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Characterization of Metal Chelation with a Mutagenic Agent, 5-Bromouracil

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The interaction of some trace metal ions with a chemical mutagen 5-bromouracil (5-BrU) has been studied spectroscopically and by magnetic measurements. 5-BrU is coordinated to the Mn(II), Ni(II), and Cu(II) ions through the N atom of $N_{(1)}H$ group and the O atom of $C_{(4)}=O$ group whereas to the Co(II), Zn(II), and Cd(II) ions through O atom of $C_{(4)}=O$ group only. The new complexes except Co(II) complex which are insoluble in water and other common organic solvents involve polymeric structures. The tentative νM-O and νM-N band assignments in the lower region favor coordination number six for all complexes.

The term, "mutation", is used to describe changes which alter the chemical structure of the gene at the molecular level.[1] There are many chemicals which can induce mutation, one of them is 5-bromouracil (5-BrU).[2] Since 5-BrU has a close structural resemblance to thymine, it is readily inserted to DNA during its replication (as 5-bromouridylic acid) in the position normally occupied by thymine. Freese initially proposed that the mutagenic action of 5-BrU resides in its enhanced capacity, as compared to thymine, to mispair with guanine.[3] Therefore, DNA replication in the presence of 5-BrU should produce transition mutations resulting from misreplication of template 5-BrU. It has been shown that this analogue does indeed exhibit enhanced mispairing with guanine during in vitro DNA replication.[4,5]

Besides these 5-BrU has also been tested for its various other biological activity.[6—9] Bochkov et al. studied the reaction of dichlorodiammineplatinum(II) with 5-substituted uracil in DMSO and suggested that 5-BrU is coordinated via $N_{(3)}$ atom.[10] Many transition metal ions are known to be present in the naturally occurring nucleic acids, and it is likely that they are responsible for holding the nucleic acids in specific conformations. Since most of the cancer chemotherapeutic agents are chelating agents and many are mutagenic agents,[11] it was thought that synthetic and characterization studies of the complexes of 5-BrU and 3d metal salts would be worthwhile in order to understand the binding sites of the ligand. The aim of the present work is to study the interaction of 3d metal ions with 5-BrU and to determine the preferential coordination sites of the ligand.

Experimental

All the chemicals were of reagent grade. 5-BrU was purchased from Sisco Research Laboratories, India, and the metal nitrates were from E. Merck.

The synthetic procedures employed were as follows: 1 m mol hydrated metal nitrate was dissolved in a mixture of 1 ml distilled water and 14 ml ethanol, and the solution was heated to 60 °C under stirring. Then, 1 mmol ethanolic solution of 5-BrU was added and the resultant mixture was constantly stirred and heated to 70 °C allowing about two third of the solvent to evaporate. The pH of the remaining solution was raised to about 5—7 by adding alkali to precipitate the new complexes. They were filtered, washed several times with absolute ethanol, finally with ether and dried in an oven at 50—60 °C.

Analyses: C, H, and N were analyzed using a Perkin-Elmer model 240 C elemental analyzer in the Department of Chemistry, B. H. U., Varanasi. The metal ions were determined by dissolving the complexes in dilute HNO_3 and titrating against EDTA.[12]

Physical Measurements. The molar conductivity was measured in pyridine by LBR/B type Conductivity-Meter. The IR spectra (4000—200 cm^{-1}) were registered in solid state (Nujol mull) on a Perkin-Elmer model 783 spectrophotometer. Nujol mull electronic spectra of cobalt(II) and nickel(II) complexes were obtained with a Hitachi 330 spectrophotometer whereas those of manganese(II) and copper(II) with a Hitachi 320 spectrophotometer. Magnetic susceptibilities were measured at room temperature by a Faraday magnetic susceptibility balance. Powder X-ray diffraction pattern was taken by a Philips X-ray diffractometer PW 1710 using Cu $K\alpha$ radiation.

Results and Discussion

The composition of the complexes, analytical data, and other physical properties are listed in Table 1. All the complexes displayed 1 : 1 stoichiometry except cobalt(II) which forms 1 : 2 complex. They were insoluble in water and other common organic solvents viz. ethanol, methanol, acetone, ether, carbon tetrachloride, chloroform, benzene, acetonitrile, pyridine, tetrahydrofuran, *N,N*-dimethylformamide, dimethyl sulfoxide etc. except cobalt(II) complex which was soluble in pyridine and *N,N*-dimethylformamide at room temperature. The insolubility of the complexes in various solvents, made impossible the studies like NMR, solution electronic spectra and molar conductivity etc. However, the solubility of cobalt(II) complex in pyridine and *N,N*-dimethylformamide allowed for conductivity measurement.

