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Study of Physiological and Pharmacological Properties of Hydrochlorothiazide Schiff Base and its Metal Ion Complex

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Abstract:- The current study focuses on the synthesis and the biological evaluation of Schiff base metal complex produced from the diuretic drug Hydrochlorothiazide. The bidentate ligand is produced by inserting salicylaldehyde into a 1:1 molar ratio and condensing 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ^6,2,4-benzothiadiazine -7-sulfonamide. Complex of Co(II) with general formula ML2 have been produced using this bidentate ligand. We examined the antibacterial properties of the produced compounds. The results that were achieved were compared with the original medicine, hydrochlorothiazide. The compounds exhibited enhanced antibacterial assay, with the Co(II) complex demonstrating especially strong activity. The ligand and its complex Co(II) were also screened for the invivo Diuretic techniques.

Keywords:- *Antimicrobial Assay, Diuretics, Diuretic Activity, Hydrochlorothiazide, Metal Ion Complex.*

I. INTRODUCTION

Diuretics have various therapeutic advantages and are essential in medical treatments because they effectively remove excess water and salt from the body. They are frequently given to treat hypertension because they efficiently lower blood pressure by decreasing the amount of fluid in the circulatory system, which lowers the risk of cardiovascular illnesses and problems with the kidneys. [1] The fact that these disorders are common highlights their importance because they are substantial contributors to global health issues and call for continuous attempts to improve diuretic therapy in order to get better results. A major focus of study continues to be the hunt for safer diuretics with fewer side effects and the creation of customised therapy regimens based on unique patient characteristics. Understanding diseases better has made it possible to identify new diuretic targets, which could lead to better treatments. Optimising the use of diuretics and creating new, cost-effective alternatives is essential due to their cost-effectiveness, particularly in environments with limited resources. [2]

Benzothiazide is the source of thiazide diuretics, a class of diuretic medications. By inhibiting the thiazide-sensitive Na+/Cl- symporter, they prevent the kidney's distal convoluted tubules from reabsorbing sodium and/or calcium. [3] Hydrochlorothiazide is a diuretic medication used to alleviate oedema caused by mild-to-moderate congestive heart failure and to treat hypertensive illness. Because of a paradoxical effect, patients with diabetes insipidus may potentially benefit from its treatment. [4]

Because of their economic and biological significance, as well as their richness and structural variability, schiff bases are extensively applicable and play a significant role in coordinating chemistry. The study of metal complexes has shown that complexation with metal ions boosts biological activity. [5] Consequently, a thorough analysis of metal complexes of Schiff bases originating from hydrochlorothiazide medications containing salicylaldehyde has been carried out in the current work.

The bioactivity of metal complexes, particularly those made with Schiff bases of hydrochlorothiazide, which may have improved antibacterial qualities, has been studied recently. The importance of transition metal ions for biological processes has prompted studies on their antibacterial capabilities. Because of their potential for bioactivity, Schiff bases—especially those generated from sulfonamide and thiazide—have been the subject of extensive research. As a result of their critical role in developing coordination chemistry, 3d transition metal ions and Schiff bases are currently the subject of increased attention in coordination chemistry. Because of their increased biological activity and industrial significance—which can occasionally outpace the effectiveness of the original medications—metal complexes, particularly those containing Schiff base chelates, are of great interest. [6] The work to synthesise, characterise, and evaluate the pharmacological activity of the Schiff base of hydrochlorothiazide and its Co (II) complex is expanded upon in this paper.

II. MATERIALS AND METHOD

Table 1 Synthesis and Physicochemical Characteristics of Complexes

Synthesis and physicochemical characteristics of complexes					
Ligand/Complex	Ligand-metal ratio	Yield	Stability constant log K/L mol^{-1}	Molar Conductance $\Omega^{-1}cm^2mol^{-1}$	Δ F K cal $mole^{-1}$
HCT-SA	----	72%	----	----	----
$[Co(HCT\text{-}SA)_2(H2O)_2]$	2:1	61%	11.03	13.2	-15.7

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Analytical grade compounds were all that were used. We bought the diuretic drug Hydrochlorothiazide from Dhamtech Pharmaceuticals in Gujarat. We bought acetone, methanol, and metal salts from Merck Chemicals.

