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## Research Article

# Spectral, Thermal and Antimicrobial Properties of Novel Mixed Ligand-Metal Complexes Derived from Saccharinate Complexes and Azo Dye Ligand

<sup>1</sup>Ragip Adiguzel, <sup>2</sup>Nevin Turan, <sup>2</sup>Kenan Buldurun and <sup>3</sup>Hanifi Korkoca

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, Munzur University, 62000 Tunceli, Turkey

<sup>2</sup>Department of Chemistry, Faculty of Arts and Sciences, Mus Alparslan University, 49250 Mus, Turkey

<sup>3</sup>Department of Nursing, School of Health, Mus Alparslan University, 49250 Mus, Turkey

## Abstract

**Background and Objective:** Saccharin and saccharinate (sac) complexes and its anion have critical importance with biological, biochemical and pharmacological properties. The main objective of this study was to synthesize the novel mixed ligand complexes from the complexes  $[M(\text{Sac})_2(\text{H}_2\text{O})_4] \cdot 2\text{H}_2\text{O}$  [M: Mn(II), Co(II), Ni(II), Cu(II) and Cd(II)] with a second neutral ligand [L:(mmpa): 5-Methyl-4-(4-methylphenylazo)-2H-pyrazole-3-ylamine]. **Methodology:** The structures of the complexes were characterized by elemental analysis, magnetic susceptibility, FT-IR, mass and electronic spectral studies and thermal analysis. Antimicrobial (antibacterial and anticandidal) activities of the complexes were tested against *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Candida albicans* ATCC 64548. **Results:** According to the experimental results, it was determined that in Mn(II), Co(II) and Cd(II) complexes, two coordinated  $\text{H}_2\text{O}$  molecules are replaced with two neutral ligands, on the other hand, in Ni(II) and Cu(II) complexes, all coordinated  $\text{H}_2\text{O}$  molecules are replaced with four neutral ligands. It was established that the second neutral ligand was coordinated to the metal ions via nitrogen atom of  $-\text{NH}_2$  group in pyrazole ring. In antimicrobial activity, study was determined to occur significant inhibition zones (20-24 mm) against *Candida albicans* ATCC 64548 strain. **Conclusion:** As a result, mononuclear and monomeric structure in octahedral geometry including both saccharin and azo dye ligands were synthesized and their characterizations were accomplished. The result of this study shows that novel mixed ligand-saccharinate complexes can be utilized for the development of anticandidal agents.

**Key words:** Saccharin, azo dyes, saccharinate complexes, antimicrobial activity, anticandidal agents

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**Corresponding Author:** Ragip Adiguzel, Department of Chemical Engineering, Faculty of Engineering, Munzur University, 62000 Tunceli, Turkey  
Tel: +904282131794/2456 Fax: +904282131624

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Saccharin ( $C_7H_5NO_3S$ , also named 1,2-benzisothiazol-3-(2H)-one-1,1-dioxide or o-benzosulfimide) is widely used as a non-caloric artificial sweetener and commercially available as water soluble alkali salts<sup>1</sup>. The imino hydrogen of sac H is acidic and thus in solutions, the molecule can be easily converted into the corresponding nitranion, saccharinate (sac). Although metal complexes of molecular saccharin are not known, the coordination chemistry of its anion is very intriguing. Sac has different coordination sites, such as one negatively charged imino nitrogen, one carbonyl and two sulfonyl oxygen atoms<sup>2,3</sup>. The most common coordination mode of sac is ligation through the negatively charged nitrogen atom, usually observed in the aqua bis(saccharinate) complexes of first-row transition metals<sup>1</sup>. Both saccharin and saccharinate complexes have critical importance with biological, biochemical and pharmacological properties<sup>3</sup>.

On the other hand, researchers has synthesized a lot of mixed ligand saccharinate complexes with the ligands such as pyridine, 2-(Aminomethyl)pyridine, mono- and diethanolamine and nicotinamide etc<sup>3</sup>. For the synthesis of mixed-ligand saccharinate complexes,  $[M(sac)_2(H_2O)_4] \cdot 2H_2O$  are used as a starting metal saccharin-aqua complex. Since the aqua ligands in these metal complexes are labile and readily displaced by neutral ligands, new complexes are simply prepared by the direct reaction of a neutral ligand with the starting aqua-saccharinate complex in solution. The addition of the ligands to the solutions of the complexes usually results in substitution of all four aqua ligands, thereby forming stable new mixed-ligand complexes<sup>3</sup>.

The azo dyes are used widely in different areas with high dyeing strength and good fastness properties and they have the antimicrobial activity, absorption characteristics<sup>4</sup>, molecular structure and nonlinear optic. Frontier molecule optical properties of the azo dyes were also investigated by Catikkas *et al.*<sup>5</sup>, but any studies related to mixed ligand complex of azo dyes and saccharin were not found in

the literature. In the studies conducted by the research group, synthesis and characterization of some first row transition metals complexes of the azo dye ligand and some 4-(o,p-substituephenylazo)-1H-pyrazole-3,5-diamine were investigated over the last 6 years<sup>6,7</sup>.

In the present study, neutral ligand (L:mmppa: 5-Methyl-4-(4-methylphenylazo)-2H-pyrazole-3-ylamine) for the synthesis of mixed-ligand saccharinate complexes were used. The structure of the ligands and saccharin-aqua complex is shown in Fig. 1. Finally, all of the compounds synthesized were tested for their antimicrobial activities against *Escherichia coli* ATCC 35218, *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Candida albicans* ATCC 64548.

The main objective of this study was to synthesize the novel mixed ligand complexes from the complexes  $[M(Sac)_2(H_2O)_4] \cdot 2H_2O$  [M: Mn(II), Co(II), Ni(II), Cu(II) and Cd(II)] with a second neutral ligand [L:(mmppa): 5-Methyl-4-(4-methylphenylazo)-2H-pyrazole-3-ylamine].

