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SYNTHESIS, PHYSICOCHEMICAL CHARACTERISTICS, AND BIOCIDAL ACTIVITY OF SOME TRANSITION METAL MIXED-LIGAND COMPLEXES WITH BIDENTATE (NO AND NN) SCHIFF BASES

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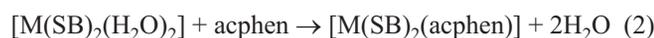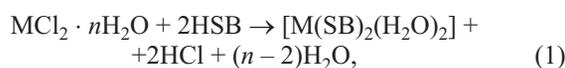* e-mail: jeenenpatel@yahoo.co.in

Mixed-ligand complexes of the type [M(SB)₂acphen], where M = Mn(II), Co(II), Ni(II), Cu(II) and Cd(II), HSB = 3,5-dibromosalicylideneaniline and acphen = bis(acetophenone)ethylenediamine, have been prepared and characterized on the basis of elemental analyses, magnetic measurements, thermogravimetry, and infrared and electronic absorption spectroscopy. All the mixed-ligand complexes exhibit an octahedral geometry. The mixed-ligand complexes show antimicrobial activities against bacteria, yeast and fungi.

Metal complexes of Schiff bases are of considerable interest and they have played a central role in the development of inorganic chemistry of chelate systems. Schiff bases and their metal complexes exhibit various biological activities, in particular, anticarcinogenic [1] and antitumor [2] because of their specific structures. Amino acid – Schiff base complexes derived from aldehydes are involved in a variety of biological processes, for example, as catalysts of transamination, racemization, and carboxylation reactions [3, 4]. In continuation of our earlier works [5, 6], we report here the synthesis and characterization of the antimicrobial activity of some transition metal mixed-ligand complexes with bidentate Schiff bases. The proposed structures of the ligands are shown in Fig. 1.

Results and discussion

Physical measurements. The magnetic moments have been obtained by Gouy's method using mercury tetrathiocyanatocobaltate(II) as a calibrant ($\chi_g = 16.44 \times 10^{-6}$ ESU units at 20°C). Diamagnetic corrections were made using Pascal's constants. The infrared (IR) spectra were recorded on a Nicolet 400D FTIR spectrophotometer. The reflectance spectra of the complexes were recorded in the 1700 – 350 nm range (as MgO discs) on a Beckman DK-2A spectrophotometer. The microanalysis for carbon, hydrogen, and nitrogen were performed on a Perkin-Elmer Model 240 elemental analyzer. The metal content in the complexes was determined by means of EDTA titration [7] after decomposing the organic matter with a mixture of perchloric, sulphuric, and nitric acids (1 : 1.5 : 2.5). Thermogravimetric analysis (TGA) was performed using a DuPont Model 951 thermal analyzer (Germany).

The analytical data, colors, yields, magnetic moments, and melting points of the ligands and complexes are presented in Table 1. The complexes were synthesized following the general procedures outlined by the following equations.

$$MCl_2 \cdot nH_2O + 2HSB \rightarrow [M(SB)_2(H_2O)_2] + +2HCl + (n-2)H_2O, \quad (1)$$

$$[M(SB)_2(H_2O)_2] + acphen \rightarrow [M(SB)_2(acphen)] + 2H_2O \quad (2)$$

The IR spectra of the mixed-ligand complexes in comparison with to of the corresponding free ligands display certain changes, which give an idea about the types of bonds and their structures. The Schiff base 3,5-dibromosalicylideneaniline exhibits a medium-intense band at 2700 cm⁻¹ due to intramolecular hydrogen-bonded (H-bonded) ν(OH) [8]. The absence of this band in the spectra of mixed-ligand complexes indicates the deprotonation of phenolic groups and the coordination of oxygen to the metal ion. In the spectrum of free ligands, a strong band at 1525 cm⁻¹ due to ν(C–O)(phenolic) shifts to higher energies by 5 – 15 cm⁻¹ for the mixed-ligand complexes, thus indicating the coordination of the phenolic oxygen atom to the metal ion [9]. This high-energy shift is expected due to the maintenance of ring currents arising from the electron delocalization in the chelate ring. The mixed-ligand complexes also exhibit ν(C–Br) bands at about 625 cm⁻¹[10]. The ν(C=N) frequency of bis(acetophenone)ethylenediamine occurs at 1640 cm⁻¹. This band shifts by 10 – 20 cm⁻¹ toward lower energies in the spectra of mixed-ligand complexes, indicating the coordination through the azomethine nitrogen [11]. The absorption in the range 1370 – 1385 cm⁻¹ is due to ν(C–CH₃) [12]. The bands in the ranges 450 – 460 and 410 – 420 cm⁻¹ are attributed to ν(M–N) [13] and ν(M–O) [14], respectively.

