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SYNTHESIS, STRUCTURE, ANTIBACTERIAL ACTIVITY AND TOXICITY OF CO(II) COMPLEX WITH 5-SULFOSALICYLIC ACID

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ABSTRACT: *In this investigation, a synthesized octahedral Bacillus subtilis, Staphylococcus aureus of 5-sulfosalicylic acid with Co(II) underwent thorough analysis of its composition and structure through elemental analysis, X-ray crystallography, infrared spectroscopy (FT-IR), and UV-visible spectroscopy. The compound, denoted as $[Co(H_2O)_6][C_7H_5O_3SO_3]_2 \cdot 2H_2O$, features a Co(II) ion arranged octahedrally with six water molecules. The bond lengths between cobalt and oxygen atoms range from 2.062(3) to 2.112(1) Å. The equatorial plane of the octahedron, defined by oxygen atoms O7, O7', O9, and O9' from the water molecules, appears almost perfectly flat with merely a 0.074(1) Å deviation. Some distortion in the octahedral geometry, induced by the Jahn-Teller effect, leads to shorter Co-O8 bonds in contrast to the Co-O7 and Co-O9 bonds.*

The antibacterial effectiveness of the complex was evaluated against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli, displaying notable efficacy specifically against Staphylococcus aureus. Furthermore, toxicity tests on the complex revealed an LD50 value of 830 mg/kg.

KEY WORDS: *5-sulfosalicylic acid, Bacillus subtilis, Staphylococcus aureus, Bacillus subtilis, Staphylococcus aureus.*

INTRODUCTION

5-Sulfosalicylic acid and its anions exhibit structural diversity and intriguing topology. Recently, there has been a growing interest in 5-sulfosalicylate ions and their metal complexes due to their antimicrobial, antifungal, and anti-inflammatory properties [1]. The 5-sulfosalicylic acid molecule contains three functional groups: SO3H, COOH, and OH. Five distinct forms of 5-sulfosalicylic acid have been identified: neutral, singly deprotonated at the sulfo group, singly deprotonated at the carboxy group, doubly deprotonated at the sulfo and carboxy groups, and triply (completely) deprotonated. The interactions of partially or fully deprotonated forms of 5-sulfosalicylic acid (H_2SSal^-, $HSSal^{2-}$, and $SSal^{3-}$) with metal ions can result in various coordination modes [2].

Cobalt, despite being present in the body in only about 1 mg, is crucial for life [3]. It is obtained from the diet, notably from green vegetables and cereals, and is frequently included in vitamin supplements [4]. A significant aspect of its biological significance lies in its association with vitamin B12 [5], also known as cobalamin, with a few other cobalt-containing enzymes having been recognized so far [6].

Although cobalt is an essential metal, it poses systemic toxicity risks, encompassing neurological, cardiovascular, and endocrine disturbances, primarily linked to free ionic Co(II). Blood concentrations exceeding 300 mg/l are deemed worrisome [7]. Cobalt's toxicity is associated with its redox properties, triggering reactive oxygen species (ROS) production, and its capacity to replace iron in metalloenzymes to create substitutionally-inert complexes [8].

SYNTHESIS OF THE COMPLEX

0.238 g (1 mmol) of the crystalline hydrate salt of $CoCl_2 \cdot 6H_2O$ was dissolved in 15 ml of water, 0.508 g (2 mmol) of a solution of 5-sulfosalicylic acid in 15 ml of absolute alcohol was slowly poured over the solution and mixed. The resulting solution was placed in a closed vial with several small holes at a constant temperature (20 °C), after 12 days, reddish-pink crystals formed at the bottom of the vessel. Yield 72%, Elemental analysis: calculated 32.43% (C), 3.04% (H), 2.36% (N), 10.81% (S), 40.54% (O); experimental 32.11% (C), 2.97% (H), 2.16% (N), 10.34% (S), 39.84% (O).

CRYSTAL STRUCTURE

The Co(II) complex is crystallized in the P-1 triclinic system. The compound $[Co(H_2O)_6][C_7H_5O_3SO_3]_2 \cdot 2H_2O$ consists of hexaaquacobalt(II) cations, 3-carboxy-4-hydroxybenzenesulfonate anions, and water molecules (Fig.1.). The cobalt(II) ion is coordinated by six water molecules in an octahedral geometry. The Co-O bond lengths range from 2.062(3) to 2.112(1) Å. The oxygen atoms O7, O7', O9, and O9' of the water molecules form the equatorial plane of the octahedron, with a minimal deviation of 0.074(1) Å from perfect planarity. The calculated distortion indices for bond lengths and angles in the CoO6 octahedron are IDd(Co-O) = 0.95% and IDa(O-Co-O) = 3.41%, indicating a slightly distorted octahedral geometry. Due to the Jahn-Teller effect, the octahedron is slightly distorted, with shorter Co-O8 bonds compared to Co-O7 and Co-O9 bonds [9]. These bond lengths and angles are comparable to similar cobalt complexes. The crystal structure features alternating layers of hexaquacobalt(II) cations and organosulfonate anions.