## MATERIALS AND METHODS

**Materials and measurements:** All reagents were purchased from commercial sources and used as supplied. The starting complex  $[M(sac)_2(H_2O)_4] \cdot 2H_2O$  (M: Mn(II), Co(II) Ni(II), Cu(II) and Cd(II)) was prepared by the method described in the literature<sup>3</sup>. In the same manner, secondary neutral ligand was obtained in compliance with the literature methods<sup>8,9</sup>.

All used solvents were of analytical grade and no further purifications were performed. The sulfate salts of above metals and starting materials for the ligand were purchased from Merck, Aldrich and Alfa Aesar. Elemental analyses were carried out with a Leco CHNS-O model 932 elemental analyzer. IR spectra were recorded with a Perkin Elmer Precisely Spectrum One spectrometer using KBr discs in the wave number range of 4000-400  $cm^{-1}$ . Electronic spectral studies were conducted with a Shimadzu model

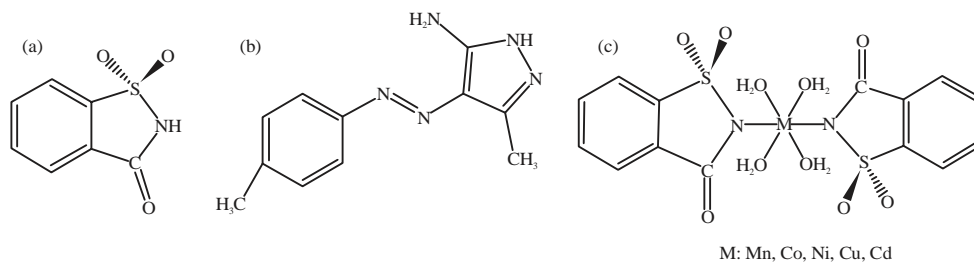
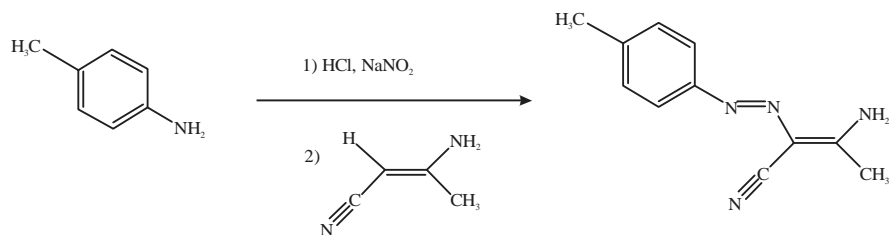
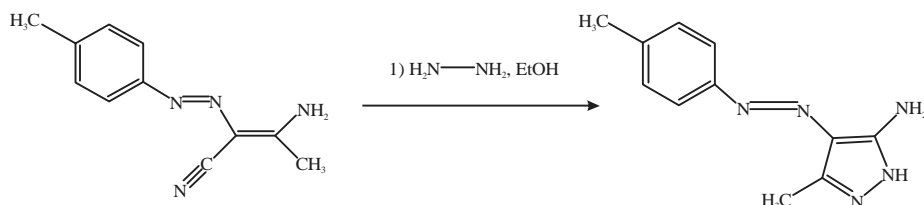


Fig. 1(a-c): Structure of saccharin, azo dye ligand (mmppa) and saccharinate-aqua-complex (a) Saccharin, (b) Azo dye (neutral ligand) and (c) Saccharinate-aqua-complex



Scheme 1: Coupling reaction in the synthesis of azo dye ligand(mmpa)



Scheme 2: Ring closure reaction in the synthesis of azo dye ligand(mmpa)

UV-1800 spectrophotometer in the wavelength of 1100-200 nm. Magnetic susceptibility measurements were performed using the standard Gouy tube technique using  $\text{Hg}[\text{Co}(\text{SCN})_4]$  as a calibrator/calibration. The LC/MS-API-ES mass spectra were recorded using an AGILENT model 1100 MSD mass spectrophotometer. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were carried out in nitrogen atmosphere with a heating rate of  $10^\circ\text{C min}^{-1}$  using Shimadzu DTG-60 AH (Shimadzu DSC 60 A) thermal analyzers. A sample size of 5-10 mg was used and sintered  $\alpha$ -alumina was used as the reference material.

**Synthesis of azo dye ligand (mmpa):** The secondary neutral ligand (L:mmpa) was synthesized in accordance with the procedure given at Scheme 1 and Scheme 2<sup>8,9</sup>. This synthesis include two steps. In other words, in the synthesis of azo dye ligand, azo coupling and ring closure reactions has been formed.

**Synthesis of the mixed ligand-metal complexes:** The azo dye ligand mmpa 0.30 g (1.40 mmol) was dissolved in 20 mL hot absolute EtOH in a 100 mL round-bottom flask. A solution of  $[\text{Mn}(\text{sac})_2(\text{H}_2\text{O})_4]\cdot 2\text{H}_2\text{O}$ , 0.37 g (0.70 mmol) in 5 mL EtOH and 0.5 mL DMF was added dropwise in 5 min periods with continuous stirring about at  $50\text{-}55^\circ\text{C}$ . In the meantime, brownish yellow solution of the ligand was turned into yellow mixture. After the addition of 5 drops of glacial acetic acid, it was left under reflux for 8 h and the brownish yellow mixture was left at room temperature. The solvent was removed completely from the mixture. The resulting precipitate in hexane and chloroform mixture was filtered and then washed

with hot EtOH and  $\text{H}_2\text{O}$  mixture. The product was dried at room temperature. Similar synthesis was performed for the mixed ligand complexes of mmpa by using  $[\text{Co}(\text{sac})_2(\text{H}_2\text{O})_4]\cdot 2\text{H}_2\text{O}$  (0.37 g, 0.70 mmol),  $[\text{Ni}(\text{sac})_2(\text{H}_2\text{O})_4]\cdot 2\text{H}_2\text{O}$  (0.19 g, 0.35 mmol),  $[\text{Cu}(\text{sac})_2(\text{H}_2\text{O})_4]\cdot 2\text{H}_2\text{O}$  (0.19 g, 0.35 mmol) and  $[\text{Cd}(\text{sac})_2(\text{H}_2\text{O})_4]\cdot 2\text{H}_2\text{O}$  (0.41 g, 0.70 mmol), L:mmpa (0.3 g, 1.40 mmol). Analysis results of the azo dye ligand and all complexes are as follows:

