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SYNTHESIS AND BIOCIDAL ACTIVITY OF IRON (II,III), COBALT (II,III) AND NICKEL (II) DIPYRIDYL COMPLEXES

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The biological activities of Fe^{2+}-, Fe^{3+}-, Co^{2+}-, Co^{3+}- and Ni^{2+}- dipyridyl complexes as well as the metal salts of $(NH_4)_2SO_4$. $FeSO_4$. $6H_2O$), $FeCl_3$. $6H_2O$), Na_3 $[Co(NO_2)_6]$, $(NH_4)_2SO_4$. $NiSO_4$. $6H_2O$, 2,2-dipyridyl and mixtures of some amino acids or some vitamins alone or mixtures of cyanocobalamine+ thiamine+ pantothenic acid, inositol + nicotonic acid +folic acid, ascorbic acid + biotin + riboflavin, vitamin B-complex have been investigated against some bacteria facultative *Bacillus stearothermophilus*, halophilic *Bacillus subtilus* strains (at 30 and 55°) and obligate Bacillus stearothermophilus (at 55°) and fungi *Aspergillus fumigatus* (at 30 and 50°), *Penicilliumnigrlacans* (at 30°) and *Humicola lanuginosa* (at 30 and 55°) species. It is concluded that dipyridyl is antibacterial and antifungal, and the bioactive effect of metal salts and their complexes increases with the increase of concentration and temperature. However, they are inactive towards bacteria and fungi species on using 50 µg concentration per disc. On the other hand, mixtures of dipyridyl and amino acids, and dipyridyl and vitamins have combined bioactive effects on bacteria and fungi species.

Key words: Biocidal activity, Metal ion, Biological system.

Introduction

The activity of various metal ions, organic compounds and metal complexes in biological systems has been explained on the basis of the different complexes species formed in the living organisms. Metal ions are found in several bacteria and fungi species, and reported to play an important role in different enzymatic and physiological reactions [1,2]. Thiazolidinones as potential antitubercular compounds [3], naphthyridine derivatives as antibacterial and antimalarial (active) compounds [4-11], violuric acid, violurates and dinitrosoresorcinolates as antibacterial and antifungal bioactive compounds [12], and pyrazol derivatives as antibacterial and antimicrobial (toxic) compounds [13,14] had been used. However, the biological activity of iron, cobalt and nickel dipyridyl complexes had not been studied before. Therefore, the study of which is of interest.

The present study reports the synthesis of the solid complexes of Fe^{2+}–, Fe^{3+}–, Co^{2+}–, Co^{3+}–, and Ni^{2+}– dipyridyl and testing the biological activity of the complexes as well as dipyridyl and the metal salts of Fe^{2+}–, Fe^{3+}–, Co^{2+}–, Co^{3+}– and Ni^{2+} –against some bacteria and fungi species.

Experimental

All the chemicals used are of Analar grade.

Preparation of Solid Complexes [12]. The solid complexes were synthesised by mixing aqueous solutions of metal salts $(NH_4)_2SO_4$. $FeSO_4$. $6H_2O$, $FeCl_3$. $6H_2O$, $CoCl_2$. $6H_2O$, Na_3 $[Co(NO_2)6]$, $(NH_4)_2SO_4$. $NiSO_4$. $6H_2O$ with alcoholic solutions of 2,2-dipyridyl in mole ratios M:L (1:1, 1:2, 1:3). The Fe^{2+}– dipy (1:1, 1:2) complexes were isolated by evaporation and addition of n-BuOH. The Fe^{3+}–, Co^{2+}–, Co^{3+}– and Ni^{2+}– dipy mixtures were evaporated near dryness, thereafter, acetone and n-BuOH were added whereby the solid complexes precipitated. The solid complexes were washed well several times using n-BuOH then dried. The isolated complexes were analysed and the chemical composition was formulated. The results obtained are cited in Table 1.

TABLE 1. RESULTS OF CHEMICAL ANALYSIS OF Fe^{2+}-, Fe^{3+}-, Co^{2+}-, Co^{3+}- AND Ni^{2+}- DIPY COMPLEXES.