- *Synthesis of Schiff Base:*

- *Synthesis of Hydrochlorothiazide – Salicylaldehyde (HCTZ-SA) Schiff Base*

Separate equimolar solutions of the pure drugs salicylaldehyde and hydrochlorothiazide were made in a 1:1 methanol water combination. For four hours, the two solutions were combined and refluxed. For four days, the reaction mixture was stored. The reaction combination produced white hydrochlorothiazide-salicyclaldehyde (HCTZ-SA) Schiff base crystals, which were filtered and cleaned with a 1:1 methanol water mixture. After that, the crystals were weighed and vacuum-dried. Melting point data was recorded. [7]

- *Ligand-Metal Ratio and Stoichiometry:*

The Monovariation approach was used for all conductometric titrations in order to verify the ligand-metal ratio. At room temperature, every titration was carried out. Plotting the results revealed that, following volume adjustments, the ligand-metal ratio was 2:1. Turner and Anderson's adaptation of Job's continuous variation method provided more confirmation of the 2:1 complex's creation. The stability constants and free energy change were also calculated. The results are given in Table 1.

- *Synthesis of Complex:*

- *Synthesis of Hydrochlorothiazide – Salicylaldehyde with Cobalt Chloride [Co (HCT-SA)2 (H2O)2]*

Separate solutions of cobalt chloride and HCT-SA were made in a 50% acetone-water combination. After combining the two solutions, a wine-red solution with a pH of 3.8 was produced. The resultant solution's pH was raised to 6.5 by dropwise adding N/5 NaOH. After refluxing for four hours, the solution was maintained at its best for a day. A crystalline substance with a bluish colour emerged in the solution. The product underwent filtration, 50% acetone washing, drying, and weighing. The yield observed was 61%. [7]

- *Characterization:*

The HCT Schiff base and its Co (II) complex were analyzed at Indian Institute of Science Education and Research, Bhopal (IISERB). Pure and dry sample of Schiff base and its Co (II) complex were sent for recording their IR spectra and also for Elemental Analysis.

- *Antimicrobial Assay:*

The antibacterial activity of both the Schiff base and Co(II) complex of hydrochlorothiazide was evaluated in conjunction with that of the parent medication, HCT. Using the agar disc-diffusion method, the antibacterial activity was evaluated against Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Discs constructed of Whatman filter paper Grade No. 1 were impregnated with the three samples' individual DMSO dissolves. Then, a lawn of Mueller-Hinton agar containing bacterial cultures was made, and on top of the lawn were the discs that had been impregnated with the three solutions—HCT, Schiff base of HCT, and the complex. The diameter of the zone of inhibition, or the area where bacteria do not grow, was measured after the plates were incubated for 18 to 24 hours. The extent of the zone correlates with susceptibility. [6]

III. RESULTS AND DISCUSSION

- *Elemental Analysis:*

The elemental analysis results are shown in Table 2, which validates the Co (II) complex's ligand metal ratio as determined by conductometric techniques. These findings are also consistent with the Co (II) complex's suggested ML2 composition (Table 2).