- L:mmpa: 5-Methyl-4-(4-methylphenylazo)-2H-pyrazole-3-ylamine), characteristic IR bands (KBr,  $\text{cm}^{-1}$ ):  $\nu(\text{NH})$ : 3458,  $\nu(\text{NH}_2)_{\text{broad}}$ : 3326,  $\nu(\text{Ar C-H})$ : 3172, 3047,  $\nu(\text{Ar-CH}_3)$ : 2842,  $\nu(\text{C=C})$ : 1537,  $\nu(\text{N=N})$ : 1603,  $\nu(\text{C=N})$ : 1500,  $\nu(\text{C-N})$ : 1388, 1293
- $[\text{Mn}(\text{sac})_2(\text{mmpa})_2(\text{H}_2\text{O})_2]$ , Yield: (76.0%). FW: 885.93  $\text{g mol}^{-1}$ .  $\mu_{\text{eff}}$  (B.M.): 4.85. Anal. Calc. for  $\text{C}_{36}\text{H}_{38}\text{N}_{12}\text{O}_8\text{S}_2\text{Mn}$ : C, 48.76, H, 4.29, N, 18.96, S, 7.22. Found: C, 49.31, H, 4.82, N, 19.49, S, 6.78. Selected IR data (KBr,  $\text{cm}^{-1}$ ):  $\nu(\text{NH})$ : 3445,  $\nu(\text{NH}_2)_{\text{broad}}$ : 3385, 3212,  $\nu(\text{Ar-C-H})$ : 3100,  $\nu(\text{Aliph-CH}_3)$ : 2855,  $\nu(\text{C=C})$ : 1539,  $\nu(\text{C=N})$ : 1598,  $\nu(\text{N=N})$ : 1604,  $\nu(\text{C-N})$ : 1395, 1260,  $\nu(\text{SO}_2)$ : 1154, 1120,  $\nu(\text{M-N})$ : 560, 516,  $\nu(\text{M-O})$ : 460, UV-VIS (in EtOH):  $\lambda_{\text{max}}$  ( $\epsilon$ ,  $\text{L mol}^{-1} \text{cm}^{-1}$ ) 261 (175.5), 358 (176), 606 (1) nm, MS [ES]: m/z 884.93 (calc.), 884.87 (found)  $[\text{M-H}]^-$ . Color: Dark yellow
- $[\text{Co}(\text{sac})_2(\text{mmpa})_2(\text{H}_2\text{O})_2]$ , Yield: (66%). FW: 889.26  $\text{g mol}^{-1}$ .  $\mu_{\text{eff}}$  (B.M.): 4.50. Anal. Calc. for  $\text{C}_{36}\text{H}_{38}\text{N}_{12}\text{O}_8\text{S}_2\text{Co}$ : C, 48.60, H, 4.27, N, 18.90, S, 7.20. Found: C, 48.01, H, 4.66, N, 18.48, S, 6.60. Selected IR data (KBr,  $\text{cm}^{-1}$ ):  $\nu(\text{H}_2\text{O})_{\text{broad}}$ : 3555,  $\nu(\text{NH})$ : 3415,  $\nu(\text{NH}_2)_{\text{broad}}$ : 3281,  $\nu(\text{Ar C-H})$ : 3164, 3128,  $\nu(\text{Aliph-CH}_3)$ : 2842,  $\nu(\text{C=C})$ :

1547,  $\nu(\text{N}=\text{N})$ : 1604,  $\nu(\text{C}=\text{N})$ : 1586,  $\nu(\text{C}-\text{N})$ : 1344, 1273,  $\nu(\text{SO}_2)$ : 1122, 1100,  $\nu(\text{M}-\text{N})$ : 536, 518,  $\nu(\text{M}-\text{O})$ : 450. UV-VIS (in EtOH):  $\lambda$  max ( $\epsilon$ ,  $\text{L mol}^{-1} \text{cm}^{-1}$ ) 231 (2000), 349 (2000), 768 (10.5) 840 (7) nm. MS [ES]:  $m/z$  888.26 (calc.), 888.30 (found)  $[\text{M}-\text{H}]^-$ . Color: Brownish yellow