IR Studies. The IR spectrum of 5-BrU is available in the literature.[13] Some characteristic IR data of 5-BrU and their complexes are presented in Table 2. In the region 3000—3500 cm^{-1} several bands appeared, which may be attributed to -NH and -OH stretching mode. The OH of water absorbs the frequencies in the 3250—3500 cm^{-1} region. The bands in this

Table 1. Analytical Data and Physical Properties of the Complexes

Complex	Color	Found (Calcd) (%)				μ_{eff}	Molar conductance at RT/ohm^{-1} cm^2 mol^{-1}
		M	C	H	N	B.M.	
$\mathrm{Mn(5\text{-}BrU)\cdot 4H_2O}$	Light brown	17.3 (17.3)	15.0 (15.1)	3.4 (3.5)	8.8 (8.8)	5.87	—
$\mathrm{Co(5\text{-}BrU)_2\cdot 4H_2O}$	Pink	11.4 (11.5)	18.8 (18.7)	2.7 (2.7)	10.9 (10.9)	4.89	1.6×10^8
$\mathrm{Ni(5\text{-}BrU)\cdot 4H_2O}$	Light green	18.3 (18.2)	15.0 (14.9)	3.4 (3.4)	8.7 (8.7)	3.11	—
$\mathrm{Cu(5\text{-}BrU)(OH)_2\cdot 2H_2O}$	Intense green	19.6 (19.6)	14.8 (14.8)	2.8 (2.8)	8.6 (8.6)	2.02	—
$\mathrm{Zn(5\text{-}BrU)(OH)_2\cdot 3H_2O}$	White	18.9 (19.0)	13.9 (13.9)	3.2 (3.2)	8.2 (8.1)	Dia.	—
$\mathrm{Cd(5\text{-}BrU)(OH)_2\cdot 3H_2O}$	White	28.8 (28.7)	12.3 (12.3)	2.8 (2.8)	7.2 (7.2)	Dia.	—

Table 2. Selected Infrared Vibrations of 5-BrU and Its Metal Complexes (cm^{-1})

Assignment	5-BrU	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)
νO-H (water)	—	3340_{w} 3450_{w}	3260_{w} $3410_{\mathrm{m,b}}$	3345_{w} $3430_{\mathrm{m,b}}$	3365_{w} 3400_{w}	3325_{w} 3440_{w}	3320_{w} 3422_{w}
νN-H	$3170_{\mathrm{m,b}}$	3120_{m}	3180_{w}	$3220_{\mathrm{w,b}}$	3132_{w}	3170_{w}	3170_{w}
$\nu C_{(2)}$=O	1705_{s}	1705_{s}	1705_{s}	1707_{s}	1701_{s}	1705_{s}	1705_{s}
$\nu C_{(4)}$=O, νC=C in phase	1680_{s}	1655_{s}	1650_{s}	1652_{s}	1664_{s}	1655_{s}	1658_{s}
$\delta N_{(1)}$-H	1517_{w}	1497_{m}	1517_{w}	1495_{m}	1497_{m}	1515_{s}	1515_{m}
νC-Br	1463_{s}	1465_{s}	1462_{s}	1463_{s}	1465_{s}	1468_{s}	1465_{m}
$\delta N_{(3)}$-H	1420_{m}	1420_{m}	1425_{w}	1418_{w}	1420_{m}	1420_{m}	1418_{m}
νC-Br	1378_{s}	1380_{s}	1380_{s}	1379_{s}	1380_{s}	1380_{s}	1380_{s}
(OH) bridging	—	—	—	—	$950_{\mathrm{m,b}}$	$935_{\mathrm{m,b}}$	$945_{\mathrm{m,b}}$
νM-O (water)	—	379_{w}	435_{m}	408_{w}	470_{w}	375_{w}	355_{w}
νM-O	—	241_{m}	230_{m}	243_{m}	244_{w}	243_{w}	244_{w}
νM-N	—	222_{m}	—	$227_{\mathrm{m,b}}$ 263_{w}	280_{m} 290_{m}	—	—

