

GREEN SYNTHESIS, CHARACTERISATION, MIC, AND BIOACTIVITY OF DOPED COBALT METAL TARTRATE COMPLEXES

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Abstract

Novel mixed transition metal complexes $\text{MnCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$ and $\text{NiCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$ are synthesized using green approach, these tartrate act as a bidentate ligand. For characterisation of complexes elemental analysis, FTIR, XRD, TGA, and SEM with EDAX techniques were used. Analytical data shows that all complexes exhibited 1:1 (metal: ligand) ratio. IR spectral data shows that bidentate ligand coordinate with metal ion as bidentate ligand through the two 'O' atoms. The TGA of the complexes showed good agreement with their suggested formulae. XRD technique shows that all the complexes found to be in polycrystalline nature. SEM shows morphological features and surface characteristics of complexes. The synthesised complexes tested for antibacterial and antifungal activity to check the physiological activity and characterized for its application in the synthesis of heterocyclic compounds as catalysts.

Keywords: Antibacterial; Antifungal; Metal tartrate; Minimum inhibitory concentration

Introduction

The discovery of cis-platin to cure cancer disease sparked widespread interest in coordination chemistry. Coordination chemistry has become a multi-disciplinary area showing numerous applications in medicinal chemistry, such as anti-bacterial and antifungal activities (Ubale *et al.*, 2016). Compared to metal ions and ligands alone, metal-ligand complexes show great biological activity, as reported with several transition metal

ions and various ligands (Parikh *et al.*, 2006; Patil *et al.*, 2016). Mixed metal complexes show various applications such as metallic enzymes (Siegbahn *et al.*, 2000) and as catalysts (Hu, 2011). Among all these complexes, metal tartrates are emerging as a pioneering class of coordination chemistry due to their antibiofilm activity (Karale *et al.*, 2016) and sonodynamic therapy (Wang *et al.*, 2009). Some tartrates of mixed metal, catalyze the synthesis of

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different heterocyclic compounds, found effective in treating neurodegenerative diseases (Walle *et al.*, 2021; Walle *et al.*, 2022). Some inner transition metal tartrates are also utilized as precursors for the synthesis of different Lanthanide oxides, magnets, and luminescent materials with excellent chemical durability and thermal stability (Wang *et al.*, 2013; Zheng *et al.*, 2014). The preparation of these types of compounds with solvents such as water is affordable and environmentally benign (Walle *et al.*, 2019; Walle *et al.*, 2021). Large-scale applications of diverse tartrate complexes have been reported (Pawar *et al.*, 2014), for example, sodium-potassium tartrate and Ca tartrate have ferroelectric and antimicrobial applications respectively (Valasek, 1921; Betallu *et al.*, 2016). Tartrates of potassium with chromium are useful in medicines (Veseleya *et al.*, 1975). Calcium-strontium mixed Laevo-Tartrate (Parikh *et al.*, 2006), and iron-manganese mixed Laevo-Tartrate is also studied. The tartrates showed ferroelectric piezo-electric applications (Valasek, 1921; Sawaguchi *et al.*, 1971; Taylor *et al.*, 1984; Kumar *et al.*, 2009; Sawant *et al.*, 2012). Many metal complexes of Schiff base ligands with nitrogen and oxygen donor atoms are reported in the literature to possess significant properties such as catalytic activity and various biological activities, including antimicrobial, antifungal, and antiviral effects. (Aripnammal and Srinivasan, 2014; Mangaiyarkkarasi and Antony, 2014; Vijayakumari *et al.*, 2014; Uzunoglu *et al.*, 2019). However, metal complexes of ligands containing oxygen donor atoms are rarely reported. Although varieties of metal-ligand complexes have been investigated for their antimicrobial activity, the literature review finds limited research on mixed metal complexes. These novel complexes were investigated for antibacterial, antifungal properties against ubiquitously observed, pathogenic bacterial strains (Fayed *et al.*, 2021) and pernicious, virulent fungal strains (Rahimifard *et al.*, 2015). In the pursuit of developing potentially active metal complexes, we prepared and characterized mixed transition metal tartrates by co-precipitation method using water and 1:1 ethanol as solvents. This approach was guided by considerations for creating innovative metal complexes.

Materials and Methods

1. Materials

All the reagents and the hydrated transition metal salts ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) were of analytical grade purchased from Loba chemicals. The double distilled water was used for the preparation of metal salt solutions and the final complexes.