- $[\text{Ni}(\text{sac})_2(\text{mmppa})_4] \cdot 1.5\text{H}_2\text{O}$ , Yield: (67%), FW: 1309.69  $\text{g mol}^{-1}$ .  $\mu_{\text{eff}}$  (B.M.): 2.49. Anal. Calc. for  $\text{C}_{58}\text{H}_{64}\text{N}_{22}\text{O}_6\text{S}_2\text{Ni}$ : C, 55.81, H, 5.13, N, 24.70, S, 5.13. Found: C, 55.66, H, 5.33, N, 24.80, S, 5.18. Selected IR data (KBr,  $\text{cm}^{-1}$ ),  $\nu(\text{NH})$ : 3457,  $\nu(\text{NH}_2)$  broad: 3325,  $\nu(\text{Ar C}-\text{H})$ : 3176,  $\nu(\text{Aliph}-\text{CH}_3)$ : 2853,  $\nu(\text{C}=\text{C})$ ,  $\nu(\text{C}=\text{N})$ : 1536,  $\nu(\text{N}=\text{N})$ : 1604,  $\nu(\text{C}-\text{N})$ : 1344, 1293,  $\nu(\text{SO}_2)$ : 1146, 1105,  $\nu(\text{M}-\text{N})$ : 560, 515. UV-VIS (in EtOH):  $\lambda$  max ( $\epsilon$ ,  $\text{L mol}^{-1} \text{cm}^{-1}$ ) 274 (650), 344 (215), 436 (240) 846 (1) nm. MS [ES]:  $m/z$  944.69 (calc.), 944.66 (found)  $[\text{M}-2\text{Saccharinate}-\text{H}]^+$ . Color: Golden yellow
- $[\text{Cu}(\text{sac})_2(\text{mmppa})_4] \cdot 0.5\text{H}_2\text{O}$ , Yield: (61%), FW: 1296.54  $\text{g mol}^{-1}$ .  $\mu_{\text{eff}}$  (B.M.): 1.71. Anal. Calc. for  $\text{C}_{58}\text{H}_{61}\text{N}_{22}\text{O}_{6.5}\text{S}_2\text{Cu}$ : C, 53.66, H, 4.76, N, 23.76, S, 4.94. Found: C, 53.64, H, 4.85, N, 23.80, S, 4.87. Selected IR data (KBr,  $\text{cm}^{-1}$ ),  $\nu(\text{NH})$ : 3453, 3430,  $\nu(\text{NH}_2)$  broad: 3307,  $\nu(\text{Ar C}-\text{H})$ : 3176, 3132,  $\nu(\text{Aliph}-\text{CH}_3)$ : 2855,  $\nu(\text{C}=\text{C})$ ,  $\nu(\text{C}=\text{N})$ : 1536,  $\nu(\text{N}=\text{N})$ : 1604,  $\nu(\text{C}-\text{N})$ : 1389, 1270,  $\nu(\text{SO}_2)$ : 1141, 1105,  $\nu(\text{M}-\text{N})$ : 542, 515. UV-VIS (in EtOH):  $\lambda$  max ( $\epsilon$ ,  $\text{L mol}^{-1} \text{cm}^{-1}$ ) 236 (650), 359 (114), 438 (16) 606 (1) nm, MS [ES]:  $m/z$  931.54 (calc.), 931.48 (found)  $[\text{M}-2\text{Saccharinate}-\text{H}]^+$ . Color: Brown
- $[\text{Cd}(\text{sac})_2(\text{mmppa})_2(\text{H}_2\text{O})_2] \cdot 0.5\text{H}_2\text{O}$ , Yield: (81%), FW: 951.43  $\text{g mol}^{-1}$ . Anal. Calc. for  $\text{C}_{36}\text{H}_{39}\text{N}_{12}\text{O}_{8.5}\text{S}_2\text{Cd}$ : C, 45.40, H, 4.12, N, 17.66, S, 6.73. Found: C, 45.53, H, 4.13, N, 17.56, S, 6.66. Selected IR data (KBr,  $\text{cm}^{-1}$ ),  $\nu(\text{H}_2\text{O})$  broad  $\nu(\text{NH})$ : 3497, 3443,  $\nu(\text{NH}_2)$  broad: 3271,  $\nu(\text{Ar C}-\text{H})$ : 3168,  $\nu(\text{Ar}-\text{CH}_3)$ : 2855,  $\nu(\text{C}=\text{C})$ : 1546,  $\nu(\text{C}=\text{N})$ : 1580,  $\nu(\text{N}=\text{N})$ : 1606,  $\nu(\text{C}-\text{N})$ : 1395, 1275,  $\nu(\text{SO}_2)$ : 1147, 1113,  $\nu(\text{M}-\text{N})$ : 537,  $\nu(\text{M}-\text{O})$ : 454. UV-VIS (in EtOH):  $\lambda$  max ( $\epsilon$ ,  $\text{L mol}^{-1} \text{cm}^{-1}$ ) 236 (236.5), 361 (299.5), 540, 582 (3) 799 (0.5) nm. MS [ES]:  $m/z$  953.43 (calc.), 953.39 (found)  $[\text{M}+2\text{H}]^{2+}$ . Color: Brownish yellow

**Antimicrobial activity studies:** The disk diffusion method was used for determining the antimicrobial activity of ligand and complexes. Antibacterial activity against Gram positive bacteria (*Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 25923) and Gram negative bacteria (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 *Klebsiella pneumoniae* ATCC 700603) was investigated. Anticandidal activity against *Candida albicans* ATCC 64548 was investigated. Mueller Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, UK) was used for all strains, except Mueller Hinton agar with 5% defibrinated sheep blood was used for *Enterococcus faecalis* ATCC 51299. Also,

Mueller Hinton agar supplemented with 2% glucose and methylene blue ( $0.5 \mu\text{g mL}^{-1}$ ) was used for *Candida albicans* ATCC 64548. The media were melted at  $100^\circ\text{C}$  and autoclaved at  $121^\circ\text{C}$  for 15 min. After they were cooled down to  $45-50^\circ\text{C}$ , they were poured into plates of 9 cm diameter in quantities of 20 mL. They were left on a flat surface to solidify and the surface of media was dried at  $37^\circ\text{C}$ . Then, colony growth method was used for the preparation of the inoculum in Mueller Hinton broth to a turbidity equivalent to a 0.5 McFarland ( $10^8 \text{CFU mL}^{-1}$ ). The organisms were streaked on petri dishes using sterile cotton swab. The surface of the media was allowed to dry 3-5 min at room temperature. The  $10 \text{mg mL}^{-1}$  (in DMSO, E. Merck) of the compounds impregnated blank discs (Oxoid Ltd., Basingstoke, Hampshire, UK) were applied to the surface of inoculated plates. The Mueller Hinton agar plates were incubated at  $35 \pm 2^\circ\text{C}$  for 18-24 h. The plates were examined and the diameter of the inhibition zone was measured. Antibiotic ampicillin ( $10 \mu\text{g}$ , Bioanalyse<sup>®</sup>) and gentamicin ( $10 \mu\text{g}$ , Bioanalyse<sup>®</sup>) were used as the standard. Fluconazole disk ( $25 \mu\text{L}$ , OXOID<sup>®</sup>) was used as standard for anticandidal test<sup>10</sup>.