region do not appear in the spectrum of 5-BrU, but appear in the spectra of their complexes indicating the presence of water molecules.[14] The presence of νM-O (water) bands in the lower region of these compounds confirm the binding of water to the complexes.[15] The νN-H bands of 5-BrU (3170 cm^{-1}) shifts to lower frequency region in the Mn(II), Ni(II), and Cu(II) complexes but remain unaffected in Co(II), Zn(II), and Cd(II) complexes, indicating that NH group is involved in coordination with Mn(II), Ni(II), and Cu(II) ions. The spectra of Mn(II), Ni(II), and Cu(II) compounds show sizeable shifts of $N_{(1)}$H deformation mode to lower wavenumbers. This type of IR behavior may be suggestive the participation of the $N_{(1)}$H nitrogen in the coordination of these systems. The $C_{(2)}$=O and $C_{(4)}$=O stretches have been assigned in 5-BrU to strong bands at 1705 and 1680 cm^{-1}, respectively. The $\nu C_{(4)}$=O band is shifted to lower wavenumbers upon complexation but $\nu C_{(2)}$=O band remains unchanged suggesting that the metal ions coordinate through $C_{(4)}$=O group and not with $C_{(2)}$=O group of 5-BrU. The bands due to νC-Br and $\delta N_{(3)}$H of 5-BrU are unaffected in all the complexes and this excludes the participation of these groups in the complexation.

The metal-oxygen stretching vibrations appear in the region 225—245 cm^{-1} for six, and at 276 cm^{-1} for four coordination number,[16,17] the presence of characteristic bands in the region 225—245 cm^{-1} strongly suggest that the metal ions coordinate through oxygen atom of 5-BrU and favor coordination number six for all complexes. The Mn(II), Ni(II), and Cu(II) complexes show νM-N bands in the lower region[14] which suggests that the 5-BrU behaves as bidentate ligand in these compounds.

In the complexes of Cu(II), Zn(II), and Cd(II) metal ions, the presence of a medium broad band in the region 950—930 cm^{-1} may be assigned as OH bridging.[15] Taking into consideration of these bands and insolubility of the complexes in various organic solvents, the polymeric structures are favored for all the compounds except for Co(II), which is soluble in pyridine and *N*,*N*-dimethylformamide. The polymerization occurs through OH bridging group in Zn(II) and Cd(II) complexes and through $N_{(1)}$H and $C_{(4)}$=O group of 5-BrU in Mn(II) and Ni(II) compounds whereas through OH bridging as well as through $N_{(1)}$H and $C_{(4)}$=O group of 5-BrU in Cu(II) complexes.