Procedure for synthesis of metal tartrates

The cobalt-nickel tartrates and cobalt-manganese tartrates were synthesized using the co-precipitation method. 1 Mili mole solution of each manganese sulfate and nickel sulfate hexahydrate was prepared. To each of these solutions, 2 millimoles of cobalt sulfate heptahydrate was added. To prevent the hydroxide formation of metal ions and to maintain the slightly acidic condition of the solution, dilute H_2SO_4 is added. Aqueous solution of tartrate (10%) is added slowly to this mixture of metal salts with constant stirring to get complete precipitation of complexes. Then acetone is poured in equal proportion and stirring is continued for the next 30 minutes. After this solution was allowed to stand for some time and cooled. A pinkish-colored precipitate appeared in the solution. The precipitated complex was filtered and washed with acetone. The complex dried under normal atmospheric conditions and stored in a desiccator for further use. (Parikh *et al.*, 2006; Patil *et al.*, 2016).

2. Characterization techniques

XRD of complexes was recorded with - Bruker D8 Advance ($\lambda = 1.5405 \text{ \AA}$). IR spectra of complexes were recorded on a Thermo Nicolet Spectrophotometer from $4000\text{--}100 \text{ cm}^{-1}$. Thermo Fisher C/H/N/S analyzer confirmed C, H percentage in complexes. SEM data of Co, Ni, and Mn were obtained on the XMX N instrument. These synthesized and characterized complexes were checked for their antibacterial activity, antifungal, and MIC activity.

Results and Discussions

1. X-ray diffraction

The XRD analysis of these complexes was performed using the Bruker D8 Advance ($\lambda = 1.5405 \text{ \AA}$) instrument to obtain information about their physical properties. The diffractograms (Figure 1) showed the polycrystalline nature of the complexes. Particle size was determined using Scherrer's formula (as shown in Table 1), where D represents the crystallite size, B is the full-width half maximum, and λ is the X-ray wavelength.

Table 1. Complex compositions and observed particle size: ($D = 0.89\lambda / B \cos \theta$)

Complex	Molecular Wt.	% Yield	Particle size
$\text{MnCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	724.798	94.705	600 $\text{\AA}$
$\text{NiCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	728.553	98.40	541.80 $\text{\AA}$

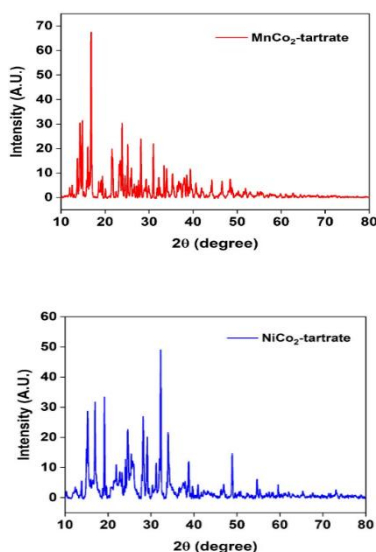


Figure 1. X-ray diffraction of complexes

X-ray pattern revealed a series of peaks corresponding to specific lattice planes within the crystal structure (PDF: 2217854. cif). By analyzing the peak positions other structural parameters of complexes can be determined. The comparison with standard reference patterns from databases like the powder diffraction file (PDF) helps in identifying the exact phases present.

2. Elemental (C/H/N/S) analysis

Thermo Fisher C/H/N/S Analyzer did carbon and hydrogen analysis, these are calculated in weight percent of tartrate precursors for metals, and percentages of C and H are in good agreement with the theoretical (calculated) values. The data is shown in Table 2. Analytical data shows that metal chelates have 1:2:3 stoichiometry. So, the general molecular formula of these novel complexes is specified as $[MM']_2(C_4H_4O_6)_3 \cdot 6H_2O$ (where M = Mn or Ni, M'=Co).