## RESULTS AND DISCUSSION

**Infrared spectra:** IR absorptions of mixed ligand complexes that included saccharinate ion and azo dye ligand along with assignments are given in experimental section. All complexes are air stable and freely soluble in coordinating solvents such as EtOH or DMF. In the IR spectrum of the azo dye ligand,  $\nu(\text{NH}_2)$  stretching vibration band was observed at  $3326 \text{cm}^{-1}$  as a broad peak for the isolated mixed ligand Mn(II) complex and two broad peaks at  $3385$  and  $3212 \text{cm}^{-1}$  were found and for Co(II), Ni(II), Cu(II) and Cd(II) complexes, one peak for each complex was appeared at  $3281$ ,  $3325$ ,  $3307$  (broad) and  $3271 \text{cm}^{-1}$ , respectively<sup>7,11</sup>.

In addition to these results, the  $\nu(\text{C}=\text{N})$  vibration peak for pyrazole ring in mmppa ligand observed at  $1500 \text{cm}^{-1}$  but for Mn(II), Co(II), Ni(II), Cu(II) and Cd(II) complexes, this peak shifted to higher frequencies of  $1598$ ,  $1586$ ,  $1536$ ,  $1536$  and  $1580 \text{cm}^{-1}$ , respectively<sup>7,12</sup>. As a result of coordination of amino group over N atom to metal ions of mmppa ligand, electron density of pyrazole ring decreased. Therefore, shifting of  $\nu(\text{NH}_2)$  vibration to low frequency and shifting of  $\nu(\text{C}=\text{N})$  vibration to high frequency were expected<sup>7,11-13</sup>. These two results support that  $\text{NH}_2$  group of the mmppa ligand coordinated over N atom to metal ion and behaved as a monodentate ligand. On

the other hand, not observing  $\nu(\text{H}_2\text{O})$  vibration peak in the Ni(II) and Cu(II) complexes confirms that all four coordination water molecules were replaced with the mmpa ligands.

The  $\nu(\text{OH})$  vibration band of  $\nu(\text{H}_2\text{O})$  at Co(II) and Cd(II) complexes was observed at 3555 and 3497  $\text{cm}^{-1}$ , respectively as broad and medium intense bands<sup>14,15</sup>. However,  $\nu(\text{H}_2\text{O})$  vibration band was not observed at Mn(II) complexes and this is possibly due to partially covering this band with  $\nu(\text{NH}_2)$  bands.  $\nu(\text{M-O})$  vibration peak from coordinated  $\text{H}_2\text{O}$  for Mn(II), Co(II) and Cd(II) complexes were observed at 460, 450 and 454  $\text{cm}^{-1}$ , respectively and similar values were also found in the literature<sup>16,17</sup>.  $\nu(\text{M-O})$  vibration peak for Ni(II) and Cu(II) complexes provides supporting evidence that no coordination water exists in the complex. The peaks for  $\nu(\text{M-N})$  vibration showing that coordination was done over N atom for both N atoms of saccharinate ion and  $-\text{NH}_2$  group of mmpa ligand were observed in the Mn(II), Co(II), Ni(II), Cu(II) and Cd(II) complexes at 560, 516, 536, 518, 560 515, 542, 515 and 537  $\text{cm}^{-1}$ , respectively similar values were found also in the literature<sup>13,16,18</sup>.

**Electronic spectra and magnetic properties:** The electronic spectra (UV-Vis) of the metal complexes were obtained from  $1 \times 10^{-5}$  M EtOH solution. The electronic spectra of the complexes display a number of well-resolved bands at 261, 358, 606 nm for Mn(II), at 231, 349, 768, 840 nm for Co(II), at 274, 344, 436, 846 nm for Ni(II), at 236, 359, 438, 606 nm for Cu(II) and at 236, 361, 540, 799 nm for Cd(II). The intense bands in the 236-344 nm range are assigned to the intraligand transitions of both sac and mmpa ligands and the bands with low intensity between 349 and 840 nm are due to d-d transitions.

The magnetic moment of 4.85 BM for Mn(II) complex suggests a high spin Mn(II) center with five unpaired electrons. Its electronic spectrum shows three bands at 261, 358 and 606 nm which may be assigned to intraligand/charge transfer transition<sup>19,20</sup>. The electronic spectra of the Cu(II) complexes exhibit intra ligand bands attributable to  $\pi \rightarrow \pi^*$  transition in the 359 and 438 nm and a d-d band in the 606 nm<sup>21</sup>. The room temperature magnetic moments are 1.80 BM for Cu(II) complex and it is consistent with the calculated value (1.73 BM) of the spin-only magnetic moment of the  $d^9$  configuration of Cu(II)<sup>18</sup>. The electronic spectrum of Co(II) complex shows several intense bands between 231 and 349 nm, which are assigned to the intra ligand transitions in mmpa and sac while the broad band at 768 nm is assigned

to d-d transitions for the octahedral Co(II) complex<sup>22,23</sup>. The room temperature magnetic moment measurements show that Co(II) complex is paramagnetic with a  $\mu_{\text{eff}}$  of 4.50 BM, which is significantly higher than the calculated spin-only value of 3.87 BM and this suggests an effective orbital contribution for the high-spin  $d^7$  octahedral configuration<sup>24</sup>. The Cd(II) complex is diamagnetic since it has no unpaired electrons. Cd(II) complex did not display any peak in the visible region but only exhibited the ligand transitions. Ni(II) complexes show mainly two maxima in the 436-846 range. The Ni(II) complex is found to have a room temperature magnetic moment value of 2.49 B.M., which is in the normal range observed for octahedral Ni(II) complexes<sup>23,25</sup>.