X-Ray Powder Diffraction Studies. Table 3 shows

Table 3. X-Ray Data of the Complexes

Powder pattern line	2 θ	d Value	Relative intensity	Q_{obs}	Q_{cal}	*hkl*
$\mathrm{Mn(5\text{-}BrU)\cdot 4H_2O}$						
1	13.928	6.3581	46	0.0247	0.0247	100
2	14.548	6.0887	80	0.0269	0.0269	010
3	19.822	4.4789	93	0.0498	0.0498	001
4	25.350	3.5134	171	0.0810	0.0807	030
5	28.173	3.1674	63	0.0996	0.0996	002
6	29.456	3.0323	139	0.1087	0.1076	040
7	32.681	2.7400	125	0.1331	0.1323	140
8	37.228	2.4151	39	0.1714	0.1729	700
9	41.173	2.1924	54	0.2080	0.2086	350
10	41.732	2.1643	51	0.2134	0.2130	170
11	43.799	2.0668	35	0.2341	0.2341	052
12	48.551	1.8751	31	0.2844	0.2849	560
13	49.082	1.8561	46	0.2902	0.2915	290
14	49.621	1.8372	37	0.2962	0.2976	630
15	50.992	1.7909	34	0.3117	0.3118	570
16	52.291	1.7495	42	0.3267	0.3267	523
$\mathrm{Co(5\text{-}BrU)_2\cdot 4H_2O}$						
1	14.046	6.3050	121	0.0251	0.0251	100
2	14.832	5.9726	219	0.0280	0.0280	010
3	20.379	4.3577	369	0.0526	0.0526	001
4	25.826	3.4497	585	0.0840	0.0840	030
5	28.226	2.1616	163	0.1000	0.1004	400
6	29.868	2.9914	355	0.1117	0.1120	040
7	31.824	2.8118	134	0.1264	0.1255	500
8	33.191	2.6991	230	0.1372	0.1371	140
9	36.550	2.4584	131	0.1654	0.1651	150
10	37.541	2.3958	126	0.1742	0.1757	700
11	40.440	2.2305	177	0.2010	0.2008	800
12	41.551	2.1733	103	0.2117	0.2124	440
13	42.602	2.1221	81	0.2220	0.2231	170
14	45.986	1.9736	76	0.2567	0.2568	820
15	48.758	1.8676	60	0.2867	0.2857	304
16	49.582	1.8385	111	0.2958	0.2944	034
17	50.560	1.8052	80	0.3068	0.3060	802
18	51.138	1.7862	55	0.3234	0.3128	840
19	52.990	1.7280	81	0.3349	0.3340	812
20	55.400	1.6584	73	0.3636	0.3644	623
21	57.353	1.6065	42	0.3874	0.3861	704
22	60.417	1.5321	56	0.4260	0.4276	046
$\mathrm{Ni(5\text{-}BrU)\cdot 4H_2O}$						
1	11.629	7.6096	40	0.0172	0.0172	100
2	13.855	6.3915	49	0.0244	0.0244	010
3	14.714	6.0203	111	0.0275	0.0275	001
4	16.254	5.4531	36	0.0336	0.0344	200
5	20.267	4.3815	121	0.0520	0.0516	300
6	22.044	4.0323	54	0.0615	0.0619	201
7	23.707	3.7531	153	0.0709	0.0722	102
8	25.627	3.4760	809	0.0827	0.0825	003
9	26.743	3.3334	75	0.0899	0.0894	202
10	28.038	3.1824	65	0.0987	0.0976	040
11	29.835	2.9946	203	0.1115	0.1100	004
12	31.882	2.8069	135	0.1269	0.1276	610
13	32.980	2.7159	184	0.1355	0.1348	520
14	33.321	2.6889	73	0.1383	0.1376	800
15	35.779	2.5096	41	0.1587	0.1582	602
16	36.506	2.4613	90	0.1650	0.1650	006
17	37.442	2.4019	82	0.1733	0.1736	350
18	39.804	2.2646	151	0.1949	0.1952	080
19	40.439	2.2305	106	0.2010	0.2008	640
20	41.450	2.1784	61	0.2107	0.2107	035
21	42.363	2.1335	48	0.2196	0.2196	090
22	42.659	2.1194	76	0.2226	0.2235	505
23	44.137	2.0518	63	0.2375	0.2372	108
24	46.163	1.9664	40	0.2586	0.2579	705
25	48.690	1.8701	67	0.2859	0.2854	706
26	49.395	1.8450	79	0.2937	0.2932	308
27	50.514	1.8067	78	0.3063	0.3060	508
28	51.285	1.7814	64	0.3151	0.3156	780
29	52.790	1.7341	132	0.3325	0.3328	880
30	55.326	1.6605	161	0.3626	0.3620	438
31	60.393	1.5327	53	0.4256	0.4252	278
32	61.946	1.4980	41	0.4456	0.4449	947
33	64.145	1.4518	57	0.4744	0.4737	587
34	65.967	1.4161	36	0.4986	0.4978	886
35	68.108	1.3767	38	0.5276	0.5284	878
36	72.822	1.2987	54	0.5929	0.5944	998
$\mathrm{Cu(5\text{-}BrU)(OH)_2\cdot 2H_2O}$						
1	22.659	3.9242	177	0.0649	0.0649	100
2	23.302	3.8173	152	0.0686	0.0686	101
3	24.922	3.5726	186	0.0783	0.0796	102
4	27.743	3.2155	371	0.0967	0.0981	103
5	36.450	2.4649	223	0.1645	0.1630	113
6	38.340	2.3476	202	0.1814	0.1812	007
7	39.084	2.3046	199	0.1882	0.1889	114
8	46.826	1.9401	212	0.2656	0.2633	201
9	62.872	1.4781	204	0.4577	0.4576	216
10	66.264	1.4104	171	0.5027	0.5057	217
11	78.959	1.2125	162	0.6802	0.6822	313
$\mathrm{Zn(5\text{-}BrU)(OH)_2\cdot 3H_2O}$						
1	12.354	7.1644	56	0.0194	0.0194	100
2	16.268	5.4485	55	0.0336	0.0336	010
3	22.287	3.9888	101	0.0628	0.0628	001
4	26.993	3.3031	123	0.0916	0.0918	310
5	28.599	3.1212	122	0.1026	0.1016	201
6	31.814	2.8128	106	0.1263	0.1256	002
7	41.511	2.1754	65	0.2113	0.2120	440
8	44.480	2.0368	70	0.2410	0.2404	260
$\mathrm{Cd(5\text{-}BrU)(OH)_2\cdot 3H_2O}$ Amorphous						