Table 2. Elemental analysis

Complex Formula		MnCo ₂ (C ₄ H ₄ O ₆) ₃ ·6H ₂ O	NiCo ₂ (C ₄ H ₄ O ₆) ₃ ·6H ₂ O
Complex Wt. (g)		724.798	728.553
C	Obs	19.432	17.255
	Cal	19.867	19.765
H	Obs	3.260	3.679
	Cal	3.3112	3.294
Co	Obs	20.19	11.78
	Cal	16.26	16.17
Metal	Obs	8.88	5.92
	Cal	7.579	8.056

3. IR spectral study

IR spectra of both complexes were recorded on a Thermo Nicolet Spectrophotometer covering the range from 4000-100 cm^{-1} as shown in Figure 2. IR

spectra were employed to verify the bidentate coordination of metal ions at donor sites of the ligand tartrate. The assigned absorption frequencies correspond to their typical vibrational modes. The coordination of both COO⁻ groups in the tartrate molecule to metallic ions is revealed by shifting the strong intensity band in the tartrate at 1750 cm^{-1} to lower wave numbers at 1598 cm^{-1} and 1607 cm^{-1} in complexes. The presence of hydration in the complexes is indicated by the prominent and intense trough positioned at 3335 cm^{-1} and 3457 cm^{-1} and is attributed to the OH⁻ vibrational frequency of water and secondary alcohol groups. M-O bonding is indicated by bands at 521 cm^{-1} and 495 cm^{-1} . The bands that correspond to the C-O bonding in the complexes range between 1386 and 1381 cm^{-1} .

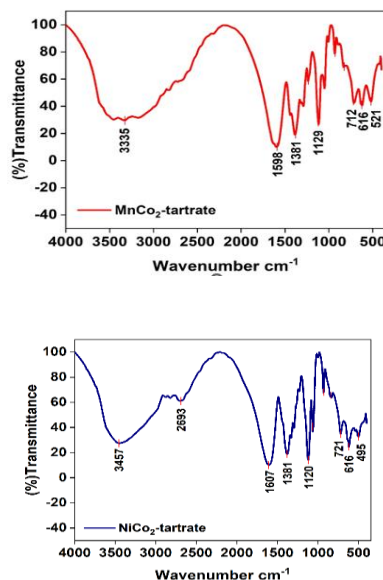


Figure 2. IR spectra of complexes

4. SEM analysis

The JSM obtained SEM micrographs of complexes- 6390 instruments (from Joint Engineering and Optical Limited manufacturers). SEM images Figure 3 (a) and 3(b) were observed at high magnifications of (10000×) to provide information about the size, shape, dispersion, structure, and morphology of the complexes. The micrographs revealed the presence of small, uniformly distributed crystals with particles of complexes having varying sizes. Most of the particles of Co Mn Complex and Co Ni Complex were found in the range of 13.11 μm and 2.23 μm in diameter respectively. The overall observation suggests an irregular shape of particles with a relatively small diameter variation. The literature survey showed its SEM data which matches the given data (Pawar *et al.*, 2014).

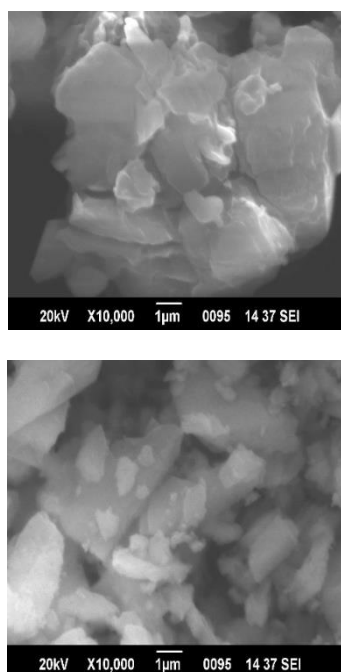


Figure 3. SEM micrograph of complexes (a) Co Mn complex (b) Co Ni complex

Antimicrobial activity

The antimicrobial activity of the compounds was evaluated using an agar well diffusion assay. After suitable treatment agar plates were incubated under proper conditions. The inhibited growth of the microbial strain was observed.

1. Antibacterial activity

Both the complexes were studied against a range of gram-positive strains such as *Staphylococcus aureus*, and *Bacillus subtilis*, and gram-negative strains like *Pseudomonas aeruginosa*, and *Escherichia coli*. Each of the samples was dissolved in Dimethyl Sulphoxide (DMSO) to prepare 0.1% concentrated solution. Pre-incubated slants on nutrient agar were incubated for 24 hours at 30-35°C. The investigation confirmed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* for both the complexes respectively (figure 4(a)). Each figure shows the inhibition zone for two different concentrations which are 5 mg/ml

and 10 mg/ml against standard streptomycin. Results of antibacterial activity revealed that for 5mg/ml concentration, complex B2 does not show inhibition for *B. subtilis*. But for 10 mg/ml concentration, it shows maximum inhibition for *E. coli* followed by *Pseudomonas aeruginosa*. Complex B3 does not show inhibition for *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 5mg/ml concentration. While it shows considerable inhibition for *Bacillus subtilis* followed by *Pseudomonas aeruginosa*, at 10 mg/ml concentration, data is given in Table 3.