3,5-Dibromosalicylideneaniline (HSB)

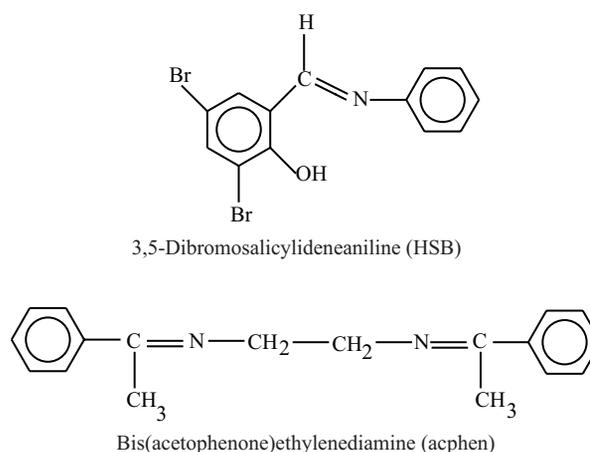Bis(acetophenone)ethylenediamine (acphen)

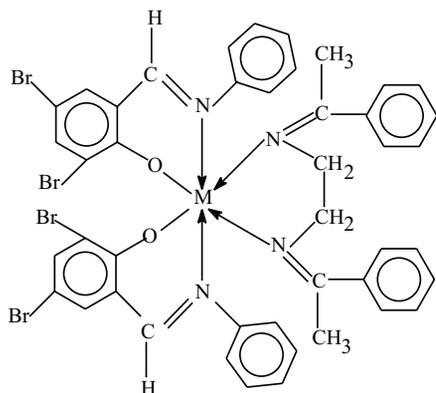**Fig. 1.** The structures of the HSB and acphen ligands.

Fig. 2. The proposed structure of the [M(SB)$_2$(acphen)] complexes with M = Mn(II), Co(II), Ni(II), Cu(II), and Cd(II).

The magnetic measurements were performed at room temperature. The magnetic moment of the Cu(II) complex is 1.82 B.M. (Bohr magneton), which corresponds to a single unpaired electron with a very slight orbital contribution and suggests an octahedral geometry [15] around the Cu(II) ion. The magnetic moment of the Co(II) complex is 3.98 B.M., which is higher than the expected value (3.87 B.M.). The difference may be due to incomplete quenching of the orbital magnetic moment by the surrounding ligand and also suggests an octahedral geometry around the Co(II) ion [16]. The magnetic moment of the Mn(II) complex is 6.05 B.M., which is within the limits of the spin-free value for five unpaired electrons and indicates that the complex represents a high-spin d^5-system with an octahedral structure [17]. The magnetic moment of the Ni(II) complex is 2.74 B.M., this result indicating that the complex probably has an octahedral geometry [18]. The Cd(II) complex is diamagnetic, as expected for a d^{10}-system.

The UV-VIS absorption spectra provide the most detailed information about the electronic structure. The reflectance spectra of the mixed-ligand complexes have been studied in the solid state. The Ni(II) complex shows three

bands at ~10300, ~17300 and ~23500 cm^{-1}, which may be due to the $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (v_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (v_2), and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (v_3) transitions, respectively, in an octahedral field of Ni(II) ion [19]. The Co(II) complex shows three bands at ~9300 (v_1), ~18200 (v_2) and ~19500 cm^{-1} (v_3), which may be assigned to the $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively, expected for an octahedral structure [20]. The electronic spectra of the Cu(II) complex exhibits a single band at ~15500 cm^{-1}, which may be assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition in an octahedral geometry [21]. The electronic spectra of the Mn(II) complex exhibits three weak absorption bands (at ~15000, ~19200 and ~25000 cm^{-1}), which may be assigned to transitions $^6A_{1g} \rightarrow {}^4T_{1g}(^4G)$ (v_1), $^6A_{1g} \rightarrow {}^4T_{2g}(^4G)$ (v_2), and $^6A_{1g} \rightarrow {}^4A_{1g}, {}^4E_g(^4G)$ (v_3), respectively, in an octahedral field of Mn(II) ion [22].

The results of thermogravimetric measurements showed that the mixed-ligand complexes did not exhibit weight loss between 50 – 180°C, which indicated that these complexes did not contain crystal water molecules and coordinated water molecules. In the interval from 200 to 310°C, the TGA curves suggested that the weight loss observed for all mixed-ligand complexes corresponds to the evaporation of acphen ligands. In the range above 310°C, the weight loss corresponds to remaining organic ligand molecules. In all cases, the remaining residue is a metal oxide. These results are in good agreement with the composition of the mixed-ligand complexes. The proposed structures of the complexes is shown in Fig. 2.