pertinent X-ray diffraction data. The indexing of the pattern was done by Ito's method.[18] The X-ray powder diffraction lines observed in Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes have been successfully indexed. The indexing pattern yields the lattice constants a=6.36, b=6.09, and c=4.48 Å for Mn(II)-5BrU, a=6.31, b=5.97, and c=4.36 Å for Co(II)-5BrU, a=7.61, b=6.39, and c=6.02 Å for Ni(II)-5BrU, a=b=3.92 and c=16.44 Å for Cu(II)-5BrU, a=7.16, b=5.45, and c=3.99 Å for Zn(II)-5BrU complex indicating orthorhombic symmetry for all these complexes except copper(II) complex which has tetragonal symmetry.

Electronic Spectra and Magnetic Properties. The

Table 4. Electronic Spectral Bands and Ligand Field Parameters

Complex	λ/nm	D_q/cm^{-1}	B'/cm^{-1}	β	β°/%
Mn(5-BrU)·4H$_2$O	432	1594.86	637.94	0.6645	33.55
	308				
	285				
Co(5-BrU)$_2$·4H$_2$O	900	1242.37	828.25	0.9032	9.68
	—				
	450				
Ni(5-BrU)·4H$_2$O	900	1111.10	428.66	0.4117	58.83
	580				
	444				
Cu(5-BrU)(OH)$_2$·2H$_2$O	680	1470.5	—	—	—

electronic spectra of the ligand and their complexes in the solid state are given in the Table 4. The magnetic moment of Mn(II) complex is 5.87 BM and its electronic spectrum exhibits bands at 285 nm, 308 nm, and 432 nm which correspond $^6A_{1g}\rightarrow{}^4A_{2g}(F)$, $^6A_{1g}\rightarrow{}^4T_{1g}(P)$, and $^6A_{1g}\rightarrow{}^4T_{2g}$ respectively in an idealized octahedral geometry.[19,20] The spectrum of Co(II) compound is characterized by main two bands at 450 nm and 900 nm which may be assigned to $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ and $^4T_{1g}(F)\rightarrow{}^4T_{2g}$ respectively. The bands due to $^4T_{1g}(F)\rightarrow{}^4A_{2g}$ transition are not observed.[21] The magnetic moment 4.89 BM is within the limits of the 'octahedral' region for Co(II) compound. The occurrence of magnetic moment value at 3.11 BM and d-d transition bands maxima at 444 nm ($^3A_{2g}\rightarrow{}^3T_1(P)$), 580 nm ($^3A_{2g}\rightarrow{}^3T_{1g}$), and 900 nm ($^3A_{2g}\rightarrow{}^3T_{2g}$) in the spectrum of Ni(II) complexes can be attributed to an octahedral geometry for the Ni(II) complex with 5-BrU.[22] Copper(II) compound shows magnetic moment value 2.02 BM; a distorted octahedral structure is suggested. The electronic spectrum of the complex is characterized by a band at 680 nm which may be due to $^2E_g\rightarrow{}^2T_{2g}(D)$ transition.[20]

On the basis of analytical data, magnetic measurements, infrared, electronic and X-ray diffraction studies, it is concluded that all these complexes have six coordination number for the metal ions. All the complexes are polymeric in nature except Co(II) complex. $N_{(1)}H$ and $C_{(4)}=O$ groups are involved in polymerization in Mn(II) and Ni(II) complexes while in Zn(II) and Cd(II) complexes polymerization takes place through OH bridging groups. In Cu(II) complex bridging occurs through OH group as well as through $N_{(1)}H$ and $C_{(4)}=O$ groups of 5-BrU. The symmetry of the complexes are found to be orthorhombic except copper(II) complex which has tetragonal symmetry with distorted octahedral configuration.

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