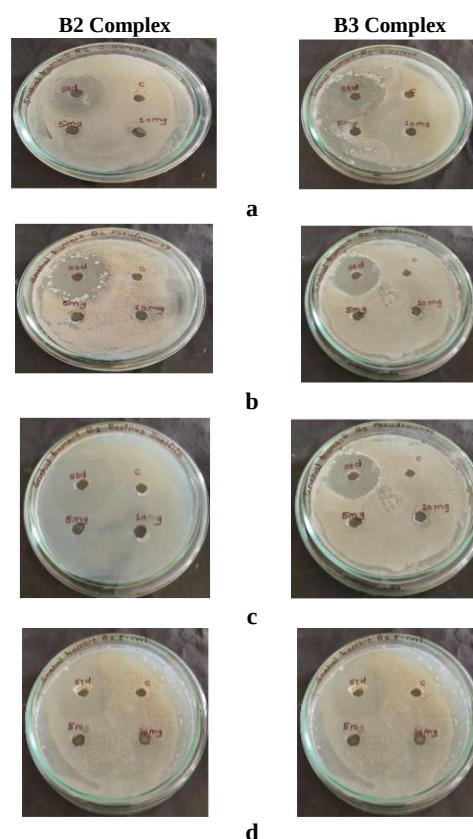


Figure 4. (a). Antibacterial activity against *Staphylococcus Aureus* ATCC no.6538, (b). Antibacterial activity against *Pseudomonas aeruginosa* ATCC no.27853, (c). Antibacterial activity against *Bacillus subtilis* ATCC no.6633 and (d). Antibacterial activity against *Escherichia coli* ATCC no. 8739

Table 3. Elemental analysis

Complexes	Sample	Conc.	Inhibition zone (in mm)			
			<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>B.subtilis</i>	<i>E.Coli</i>
$\text{MnCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	B2	5mg/ml	01	01	00	02
		10mg/ml	01	03	01	04
$\text{NiCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	B3	5mg/ml	00	00	01	01
		10mg/ml	00	03	04	02

2. Antifungal activity

The antifungal activity was evaluated with *Aspergillus niger* and *Candida albicans* in sabourauds dextrose agar medium. Both the suspensions of *Candida albicans* and *Aspergillus niger* were incubated at temperatures between 20-25°C for 72 hours and 30-35°C for 24 hours respectively. Figures 5(a) and 5(b) show the inhibition growth of *Aspergillus niger* and *Candida albicans* at 5mg/ml and 10 mg/ml concentration against standard fluconazole for B2 and B3 complexes. The data indicates that for 10 mg/ml concentration, complex B2 and B3 showed appreciable inhibition growth of *Aspergillus niger* and *Candida albicans* (Table 4).

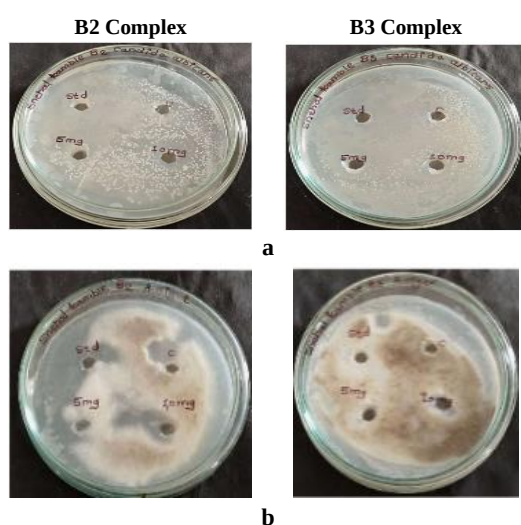


Figure 5. (a). Antifungal activity against *Aspergillus niger* ATCC no.16404 and (b). Antifungal activity against *Candida albicans* ATCC no. 10231

3. MIC activity

MIC or Minimum Inhibitory Concentration, refers to the lowest concentration of a sample that effectively prevents the growth of specific bacterial or fungal strains. The MIC of the reported complexes was determined using the broth dilution method. Samples were prepared through serial dilutions ranging from 2 mg to 0.25 mg. Initially, the cultures were incubated and then diluted to a density of 2×10^4 cells per ml, with cell counts performed using a hemocytometer. After treatment, the optical density was measured at 600 nm to assess the growth inhibition.