Pharmacological tests. A number of organic compounds have been used as antimicrobial agents, whose biological activity depends on the size, shape, and structure of the molecule. It has been found that a majority of the complexes possess higher biological activity than that of the initial organic compounds.

The results of our tests, which are presented in Table 2, show that the synthesized mixed-ligand complexes are more toxic than their parent organic ligands and metal nitrates with respect to the same microorganisms tested un-

Table 1

Yields and Physicochemical Characteristics of Mixed-Ligand Complexes and Their Components

Compound* (empirical formula)	Formula weight	Color	M.p. (°C)	$\mu_{eff.}$ (B. M.)	% Yield
Acphen C$_{18}$H$_{20}$N$_2$	264.00	Yellow	260	–	75.0
HSB C$_{13}$H$_9$Br$_2$NO	354.80	Yellow	110	–	75.0
[Mn(SB)$_2$acphen] C$_{44}$H$_{36}$Br$_4$MnN$_4$O$_2$	1026.54	Yellowish brown	290	6.05	79.0
[Co(SB)$_2$acphen] C$_{44}$H$_{36}$Br$_4$CoN$_4$O$_2$	1030.54	Light brown	295	3.98	80.0
[Ni(SB)$_2$acphen] C$_{44}$H$_{36}$Br$_4$N$_4$NiO$_2$	1030.51	Green	290	2.72	70.0
[Cu(SB)$_2$acphen] C$_{44}$H$_{36}$Br$_4$CuN$_4$O$_2$	1035.15	Greenish brown	288	1.82	75.0
[Cd(SB)$_2$acphen] C$_{44}$H$_{36}$Br$_4$CdN$_4$O$_2$	1084.00	Yellow	290	–	65.0

* HSB = 3,5-Dibromosalicylideneaniline; acphen = Bis(acetophenone)ethylenediamine

der otherwise identical conditions. The increase in antimicrobial activity of the mixed-ligand complexes may be due to the effect of the metal ion on the normal cell process. A possible mode for toxicity increase can be considered in terms of Overtone's concept [23] and the Tweedy's chelation theory [24]. According to Overtone's concept of the cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials. Therefore, liposolubility is an important factor which controls the antifungal activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to an overlap with the ligand orbital and a partial sharing of the positive charge between the metal ion and donor groups. Further, it increases the delocalization of π electrons over the whole chelate ring and thus enhances the lipophilicity of the complexes. This increased lipophilicity facilitates penetration of the complexes through lipid membranes and leads to blocking of the metal binding sites in the microbial enzymes. These complexes also disturb the cell respiration process and block the synthesis of proteins, thus suppressing the growth cell. Furthermore, the mode of action of the complex compounds may involve the formation of a hydrogen bond through azomethine groups with the active centers of cells, which results in interference with the normal cell process [25]. Thus, there is a sufficient increase in the fungicidal activity of the mixed-ligand complexes as compared to free ligands, metal nitrates, and the control dimethyl formamide (DMF).

Experimental chemical part

3,5-Dibromosalicylideneaniline (HSB). The Schiff base 3,5-dibromosalicylideneaniline was synthesized as described in [26].

Bis(acetophenone)ethylenediamine (acphen). Bis(acetophenone)ethylenediamine was prepared by refluxing a solution of 20 mmole of acetophenone (2.40 g) in 100 ml of ethanol with a solution of 10 mmole of ethylenediamine (0.60 g) in 100 ml of the same solvent for 2 – 3 h. The target Schiff base precipitated from the reaction mixture in the form of yellow crystals. The product was filtered, washed with ether, and dried in air; yield, 2.26 g (75.0%); m.p., 260°C.

Preparation of mixed-ligand complexes. The target bis(3,5-dibromosalicylideneaniline)diaquoM(II) complexes with M=Mn(II), Co(II), Ni(II), Cu(II), and Cd(II) were synthesized using a procedure described in [27]. For example, bis(3,5-dibromosalicylideneaniline)acphenNi(II) was prepared by refluxing a solution of 2 mmole of bis(3,5-bromosalicylideneaniline)diaquoNi(II) (1.48 g) in 50 ml of DMF with a solution of 2 mmol of bis(acetophenone)ethylenediamine (0.52 g) in 50 ml of the same solvent for ~1 h. Then, the solution was concentrated to 15 ml by evaporation, scratched, and cooled in a refrigerator overnight at 5°C. The obtained crystals were collected, recrystallized from chloroform, and, dried in air. The mixed-ligand complexes of Mn(II), Co(II), Cu(II), and Cd(II) were prepared similarly.

