
		S. aur.	*B.subt.*	*E. coli*	*K.pneu*
1	PFA	-	-	-	-
2	DMF	-	-	-	-
3	Ni-PFA	10	-	-	-
4	Ni-PFA-ala	15	-	-	-
5	Ni-PFA-phe	12	-	-	12
6	Ni-PFA-en	12	10	09	10
7	Ni-PFA-phen	14	11	07	11
8	Ni-PFA-ox	13	-	-	-
9	Ni-PFA-mal	12	-	-	-
10	Cu-PFA	12	10	09	10
11	Cu -PFA-ala	21	11	06	11
12	Cu -PFA-phe	20	12	06	13
13	Cu -PFA-en	27	14	10	14
14	Cu -PFA-phen	25	12	12	14
15	Cu -PFA-ox	22	13	10	12
16	Cu -PFA-mal	24	11	09	10
17	Zn-PFA	08	08	-	09
18	Zn -PFA-ala	10	-	-	-
19	Zn -PFA-phe	11	-	-	-
20	Zn -PFA-en	14	16	-	15
21	Zn -PFA-phen	12	18	-	13
22	Zn -PFA-ox	11	-	-	-
23	Zn -PFA-mal	12	-	-	-

CONCLUSION

From the present study it is concluded that the Ni(II),Cu(II) and Zn(II) binary and mixed ligand complexes are soluble in DMF and are non-electrolytes, Ni(II)and Cu(II) are paramagnetic in nature indicating presence of free electrons, from analytical data, infrared spectra and. The molecular modeling studies show the octahedral geometry for all the complexes.

Biological studies reveal that not only metals but also the binary and mixed ligand complexes induce biological activity by increase in the antioxidant levels like, Ascorbate, Glutathione and Tocopherol in the plants.

The synthesized complexes shows the better antimicrobial activity compare to the ligand and also found that there is an increase of inhibition zone on bacterial culture with the increase of the chelates weight.

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