**Thermal studies:** The thermal properties of the complexes were investigated by performing a simultaneous TGA and DTA analysis on the same sample. The TGA and DTA curves were obtained at a heating rate of  $10^\circ\text{C min}^{-1}$  under nitrogen atmosphere over the temperature range of 25-800°C. The thermal data are summarized in Table 1. The results are in good agreement with the theoretical formula suggested by the elemental analyses. The weight losses for complexes were calculated within the corresponding temperature ranges.

The TGA curves of all complexes were interpreted in one step except for Ni(II) complex. Mn(II) complex is stable up to 185.33°C and its decomposition started at this temperature. 4.15 (4.07%) weight loss was observed at 291.20°C corresponding to 2 moles of coordinated water molecules<sup>26</sup>. In the same manner, Co(II) complex was stable up to 173.23°C and its decomposition started at this temperature. 4.14 (4.05%) weight loss was observed at 200.11°C corresponding to 2 moles of coordinated water molecules<sup>26</sup>.

Ni(II) complex was decomposed in two stages. In the first stage, weight loss of 1.5 moles of water of the crystallization occurred as 2.06 (2.30%) weight loss in the temperature range of 88.32-192.34°C<sup>24</sup>. In the second stage, a weight loss of 2 moles saccharinate ions occurred as 27.69 (27.95%) in the temperature range of 192.60-272.94°C<sup>27</sup>.

The Cu(II) and Cd(II) complexes displayed the same decomposition properties. In the Cu(II) complex, weight loss of a half mole of water of crystallization and 2 moles of saccharinate ions as 28.15 (28.78%) at the temperature range of 85.10-298.64°C were observed<sup>26,27</sup>. The decomposition of Cd(II) complex is due to removal of a half mole of the crystallization water molecule and 1 mole of saccharinate ion and weight loss of 20.25 (20.18%) was measured at the temperature range of 92.12-296.08°C<sup>26,27</sup>.

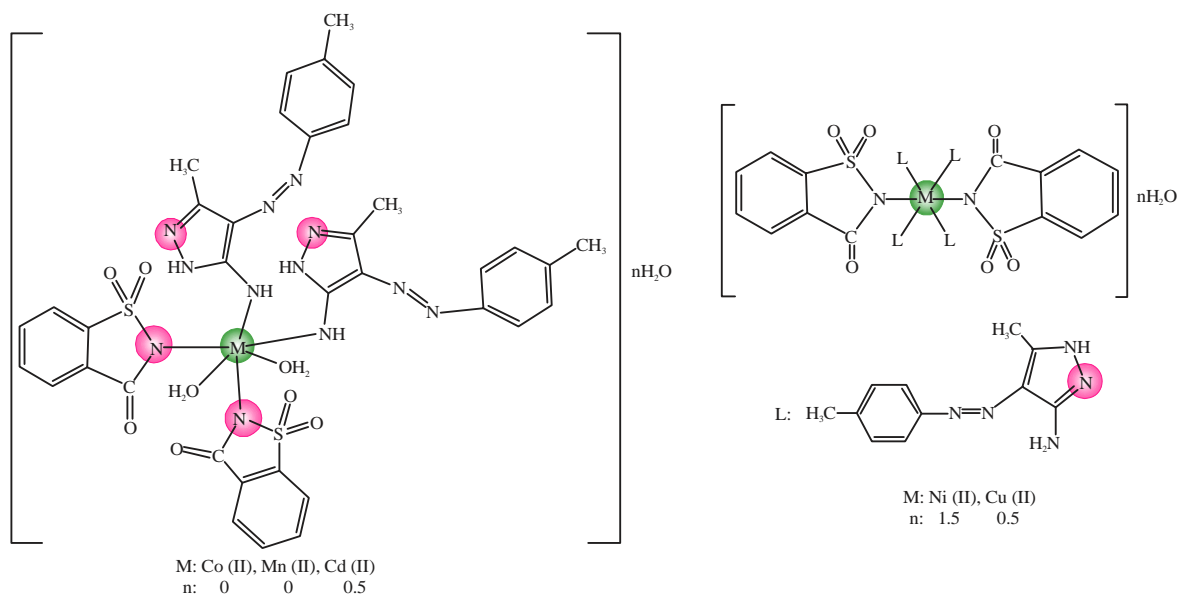


Fig. 2: Suggested structure of the octahedral mixed ligand-metal complexes

Table 1: Proposed decomposition steps and the respective mass losses of mixed ligand-metal complexes

Compounds molecular weight (g mol <sup>-1</sup> )	Mass loss (%), found (Calc.) Decomposition groups		Total mass loss (%) found (Calc.)
	First step (°C)	Second step (°C)	
[Mn(sac) <sub>2</sub> (mmppa) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] 885.93	185.33-291.20 4.15 (4.07) 2H <sub>2</sub> O (coord.)		4.15 (4.07)
[Co(sac) <sub>2</sub> (mmppa) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] 889.26	173.23-200.11 4.14 (4.05) 2H <sub>2</sub> O (coord.)		4.14 (4.05)
[Ni(sac) <sub>2</sub> (mmppa) <sub>4</sub> ]-1.5H <sub>2</sub> O 1309.69	88.32-192.34 2.30 (2.06) 1.5H <sub>2</sub> O	192.60-272.94 27.69 (27.95) 2 Saccharinate ions	29.99 (30.01)
[Cu(sac) <sub>2</sub> (mmppa) <sub>4</sub> ]-0.5H <sub>2</sub> O 1296.54	85.10-298.64 28.15 (28.78) 0.5H <sub>2</sub> O+2 Saccharinate ions		28.15 (28.78)
[Cd(sac) <sub>2</sub> (mmppa) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]-0.5H <sub>2</sub> O 951.43	92.12-296.18 20.25 (20.18) 0.5H <sub>2</sub> O+Saccharinate ion		20.25 (20.18)

