Physico-chemical and Biological Studies of Some Metal Complexes with Fluorinated Chloramphenicol Derivative

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Physico-chemical and Biological Studies of Some Metal Complexes with Fluorinated Chloramphenicol Derivative

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Abstract. Due to the prevalence of FF, its complexes with (CoII, NiII, and CuII) were prepared, characterized, and the effect of these metal ions on the biological effectiveness of FF was studied. Elemental analysis; IR and 1H-NMR were used to determine the structures and mode of bonding, TGA, and DSC techniques were used to make thermal analysis for the prepared complexes. TGA was used to study the mechanisms of the thermal decomposition for florfenicol complexes. The prepared complexes are thermally stable and started to decompose after 200oC. Broido's method was used to determine the activation energy for the prepared compounds, while thermal transitions and kinetic studies were carried out by differential scanning calorimetry technique (DSC). The antimicrobial activity of the complexes of florfenicol with different concentrations (0.005, 0.01, 0.02 μg/mL) was examined by the agar prolixity technique against common human pathogenic Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). Comparatively, Nickel and Copper complexes showed a higher zone of inhibition against the tested pathogens. The antibiotic complexes were more active than the ligand itself for some breeds.

Keywords: Florfenicol, IR, ^{1}H-NMR, Thermal Studies (TGA and DSC), Antimicrobial Activities.

1. Introduction

Three compounds belong to the amphenicol family: thiamphenicol, florfenicol, and chloramphenicol. The tissue penetration due to its lipophilicity. Chloramphenicol's effects on the body include aplastic anemia, gray baby syndrome, agranulocytosis, hypersensitivity, and human cancer. Regulators have established MRLs (minimum necessary performance levels) and MRLs (maximum residue limits) for Amphenicol family residues in some animal-derived foods because these residues are easy to get, relatively inexpensive, and have adverse effects [3,4]. Amphenicols, belonging to the Amphenicol family, are phenylpropanoid-structured, broad-spectrum antibiotics that work against various Gram-positive and Gram-negative bacteria, including most anaerobic bacteria. The range of florfenicol MRLs is 100 IU/kg for poultry muscle and 3000 IU/kg for bovine, ovine, and caprine liver [5,6]. fluorinated (Cm) derivative of florfenicol (FF) which is a synthetic drug and also a die-stereoisomer has antimicrobial activity. FF is considered one of the most widespread medicines, as tens of thousands of tons are used annually, and its poor solubility in water limits its clinical uses [1,2]. Florfenicol shows good Florfenicol is similar in structure to chloramphenicol but lacks the para-n-nitro group, which is associated with aplastic anemia in humans. Florfenicol is approved for the treatment of cattle, poultry, and aquaculture, as well as for the treatment of other domestic and non-domestic

species. FF could be a broad-spectrum bactericidal antibiotic that acts against the 50S rDNA of bacteria. In cattle, FF is moderately bioavailable after intramuscular injection (79%) and has good tissue distribution into the central nervous system [7,8]. As a result of the widespread of FF, its complexes with (CoII, NiII, and CuII) that have an important biological effect with this drug were prepared. The influence of these metal ions on the biological efficacy of the FF was examined.

2. Experimental

2.1 Preparation:

The solution of the antibiotic was prepared and mixed with an aqueous solution of a metal with a molar ratio (of 1:1). For two hours, the resultant mixture was heated under reflux. A solid product formed after the concentrated solution was let to stand at room temperature for the entire night. This solid product was subsequently filtered and dried.

2.2 Elemental analysis:

The Cairo University central lab performed the CHNS investigation of the antibiotic and its complexes. The Volhard method was utilized to assess the complexes' chloride contents. Table 1 contains both the complexes' analytical and physical measurements.

Table 1. The elemental analysis, melting point, and color of Florfenicol and its complexes

Compound	Color	Calculated (Found)%					
		Cl	M	S	N	H	C
$C_{12}H_{14}Cl_2FNO_4S$	White	19.83	------	8.94	3.91	3.91	40.22
$Cu(L)_3.2OH.4H_2O$	Pale	17.12	5.11	7.72	3.38	3.38	34.72
	Blue	(16.92)	(4.81)	(7.54)	(3.31)	(3.23)	(35.10)
$Co(L)_2.2H_2O$	Dark	17.51	7.27	7.89	3.45	3.45	35.51
	Green	(17.02)	(7.89)	(7.71)	(3.22)	(3.33)	(36.01)
$Ni(L)_3.2OH.4H_2O$	Pale	17.18	4.74	7.74	3.39	3.39	34.85
	Green	(16.83)	(4.22)	(7.68)	(3.32)	(3.31)	(34.26)

All complexes exhibit a melting point greater than 300°C

2.3 Measurements

2.3.1 Infrared spectra: The Shimadzu 4800s FTIR spectrometer Maxima, manufactured in Japan, was utilized to conduct the data analysis. The KBr disk technique was employed during the process

2.3.2 Thermal analyses measurements: The experiments were conducted using Shimadzu DSC-60A Maxima and Shimadzu TGA 50 GALM instruments from Japan. The measurements were carried out in a nitrogen atmosphere at a rate of 10 °C per minute.