Table 2 Analytical Data of Ligand and Complex

Analytical data of ligand and complex

Ligand/Complex	Elemental analysis % Found (calculated)						Melting point°C
	C	H	N	S	Cl	Metal	
HCT-SA	41.22 (41.84)	2..14 (2.98)	10.01 (10.45)	15.31 (15.94)	8.91 (8.84)	----------	255
$[Co(HCT\text{-}SA)_2(H2O)_2]$	37.22 (37.50)	2.28 (2.90)	9.81 (9.38)	14.11 (14.29)	7.11 (7.92)	6.92 (6.58)	242

- *IR Spectra:*

The characteristic infrared spectra of the Schiff base of hydrochlorothiazide and its Co (II) complex showed potential donor sites that have the tendency to coordinate with the metal ions: azomethine linkage (C=N) at 1559 cm-1 and 1560 cm-1, respectively; benzothiadiazine secondary amine (NH-C-NH) at 1150 cm-1 and 1148 cm-1, respectively; and aldehydic hydroxyl group (Ar-OH) at 2346 cm-1 and 2345 cm-1, respectively. The amine/amide (N-H) vibration in the HCT-SA and Co(II) complex infrared spectra were recorded at 3361 cm-1 and 3362 cm-1, respectively. The hydrogen bonded alcohol/phenol linkage (-OH) was responsible for the appearance of a new, sharp band at 3266 cm-1 for HCT-SA and 3265 cm-1 for Co(II) complex. These were both strong peaks. In the metal complex, a new band emerged between 609 cm-1 because of the M–N vibration, which shows that the imine nitrogen atom coordinates with the metal ions. The M–O linkage was indicated by a prominent peak that emerged at 1316 cm-1 in the Co(II) complex graph. For both adducts, the nitrile peaks coincided at 2160 cm-1. For the HCT-SA and Co(II) complexes, the aromatic ring of C=O displayed a faint peak at 1655 cm-1 and a strong peak at 1540 cm-1, respectively. The HCT-SA graph's aromatic ring of C–H peaked at 795 cm-1, while the Co(II) complex graph's peaked at 805 cm-1.

- *Antimicrobial Assay:*

The Schiff base of HCTZ and its Co(II) complex were assayed for Antimicrobial activity alongside the parent drug Hydrochlorothiazide, against three bacteria – *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* using the agar disc-diffusion method. The zone of inhibition was measured and the results are depicted in Table 3.

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Table 3 Antimicrobial Activity of the Drug Hydrochlorothiazide, its Schiff base and its Co(II) complex.

Compounds	**Zone of Inhibition (mm)**		
	E. coli	***P. aeruginosa***	***S. aureus***
HCTZ	—	—	—
HCT-SA	5 mm	4 mm	4 mm
$[Co(HCT\text{-}SA)_2(H_2O)_2]$	—	8 mm	5 mm

The parent drug Hydrochlorothiazide does not show any activity against any of the three bacterial strains. Whereas, the Schiff base of Hydrochlorothiazide is active against all bacterial strains and shows average activity. On the other hand, the Co(II) complex shows relatively good activity as compared to HCT-SA, except it does not show zone of inhibition against E. coli.

- *In-Vivo Diuretic Activity:*

The diuretic activity was tested on species of Albino mice. The results have been depicted in Table 4.

Table 4 Summary of Diuretic Activity of Adducts

S. No.	**Group**	**Average Volume of Urine (µl)**			**Final Value**
		1:00pm	**3:00pm**	**5:00pm**	
1.	Control	113.94	167.22	118.01	113.06±29.656
2.	HCT	155.66	208.09	266.91	210.22±55.656
3.	HCT-SA	175.75	235.48	289.10	233.44±56.702
4.	Co (II) Complex	386.66	375.29	378.57	380.17±5.852

IV. CONCLUSION

The successful synthesis of the hydrochlorothiazide schiff base and its Co(II) complex was achieved. Both adducts have been reported to be stable at room temperature, insoluble in water, and somewhat soluble in DMSO based on their physicochemical properties. The complex's paramagnetic character is supported by the magnetic moment measurements. The complex's molar conductance value reveals that it is not electrolytic. The elements C, H, Cl, N, S, and Co were verified to be present in the structure by the elemental analysis. Moreover, infrared spectra supported the suggested structure. The test for the antibacterial activity was successful. Comparing both adducts to the original drug, increased activity was seen.

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