**Mass spectra:** The mass spectra of the Mn(II), Co(II), Ni(II), Cu(II) and Cd(II) complexes contain peaks attributable to the related molecular ions,  $m/z$ : 884.87 [M-H]<sup>-</sup>,  $m/z$ : 888.30 [M-H]<sup>-</sup>,  $m/z$ : 944.66 [M-2 Saccharinate-H]<sup>+</sup> (cationic complex),  $m/z$ : 931.48 [M-2 Saccharinate-H]<sup>+</sup> (cationic complex) and  $m/z$ : 953.39 [M+2H]<sup>2+</sup>, respectively<sup>7,28</sup>. The experimental and theoretical mass spectra data of the complexes were previously given in the experimental section. The values which have high abundance in the mass spectra were reported. The suggested structures of all complexes, which were obtained according to spectroscopic and thermal analyses are given in Fig. 2.

**Antimicrobial evaluation:** Antimicrobial activities of the complexes were tested against *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *Candida albicans* ATCC 64548. While it is determined that the compounds synthesized in this study affected the *Staphylococcus aureus* ATCC 25923 strain moderately, it did not show any significant antibacterial effect against other strains. But significant inhibition zones (20-24 mm)<sup>29,30</sup> were determined to occur against *Candida albicans* ATCC 64548 strain used in the study (Fig. 3). The results are displayed in Table 2.

Table 2: Antimicrobial activity test data of all mixed ligand-metal complexes  
 Inhibition zone diameter (mm)( blank disk diameter, 6 mm)

Chemicals test microorganisms	Gram-positive bacteria		Gram-negative bacteria				Yeast
	<i>Enterococcus faecalis</i> : ATCC 51299	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 35218	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Klebsiella pneumoniae</i> ATCC 700603	<i>Candida albicans</i> ATCC 64548	
<b>Complexes</b>							
Mn (II)	7	16	8	0	0	24	
Co (II)	9	13	7	0	0	21	
Ni (II)	7	15	8	0	0	24	
Cd (II)	10	13	11	0	11	20	
Cu (II)	7	19	7	0	0	22	
Ampicillin	10	27	0	0	0		
Gentamicin	0	21	23	21	13		
Fluconazole						27	