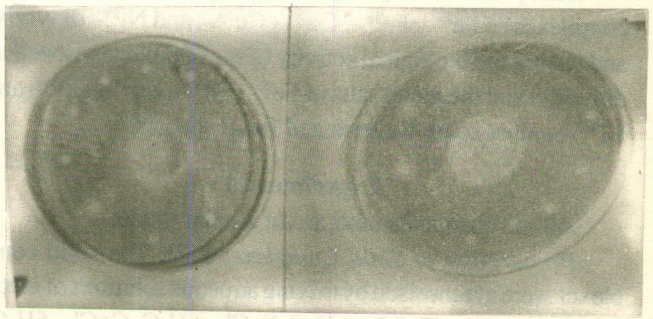
Compound	Analysis % Found/(Calcd)				
	C	H	N	Cl	M
$FeC_{10}H_8N_2.SO_4.6H_2O$	28.41	5.27	6.92	–	13.95
	(28.86)	(4.85)	(6.73)	–	(13.42)
$Fe(C_{10}H_8N_2)_2.SO_4.6H_2O$	41.38	5.20	9.90	–	9.44
	(41.97)	(4.93)	(9.97)	–	(9.76)
$FeC_{10}H_8N_2.Cl_3.H_2O$	35.38	2.70	8.13	31.45	16.45
	(35.70)	(3.0)	(8.33)	(31.62)	(16.60)
$Fe(C_{10}H_8N_2)_2.Cl_3.4H_2O$	43.19	3.96	9.89	19.55	10.74
	(43.94)	(4.39)	(10.24)	(19.46)	(10.22)
$Fe(C_{10}H_8N_2)_3.Cl_3.6H_2O$	48.47	4.38	11.72	14.50	7.72
	(48.76)	(4.91)	(11.37)	(14.40)	(7.56)
$CoC_{10}H_8N_2.Cl_2.3H_2O$	34.52	3.63	8.14	20.73	17.37
	(35.31)	(4.10)	(8.24)	(20.85)	(17.33)
$Co(C_{10}H_8N_2)_2.Cl_2.3H_2O$	47.95	4.64	11.10	14.30	11.72
	(48.40)	(4.47)	(11.29)	(14.26)	(11.87)
$CoC_{10}H_8N_2.(NO_2)_3.2H_2O$	31.05	3.07	17.85	–	15.26
	(30.87)	(3.10)	(18.00)	–	(15.14)
$Co(C_{10}H_8N_2)_2.(NO_2)_3.2H_2O$	43.35	3.63	17.62	–	11.02
	(44.05)	(3.70)	(17.98)	–	(10.81)
$Co(C_{10}H_8N_2)_3.(NO_2)_3.4H_2O$	48.95	4.64	16.80	–	8.26
	(48.86)	(4.37)	(17.09)	–	(7.99)
$NiC_{10}H_8N_2.SO_4.6H_2O$	29.22	4.38	6.59	–	14.15
	(28.66)	(4.77)	(6.68)	–	(14.01)
$Ni(C_{10}H_8N_2).SO_4.6H_2O$	14.52	4.56	9.62	–	10.25
	(14.76)	(4.90)	(9.74)	–	(10.21)

The antimicrobial activity of 2,-2-dipyridyl, metal salts [$(NH_4)_2SO_4 . FeSO_4 . 6H_2O$, $CoCl_2 . 6H_2O$, Na_3 [Co $(NO_2)6$], $(NH_4)_2SO_4 . NiSO_4 . 6H_2O$] and solid complexes using 5, 10, 50 and 100 µg concentrations per disc were tested and evaluated in vitro against facultative thermophilic Bacillus stearothermophilus (F.B. st.) L.N. 94, *Bacillus subtilus* (B. sub.) [incubation temperatures 30 and 55° to study the effect of compounds at mesophilic conditions (30°) and at thermophilic conditions (55°)] and Obligate thermophilic Bacillus stearothermophilus (O.B.st) L.N. 90 (at 55°) using nutrient agar medium (nutrient agar medium was prepared by dissolving 2g of beef extract "Difco" in few ml of hot water and then mixed in a mortar, and triturated with 5g peptone, 2.5g NaCl and 20g agar, and the volume was made lL. The pH was adjusted to 7.2 and the medium was autoclaved at 1.5 atm for 15 min), while in the case of *Aspergillus fumigatus* (A.f.) (at 30 and 50°), *Penicillium nigrlacans* (P.n.) (at 30°) and *Humicola lanuginosa* (H.l.) (at 30 and 50°) Czapecks agar media were employed following Gould Bowie method [16]. (The media were prepared by dissolving mixture of 9g glucose, 7g $NaNO_3$, lg KH_2PO_4, 0.5g $MgSO_4 . 7H_2O$, 0.5g KCl and 20 g agar in lL bidistilled water, and the media were diffused on filter paper disks). The combined effects of amino acids or water soluble vitamins with free ligand in vitro were also carried out following Lamana and Shapiro technique [17].