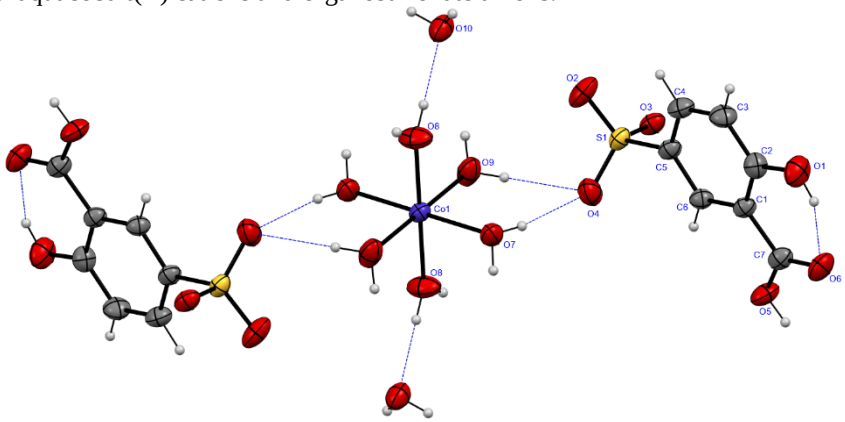

Figure 1. Molecular structure of $[Co(H_2O)_6][C_7H_5O_3SO_3]_2 \cdot 2H_2O$

The phenyl rings of the anions are nearly perpendicular to the b,c-plane. Within the sulfonate layer, adjacent organic molecules have sulfite groups pointing in opposite directions. Hydrogen bonding plays a crucial role in stabilizing the structure. Water molecules form short, linear O-H···O hydrogen bonds with sulfonate oxygen atoms. Additionally, phenyl-phenyl interactions between organic ligands contribute to the formation of one-dimensional chains along the [100] direction. The shortest cobalt-cobalt distance is 7.66 Å.

INFRARED SPECTROSCOPY

The infrared spectroscopic analysis of the cobalt coordination compound is shown in Table 1. The spectra indicate that the valence vibrations of the –OH group from water molecules in the compound were observed in the 3350 cm^{-1} region. Additionally, the asymmetric and symmetric valence vibrations of the carbonyl group were detected at 1699 cm^{-1} and 1663 cm^{-1}, respectively. The bonds between the metal and the ligand were noted in the 571 cm^{-1} region (Figure 2) [10].

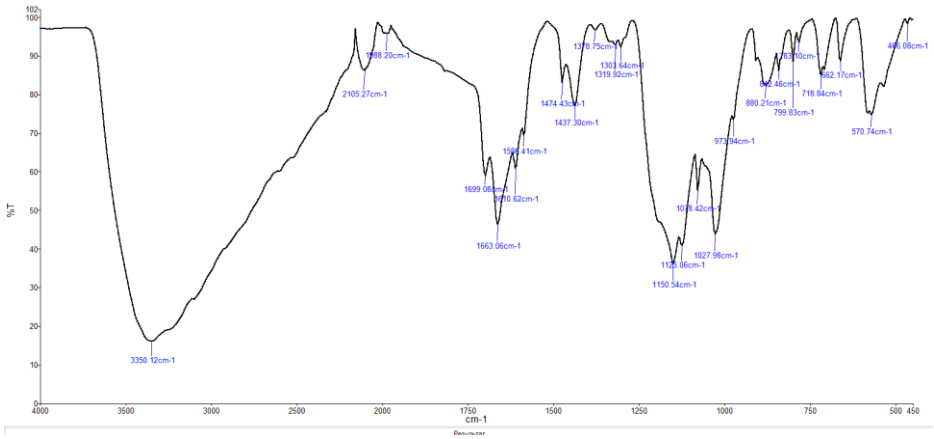

Figure 2. Infrared spectrum of the compound

Table 1. Infrared spectra of the compound with absorption peaks.