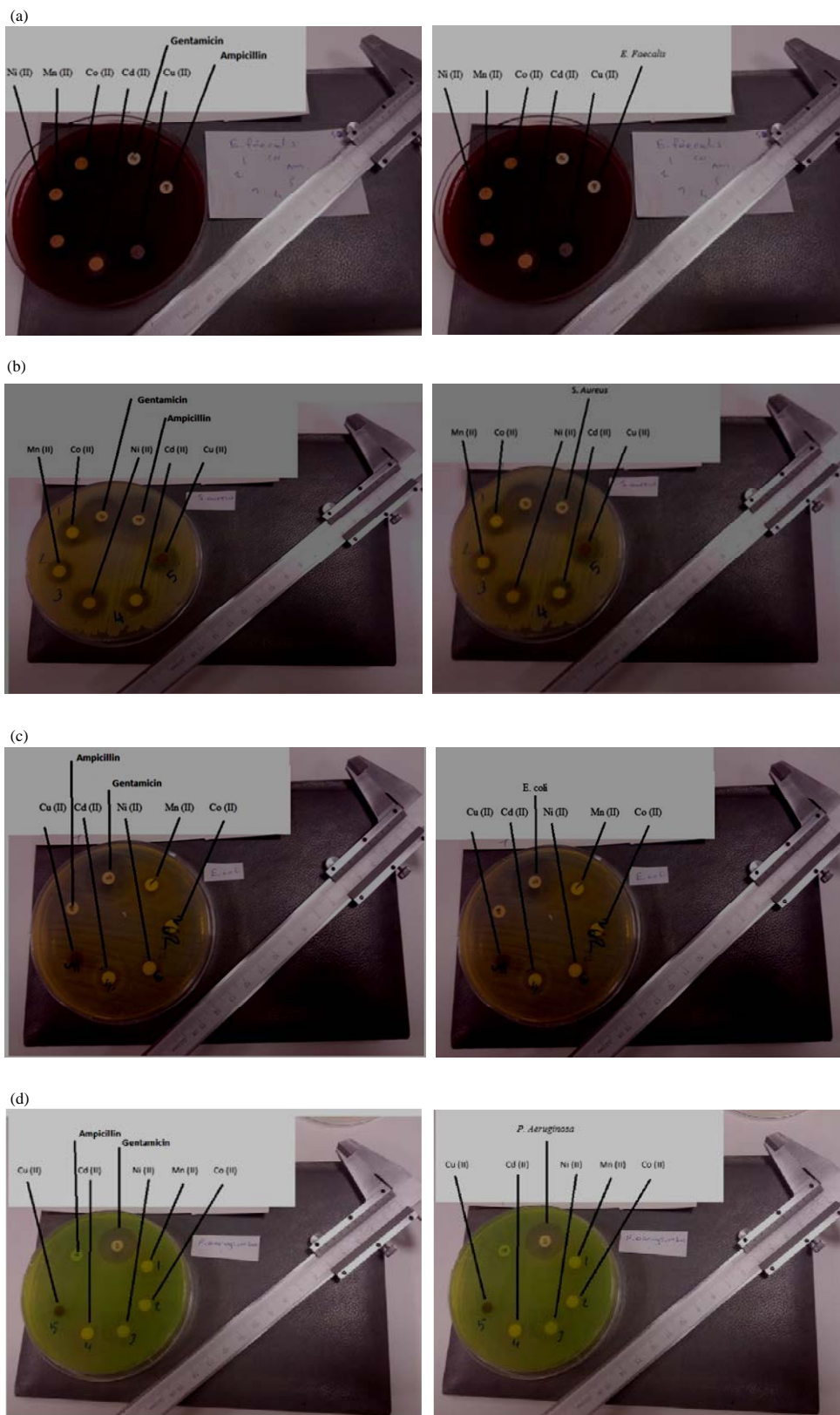


Fig.3(a-f): Continue

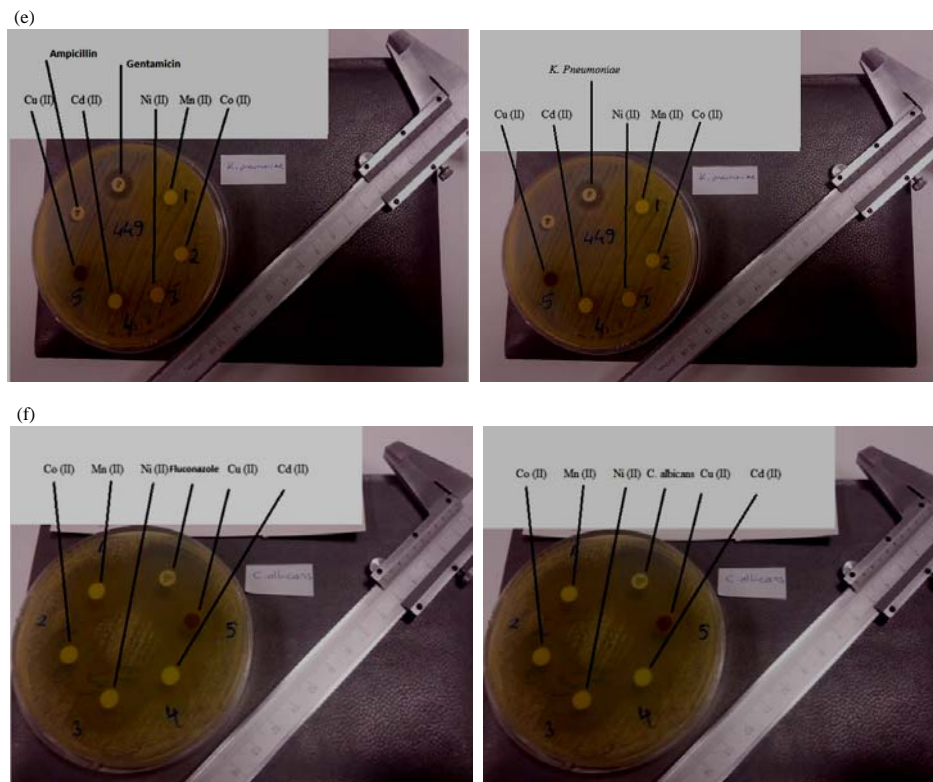


Fig. 3(a-f): Antimicrobial activity studies of all mixed ligand-metal complexes (a) *Enterococcus faecalis* ATCC 51299, (b) *Staphylococcus aureus* ATCC 25923, (c): *Escherichia coli* ATCC 35218, (d) *Pseudomonas aeruginosa* ATCC 27853, (e) *Klebsiella pneumoniae* ATCC 700603 and (f) *Candida albicans* ATCC 64548

### CONCLUSION AND FUTURE RECOMMENDATIONS

In this study, a series of new mixed-ligand saccharin complexes of Mn, Co, Ni, Cu and Cd with azo dye ligand (mmpa) were synthesized. Structures of the complexes were characterized by spectral and analytical techniques. The biological activities (antibacterial and anticandidal) of the new complexes were also investigated. The results obtained from anticandidal activity studies showed significant inhibition zones (20-24 mm) against *Candida albicans* ATCC strains 64548 even better than results found in the literature, such as the interpretive breakpoints for fluconazole susceptibility with disk diffusion were found  $\geq 19$  mm.

The compounds can be utilized for the development of new anti candidal agents. These novel compounds are strongly recommended to future researchers in that the novel complexes of  $[\text{Mn}(\text{sac})_2(\text{mmpa})_2(\text{H}_2\text{O})_2]$ ,  $[\text{Co}(\text{sac})_2(\text{mmpa})_2(\text{H}_2\text{O})_2]$ ,  $[\text{Ni}(\text{sac})_2(\text{mmpa})_4] \cdot 1.5\text{H}_2\text{O}$ ,  $[\text{Cu}(\text{sac})_2(\text{mmpa})_4] \cdot 0.5\text{H}_2\text{O}$  and  $[\text{Cd}(\text{sac})_2(\text{mmpa})_2(\text{H}_2\text{O})_2] \cdot 0.5\text{H}_2\text{O}$  may have a very promising future in biomedical and pharmaceutical

application for their antimicrobial, antipyretic, analgesic properties and for glycemic control etc.

### SIGNIFICANCE STATEMENTS

- Due to the existence of azo dye, this study investigates mixed ligand-saccharinate complexes for their antimicrobial properties and it is of major importance in the research field. It was thought that novel synthesized complexes are very important in terms of anticandidal activity. Therefore, these complexes can be utilized for the development of new anticandidal agents
- These complexes recommended to researchers because the researchers of this study assume that if used in pharmacological applications, more of new biological properties will be ensured

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