Results and Discussion

The data obtained are recorded in Tables 1-4. The results cited in Table 2 show that dipyridyl is toxic towards bacteria and fungi species used, and toxicity increases with increasing dipyridyl concentration (5 µg dipy is nonperceptible) at constant temperature and decreases with the increase of temperature. Activity of 100 µg of the metal salts per disk against bacteria species increases slightly with increasing temperature (5 and 50 µg have no effect and may be useful for bacteria growth). However, all concentrations of metal salts (except 100 µg $CoCl_2 . 6H_2O$) used are inactive towards fungi species. The biological activity of the complexes against bacteria increases with increasing molecular weights (and dipyridyl concentration in complexes) and with the increase of temperature. However, these complexes except Co^{2+}– dipy (1:1 and 1:2) are nontoxic fungi species. Therefore, these substances may be used for overgrowth of *Aspergillus fumigatus, penicillium nigrlacans* and *Humicola lanuginosa*, particularly, *penicillium nigrlacans* which is being considered as a source for producing pencilline. The antimicrobial activity of dipyridyl is more effective than that of metal salts or complexes (Fig. 1). This may be that dipyridyl has comperatively faster diffusion through bacteria and fungi cells [18]. The biocidal activity of dipyridyl, metal salts and complexes towards bacteria species under study, may be attributed to that these compounds break the peptide linkage and lead to the formation of mixed metal-mixed ligand complexes as a result of the interaction of the released trace metals with ribonucleic acid present in the microbial cells and with the metal salts or complexes [19,20]. Inactivity (nontoxicological effects on bacteria and fungi species) of metal salts or complexes towards fungal species may be because the cell wall membranes of fungi species (Eucaryotae) are more rigid than the membranes of those of bacteria (procaryotae) or the salts or complexes containing these metals may be useful nutrients for the performance of normal metabolism and proper functioning of the living cells of the fungi species under study [21].

On the comparison of the bioactive effect of 2,-2-dipyridyl alone to that of mixtures of water soluble amino acids, individuals or mixtures of vitamins containing the same concentration of dipyridyl towards the tested bacteria and fungi species, it is found that these substances are biologically active against the fungi species, i.e., the toxicological effect of dipyridyl against fungi species is not reduced on mixing with amino acids or vitamins (Table 3). Thus, on using mixtures of amino acids with dipyridyl as in the case of dipy+group 1, and dipy+group 2, the antibacterial effects decrease than that of dipyridyl alone. However, the other mixtures, i.e., dipy+group 3, dipy+group 4 and dipy+group 5, have bioactive effects. Therefore, they can be used as antibactericides and antifungicides. Decrease of activity (10-34%) in the case of dipy+group 1 or dipy+group 2 against the tested bacteria species may be attributed to the involvement of some hydroxy (threonine and L-serine in group 1, L-tyrosine in group 2) or sulphur (L-cysteine in group 2) contained in the amino acids present in the mixtures used. The hydroxyl and the amino group present in the amino acid may bound together to form NH_2.....OH linkage which may decrease or reduce toxicity. This is in addition to the consumption of sulphur (nutrient) for growing bacteria. However, other amino acids in the two group mixtures have bioactive effects. Thus, the combined effect on



A: Penicillum nigrlcans B: Aspergillus fumigatus

Fig. 1. Spot in centre refers dipyridyl while those around the centre refer to metal-dipyridyl complexs

bacteria is positive. Dipy+group 4 mixture exhibits antibacterial effect equals 100% with O.B. st., 95% with F.B. st and 83% with B. subt. and 100% with A.f., P.n. and H.l., whereas dipy+group 5 mixture shows antimicrobial effect equals 100% with the tested bacteria and fungi species. The greater antimicrobial effect of dipy+group 3, dipy+group 4 (with F.B. st., O.B. st.) and dipy+group 5 mixtures against bacteria may be related to the combined effect of the dipyridyl and the two carboxylic groups involved in the amino acids (two carboxyl groups constitute a quite bulky ligand which have steric interference of the carboxylates) which may form strong and very stable mixed ligand complexes of lipophilic nature [19,20].

On using binary mixtures consisting of dipyridyl and

TABLE 2. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF 2,-2-DIPYRIDYL, METAL SALTS AND Fe^{2+}-, Fe^{3+}-, Co^{2+}-, Co^{3+}- AND Ni^{2+}- DIPY COMPLEXES.