Compound	ν (-OH)	ν (C=O)	ν (xalqa)	ν (S=O)	δ (S=O)	δ_{as} (C-H)	δ_s (C-H)	ν (O-M)
$[Co(H_2O)_6](5\text{-}SSK)_2 \cdot 2H_2O$	3350	1699 1663	1610 1586	1474 1437	1320 1304	1151 1125	1078 1028	571

UV-VISIBLE SPECTROSCOPY

The electron transition phenomena in the compound were investigated using UV spectrophotometry over the range of 200-800 nm. Under UV light, the following electron transfer phenomena occurred in the compound: $t_{2g} \rightarrow e_2$, $n \rightarrow \pi^*$, ligand-to-metal charge transfer (LMCT), and $\pi \rightarrow \pi^*$(Table 2 and Figure 3) [11].

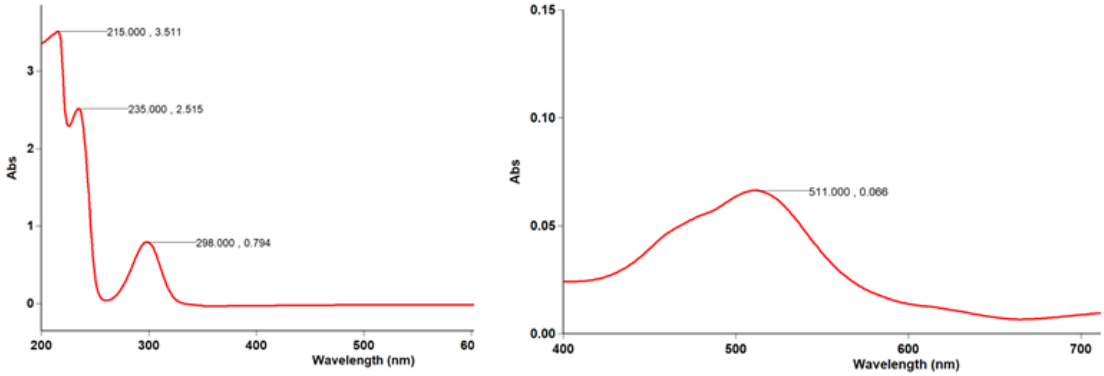

Figure 3. UV light absorption peaks in the compound.

Table 2. Electron transfer phenomenon of a coordination compound under UV light exposure.

Compound	$T_{2g} \rightarrow E_g$	$n \rightarrow \pi^*$	LMCT	$\pi \rightarrow \pi^*$
$[Co(H_2O)_6](5\text{-}SSK)_2 \cdot 2H_2O$	511	298	235	215

BIOLOGICAL ACTIVITY OF THE COMPOUND AGAINST MICROORGANISMS

First, a nutrient medium was prepared for bacterial growth and development. This involved adding 20 grams of "Meat Extract B Agar" (MPA) to one litre of water and autoclaving it under standard conditions for 2 hours.

Next, 20 ml of the prepared MPA solution was added to Petri dishes for the analysis. Standard concentration solutions of the samples under study were then applied in 500 µl drops under sterile conditions [12]. During this process, selected bacteria (gram-positive: *Bacillus subtilis*, *Staphylococcus aureus*; gram-negative: *Escherichia coli*) were evenly inoculated.

To evaluate the antibacterial activity of the compounds, the diameter of the sterile zone around the sample application site was measured after 24, 48, and 72 hours.

During the assessment of biological activity, the coordination compound was designated as KB-4. As per the test outcomes, the coordination compound exhibited significant efficacy against *Staphylococcus aureus*, a type of gram-positive bacteria*(*Table 3, Figure 4*)*.