Experimental pharmacological part

In the present study, the antimicrobial properties of the control (DMF), the ligands, the metal salts, and their corresponding mixed-ligand complexes were tested against *Salmonella typhi* (bacteria) and *Saccharomyces cerevisiae* (yeast) species using the agar diffusion method [28]. The antifungal activity of the compounds have been screened against *Lasiodiplodia theobromae* and *Fusarium oxysporum* using the potato dextrose agar method [29]. In each test, a fresh sterile nutrient agar medium (for bacteria and yeast) and potato dextrose medium (for fungi) were prepared. All the laboratory ware in need was sterilized. Each sterile petridisc was charged with 15 – 20 ml of molten media and, simultaneously, 0.05 – 0.11 ml (approx 2 – 3 drops) of freshly diluted 24-h culture of a test microbe was added. The culture and nutrient agar media were mixed thoroughly by rotatory motion of agar plate on a plane surface. It was allowed to solidify at room temperature. Then, the cups of agar wells were made with the help of a sterilized stainless steel borer (diameter, 6 mm). Solutions of test compounds were added to the agar cups/wells. They were allowed to diffuse in the media, while the plates were incubated at 37°C for 24 hours, and then the diameter of the zone of inhibition was measured. The reading and interpretation of results were done according to Cruickshank et al. [30]. The diameter of the zone of microbial growth inhibition, with allowance for the 6-mm hole diameter, was measured in millimeters by viewing the disc against a ruler.

All synthesized compounds showed significant antibacterial activity at 5×10^{-4} g/ml against the test bacteria, yeast and fungi. Since the compounds were dissolved in DMF, the results were compared to the control (pure DMF) and expressed as the size of the zone of inhibition and as the percentage inhibition versus control using the following formula:

Antimicrobial Activity of Mixed-Ligand Complexes and Their Components

Compound*	Zone of Inhibition in mm (% Inhibition)			
	S. typhi	S. Cerevisiae	L. theobromae	F. Oxysporum
DMF (control)	18	19	15	13
HSB	24.9(38)	24.9(31)	18.0(20)	14.9 (15)
Acphen	24.9(38)	20.9(10)	18.9(26)	15.9 (23)
MnCl$_2$ · 6H$_2$O	21.9(22)	22.9(21)	18.0(20)	14.9 (15)
CoCl$_2$ · 4H$_2$O	20.7(15)	20.9(11)	15.9(07)	14.0 (08)
NiCl$_2$ · 6H$_2$O	19.9(11)	20.5(08)	16.5(10)	14.3 (10)
CuCl$_2$ · 2H$_2$O	21.9(22)	21.9(16)	18.0(20)	14.9 (15)
CdCl$_2$ · 2H$_2$O	20.9(17)	20.5(08)	15.9(07)	14.0 (08)
[Mn(SB)$_2$acphen]	30.0(67)	30.7(62)	20.1(34)	19.5 (50)
[Co(SB)$_2$acphen]	28.9(61)	30.0(58)	22.0(47)	20.1 (55)
[Ni(SB)$_2$acphen]	29.1(62)	29.8(57)	22.2(48)	19.8 (53)
[Cu(SB)$_2$acphen]	30.9(72)	30.9(63)	21.9(46)	20.0 (54)
[Cd(SB)$_2$acphen]	31.1(72)	31.7(67)	24.3(62)	21.1 (63)

* HSB = 3,5-Dibromosalicylideneaniline; acphen = Bis(acetophenone)ethylenediamine

Inhibition (%) = $[(B - A)/A] \cdot 100$,

where, A and B are the readings for the control (DMF) and sample, respectively. The results of tests given in Table 2 represent the average values of three replicates.

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СИНТЕЗ, ФИЗИКО-ХИМИЧЕСКИЕ ХАРАКТЕРИСТИКИ И АНТИМИКРОБНЫЕ СВОЙСТВА КОМПЛЕКСОВ ПЕРЕХОДНЫХ МЕТАЛЛОВ С БИДЕНТАТНЫМИ (NO И NN) ОСНОВАНИЯМИ ШИФФА

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Синтезирован ряд комплексов переходных металлов типа [M(SB)₂acphen], где M = Mn(II), Co(II), Ni(II), Cu(II), и Cd(II)), HSB = 3,5-дибромсалицилиденанилин и acphen = бис(ацетофенон)этилендиамин. Установлены состав и структура комплексов. Полученные соединения исследованы методами ИК и отражательно-абсорбционной спектроскопии, термогравиметрического анализа и магнитометрии. Все комплексы характеризуются октаэдрической геометрией. Исследованы антимикробные свойства комплексов в отношении штаммов бактерий, грибков и дрожжей. Все вещества проявляют антимикробную активность, причем активность комплексов выше, чем активность индивидуальных органических компонентов (лигандов), нитратов тех же металлов и растворителя (ДМФ).