2.3.3 ^{1}HNMR measurements: NMR spectra were obtained from DMSO-d6 at 500 MHz using a Joel Lambda 300 instrument

2.3.4 Biological activity: The Clinical and Laboratory Standards Institute's criteria were followed for conducting the antibacterial activity measurements [9]. Using the agar diffusion method, the produced complexes were evaluated for their antibacterial efficacy against the human pathogenic pathogens Escherichia coli and Staphylococcus aureus. Using a sterile corn borer, 5 mm diameter wells were made in each Petri dish. For the bacterial pathogens, Mueller Hinton Agar (MHA) was employed as the medium. Each liter of the medium contained the following ingredients: Agar (17g), casein hydrolysate (17.5g), starch 1.5g, and beef (dehydrated infusion from 300) 2g were all dissolved in 1L of distilled water with a final pH at 25°C is 7.3 ± 0.1. The medium was autoclaved for 15 minutes at 121°C to sterilize it [10,11]. After that, the infected plates were incubated for 24 hours at 37°C. Two different bacteria groupings were tested biologically with Florfenicol and its metal complexes: Gram-positive (Staphylococcus) and coli ATCC8739 strain) and Gram-negative (Escherichia coli ATCC6538P).

3. Results and Discussion

3.1 IR SPECTRA

Table 2, Figure 1 presents the IR spectra of Florfenicol and its metal complexes., it shows strong bands at the range of 3445-3453 and 3318–3396 cm−1 for the stretching vibration of NH amide groups [12]. The band of OH stretching vibration appears at the range of 3450 cm−1 for Florfenicol and its complexes spectra overlapped with the stretching vibration of NH amide groups. The two sharp bands at 1683 and 1535 for the amide group appear in the spectrum of the ligand and the complexes without Noticeable changes in the position or shape of the bands indicate the amide group does not participate in the complexation. The far IR spectra display bands indicating the bonding of oxygen and chlorine with the metals at 480-482 cm−1 (M-O) and 419-425 cm-1 (M-Cl).

Table 2. Fundamental infrared bands (cm^{-1}) of Florfenicol and its (Co, Ni, Cu) metal complexes

	Ligand	Copper	Cobalt	Nickel
ν_{NH} &ν_{OH}	3453	3572-3445	3453	3453
ν_{NH}	3319	3396-3321	3320	3319
ν_{C-H}	2925-3030	2994-3031	2925-2994	2931-3018
$\nu_{C=O}$	1684	1684	1684	1683
Amide $\nu_{C=O}$	1530	1534	1534	1530
ν_{C-N}	1277	1277	1277	1279
Stretch ν_{C-O}	1144.79	1136.11	1144.79	1140
ν_{M-O}	-----	482	481	481
ν_{M-Cl}	-----	426	420	420

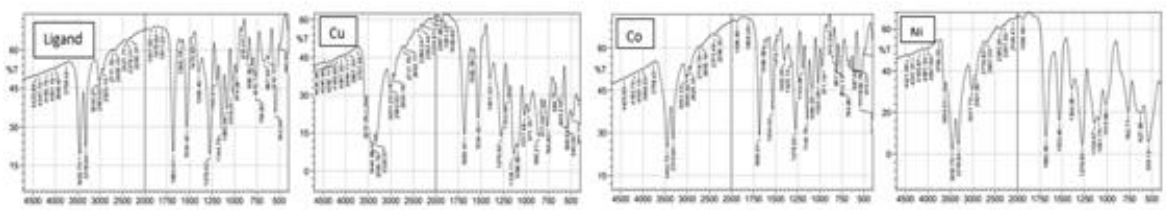

Figure 1. Infrared spectra Florfenicol and its (Co, Ni, Cu) metal complexes

3.2 ^{1}H-NMR SPECTRA

The 1H-NMR spectra of Florfenicol complexes, Table 3, shows signal appeared at δ = 7.59 ppm (doublet) due to C2-H protons at C3-H and C4-H. While the signal at 7.83 ppm (doublet) is probably for 2-C-H protons at C2-H and C5-H.

The signals for the CH3 protons at (3.34-3.35 ppm), NH at position 10 at 8.62 ppm, and for OH proton at (3.13-3.17 ppm) are appeared in all complexes.