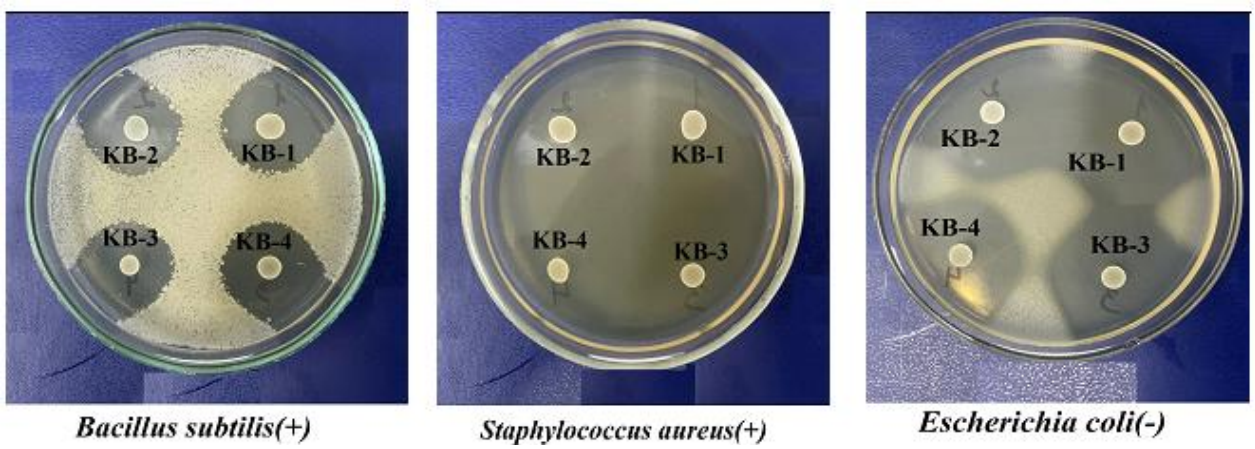

Figure 4. Antibacterial effect of a coordination compound

Table 3. Biological activity of the compound against microorganisms

	Gram positive		**Gram negative**
	B.sub	*St.aur*	*E.coli*
5-SSA	20	22	6
KB-4	21	40	30

ASSESSMENT OF THE TOXICITY LEVEL OF A COORDINATION COMPOUND

In addition, the analysis of biological activity is an integral part of assessing toxicity. The acute toxicity of the studied compounds was evaluated using the intragastric administration method, following OECD Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure. This test is outlined in Section 4 of the OECD Guidelines for the Testing of Chemicals, published by the OECD Publishing House in Paris [13].

Fixed-dose samples of the compound were administered to a group of male laboratory mice of the same sex at doses of 50, 100, 500, 1000, and 1500 mg/kg. The toxicity of all compound was monitored throughout the experiment. Male white laboratory mice with an average body weight of 20 ± 2.0 g were selected for the study.

The compounds were administered orally using a special probe at doses of 50, 100, 500, 750, 1000, and 1500 mg/kg. The control group received an equal amount of purified water instead of the compounds. On the first day of the study, the general condition of the animals in both the experimental and control groups was monitored hourly, with particular attention given to possible tremors and mortality (Table-4).

At the end of the experiment, the median lethal dose (LD50) and toxicity class of the tested substances were determined. LD50 refers to the dose at which 50% of the tested animals died, while the toxicity class indicates the degree of toxicity of the substance based on the obtained results.

-50-100 mg/kg doses: All test substances were administered orally to mice at doses of 50 and 100 mg/kg by gavage. Following administration, the mice exhibited rapid breathing for approximately 5 minutes, followed by a 20-30 minute

period of hoarding behavior. These effects lasted for 40-45 minutes. The mice fully recovered within 2-4 hours, and no signs of acute toxicity or death were observed during the 14-day observation period.

- 500 mg/kg dose: After administration of 500 mg/kg of the samples, the mice displayed rapid breathing and hoarding behavior after about 5 minutes, with their eyes narrowing shortly thereafter. Acute toxicity effects were noted, but no deaths occurred.

-750 mg/kg dose: Following the administration of 750 mg/kg, the mice showed rapid breathing and hoarding after approximately 5 minutes, and tremors along with narrowed eyes were observed shortly thereafter.

-1000 mg/kg dose: The mice exhibited rapid breathing, tremors, hoarding, narrowed eyes, and decreased movement. By the seventh day of the experiment, 50% of the mice treated with KB-4 experienced acute poisoning and died.

Table-4. The toxicity level of the compound.

Groups	Animal type, gender	Dose mg/kg, ml	Number of animals/dead animals in the group	Average animal weight (g) (1 day)	Average animal weight (g) (7 days)	Average animal weight (g) (14 days)	LD50 with confidence interval
KB-4	Laboratory white mouse, male	50	6/0	20	22	24	830 mg/kg
		100	6/0	21	22	24	
		500	6/0	21	20	23	
		750	6/0	21	18	21	
		1000	6/3	20	19	20	
		1500	6/6	21	-	-	

CONCLUSION

The synthesis and structural examination of the octahedral aqua complex of 5-sulfosalicylic acid with Co(II) are detailed. The compound's structure and composition were scrutinized employing techniques like elemental analysis, X-ray crystallography, and spectroscopy. This complex exhibited antibacterial properties against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli, with notable effectiveness specifically against Staphylococcus aureus. Furthermore, its toxicity was assessed, revealing an LD50 value of 830 mg/kg.

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