	Disc potency (μg)	Inhibition zone diameter (mm)									
		Antibacterial						Antifungal			
		F.B.st		O.B.st	B.sub			A.f.		P.n.	H.l.
		30	55°	55	30	55°	30	50	30	30	55°
Dipyridyl	50	11	10	--	9	14	9	8	12	10	--
	100	30	20	21	25	22	37	43	45	40	25
$(NH_4)_2SO_4FeSO_4.6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	10	13	9	--	--	--	--	--	--	--
Fe. dipy.SO_4. $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	8	8	9	8	9	--	--	--	--	--
$Fe(dipy)_2.SO_4$. $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	9	8	11	9	10	--	--	--	--	--
Fe Cl_3 . $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	8	8	9	8	9	--	--	--	--	--
Fe. dipy.Cl_3. H_2O	50	--	--	--	--	--	--	--	--	--	--
	100	6	13	12	7	8					
Fe. $(dipy)_2.Cl_3$. $4H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	6	6	10	10	12	--	--	--	--	--
Fe. $(dipy)_3.Cl_3$. $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	10	11	13	10	14	--	--	--	--	--
Co Cl_2 . $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	16	20	20	13	15	12	15	--	16	15
Co. dipy. Cl_2. $3H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	10	12	15	9	13	8	20	--	10	17
Co. $(dipy)_2$. Cl_2. $3H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	14	18	20	12	15	10	30	--	9	20
Na_3 $Co(NO_2)_6$	50	--	--	--	--	--	--	--	--	--	--
	100	8	10	15	11	14	--	15	--	--	--
Co. dipy. $(NO_2)_3$. $2H_2O$	50	--	6	--	6	7	--	--	--	--	--
	100	10	14	13	9	10	--	13	--	--	--
Co. $(dipy)_2$. $(NO_2)_3$. $2H_2O$	50	7	8	--	10	8	--	--	--	--	--
	100	17	19	18	11	14	--	20	--	--	--
Co. (dipy). $(NO_2)_3$. $4H_2O$	50	8	9	--	12	--	--	--	--	--	--
	100	18	24	24	13	16	--	24	--	--	--
$(NH_4)_2SO_4$. $NiSO_4$. $6H_2O$	50	--	--	--	--	--	--	--	--	--	--
	100	12	14	15	16	18	13	--	--	--	--
Ni dipy SO_4 . $6H_2O$	50	--	--	--	--	--	--	--	--	--	--

Sign (--) shows no antimicrobial activity.
The tested compounds at 10 μg concentration exhibit no antimicrobial activity.

TABLE 3. BIOACTIVE EFFECTS OF DIPYRIDYL AND MIXTURES OF AMINO ACIDS ON BACTERIA AND FUNGI SPECIES. [DIPY] = 100 μG, [AMINO ACIDS] = 50 μG T=55°

Mixture	percentage of inhibition of bacterial and fungal growth					
	F.B.st	O.B.st	B.sub.	A.f.	p.n.	H.l.
Dipy (control)	100	100	100	100	100	100
Dipy + Group 1	90	66.6	76.6	100	100	100
Dipy + Group 2	80	76.6	66.6	100	100	100
Dipy + Group 3	100	100	100	100	100	100
Dipy + Group 4	95	100	83	100	100	100
Dipy + Group 5	100	100	100	100	100	100

Group 1 = L - alanine, L - glycine, L - serine, L - cysteine, L - methionine; Group 2 = DL - phenylalanine, L - tryptophan, L - tyrosine, L - histidine; Group 3 = L - aspartic acid, L - glutamic acid, L - proline; Group 4 = L - arginine, L - lysine; Group 5 = Dl - valine, L - isoleucine, L - leucine.

TABLE 4. BIOACTIVE EFFECTS OF MIXTURES OF DIPYRIDYL AND INDIVIDUALS OF VITAMINS OR GROUPS OF VITAMINS ON BACTERIAL AND FUNGAL SPECIES [DIPY] = 100 μG, [VITAMIN] = 50 μG, T = 35°

Compounds	Toxicity percent of bacterial and fungal growth					
	F.b.st	O.b.st	B.sub.	A.f.	P.n.	H.l.
Dipy (control)	100	100	100	100	100	100
Dipy + R	100	100	100	100	100	100
Dipy + F	100	100	100	100	100	100
Dipy + C	80	36	42	100	100	100
Dipy + T	100	100	100	100	100	100
Dipy + N	100	100	30	100	100	100
Dipy + B	100	100	100	100	100	100
Dipy + P	100	20	100	100	100	100
Dipy + A	100	100	100	100	100	100
Dipy + C + T + P	95	43	50.5	100	100	100
Dipy + I + N + F	71	82	83	100	100	100
Dipy + A + B + R	100	100	96	100	100	100
Dipy + V	76	92	53	100	100	100

Dipy = dipyridyl, R = riboflavin, F = folic acid, C = cyanocobalamine, T = thiamine; N = nicotonic acid, B = biotin, P = pantothenic acid, A = ascorbic acid, I = inositol, V = vitamin B-complex.

individuals of vitamins or dipyridyl and more than one vitamin (Table 4), the toxicity of dipy+riboflavin, dipy+thiamine, dipy+nicotonic acid, dipy+biotin, dipy+pantothenic acid (except with O.B. st. species), dipy+ascorbic acid, or mixture of dipy+ascorbic acid+riboflavin+biotin is very strong and as same as that of dipyridyl. However, with others are more or less toxic. Activity of cyanocobalamine+dipy towards F.B. st. decreases by 20% than that of dipy with O.B. st. and B. sp, activity is 36 and 42% respectively. This indicates that cyanocobalamine or pantothenic acid has a marked reducing effect as a result of being considered as enzymatic and metabolic constituents.

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