MIC of B2 ($\text{MnCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$) complex

The analysis of MIC activity against bacteria and fungi was carried out at different concentrations, ranging from 10 ug/ml to 80 ug/ml. This study elucidated that the B2 complex showed inhibition at low concentrations (10 ug/ml) for *E. coli* bacteria and *A. niger* fungus. For bacterial strains, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and fungus *Candida albicans* it required 20 ug/ml concentration to inhibit 50% of its growth (Table 5).

MIC of B3 ($\text{NiCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$) complex

Sample B3 was assessed for MIC activity against bacterial and fungal strains for varying concentrations (Table 6). This analysis concluded that the B3 sample required 10µg/ml for *E. coli*, 20 µg/ml for strains *S. aureus*, *B. subtilis*, and 40 µg/ml *P. aeruginosa* to inhibit 50% growth of bacteria. MIC assay regarding fungi showed that for both the fungal strains *C. albicans* and *A. niger* 10µg/ml (which is a low concentration) of B3 sample is required to inhibit 50% growth of fungi.

Table 4. Antifungal activity

Complexes	Sample	Conc.	Inhibition zone (in mm)	
			<i>C. albicans</i>	<i>A.Niger</i>
$\text{MnCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	B2	5mg/ml	03	01
		10mg/ml	06	05
$\text{NiCo}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot 6\text{H}_2\text{O}$	B3	5mg/ml	01	01
		10mg/ml	03	03

Table 5. Minimum inhibitory concentration B2 complex

Conc.	80 (ug/ml)	40 (ug/ml)	20 (ug/ml)	10 (ug/ml)
<i>E. coli</i>	-	-	-	-
<i>S. aureus</i>	-	-	+	+
<i>P.aeruginosa</i>	-	-	-	+
<i>B. subtilis</i>	-	-	-	+
<i>C.albicans</i>	-	-	-	+
<i>A. niger</i>	-	-	-	-

("+" -Turbidity/ growth, "-" No turbidity/no growth)

Table 6. Minimum inhibitory concentration B3 complex

Conc.	80 (ug/ml)	40 (ug/ml)	20 (ug/ml)	10 (ug/ml)
<i>E. coli</i>	-	-	-	-
<i>S. aureus</i>	-	-	-	+
<i>P.aeruginosa</i>	-	-	+	+
<i>B. subtilis</i>	-	-	-	-
<i>C.albicans</i>	-	-	-	-

("+" -Turbidity/ growth, "-" No turbidity/no growth)

Conclusion

This research article builds a base for developing novel mixed metal complexes that deserve applications across various fields. In this study, we successfully synthesized mixed Co-M tartrate coordinated complexes using the co-precipitation method. The resulting complexes have the formula Co_2ML_3 (where M = Mn, Ni, and L = $\text{C}_4\text{H}_4\text{O}_6^{2-}$). The results revealed as follows:

1. A simple, less time-consuming, environment-friendly inexpensive method was used for the synthesis of complexes.
2. The yield of both complexes is significant as compared to previously studied tartrate complexes.
3. XRD spectra confirmed the polycrystalline nature of complexes and calculated the grain size of complexes.
4. Our results regarding Infrared spectra verify the bidentate coordination of metal and ligand in complexes and SEM images showed the morphology of tartrate complex particles.
5. Complexes showed moderate inhibiting growth of bacterial strains and good inhibiting growth of fungal strains.
6. MIC study demonstrates that Co_2Mn tartrate complex exhibited potent antibacterial activity against *E. coli* and *P. aeruginosa* as well as fungi, *Candida albicans*, and *Aspergillus niger*.
7. For the Co_2Ni tartrate complex MIC results were found effective against *E. coli* bacteria and fungi, *C. albicans*, and *A. niger* at low concentrations. However, further studies are needed to evaluate the potential clinical applications and toxicity for therapeutic use.

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