Table 3. ^{1}H-NMR spectra for Florfenicol metal complexes

	H_7	H_1	H_8, H_9	H_6	H_{11}	H_3, H_4	H_2, H_5	H_{10}
Cu	3.17	3.34	4.29-4.96	6.15	6.42	7.59	7.83	8.62
Co	3.13	3.35	4.25-4.96	6.15	6.43	7.59	7.83	8.62
Ni	3.13	3.34	4.25-4.96	6.15	6.42	7.59	7.83	8.62

3.3 Differential scanning calorimetry (DSC): The prepared complexes were analyzed using a DSC graph to determine the glass transition, melting, and crystallization temperatures [13], Table 4, Figure 2.

Table 4. Thermal transitions of the prepared complexes.

Complex	Peak (type)	Point of reaction (ºC)	Tc (ºC)	Tm (ºC)	Tg (ºC)	Enthalpy (ΔH) (J/g)
Cu complex	a(endo)	150.89	152.87	——	158.36	-67.57
	b(exo)	214.69	——	226.43	——	122.62
	c(endo)	327.28	32.83	——	338.83	-11.49
Co complex	a(endo)	151.16	153.37	-------	158.37	-244.21
	b(exo)	231.58	——	245.46	——	203.09
Ni complex	a(endo)	150.16	152.20	-----	157.57	-231.38
	b(exo)	239.70	——	264.95	——	129.56

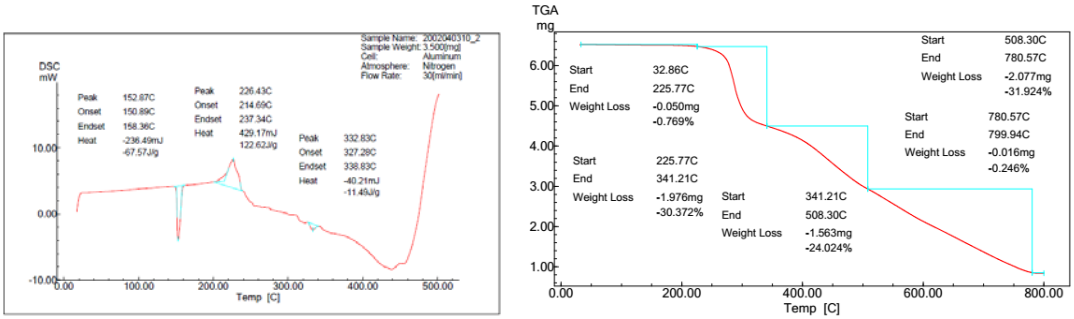

Figure 2. Thermal analysis data TGA and DSC Cu complex

The change in capacity across a broad temperature range is described by the Debye model [14]. This empirical formula can be used to express the capacity, C_p. $C_p = aT + b$ (1)
"a" and "b" parameters were determined in Table 5.
Additional applications utilizing the Debye model on the complexes are presented within the parameters of the subsequent equations:

$$C_p \cong C_y = \alpha T^3 + \gamma T \quad (2) \qquad \frac{C_p}{T} = \alpha T^2 + \gamma \quad (3)$$

γ and α represent the coefficients of lattice and electronic heat capacities, respectively. It is assumed that C_p, the heat capacity at constant volume, is equal to C_v. Straight lines with slopes α and intercepts γ should be obtained in Table 6 when plotting Cp / T against T^2.

3.3.1 Determination of kinetic parameters through thermal decomposition analysis: Thermogravimetric data was used to calculate the kinetic parameters of solid-state processes involving weight gain or loss [15]. The activation energy linked to each stage of decomposition was calculated using Broido's approach [16]. The activation energy (Ea) can be found using the following equation:

$$lnln\left(\frac{1}{Y}\right) = \left(\frac{-E_a}{R}\right)\frac{1}{T} + constant \quad (4) \qquad \text{where} \quad Y = \frac{W_t - W_\infty}{W_0 - W_\infty}$$

Here, Y represents the fraction of the number of initial molecules that have not yet decomposed; Wt denotes the weight at any given time t; W∞ represents the weight at infinite time (which is equal to zero); and W0 represents the initial weight. The kinetic parameters of the prepared complexes are given in Table (7).

Table 5. The slopes "a" and intercept "b" for DSC curves of the complexes.

Complex	a_1	a_2	a_3	a_4	a_5
	$[b_1]$	$[b_2]$	$[b_3]$	$[b_4]$	$[b_5]$
Cu complex	-0.0493	-29.593	5.0137	0.5313	-1.4228
	2.6178	16991	-2165.5	-223.47	754.82
	319-393 ºK	429-431 ºK	431-438 ºK	469-496 ºK	501-521 ºK
Co complex	7.0296	0.383	-1.4905		
	-3064.6	-153.64	796.66		
	431-440 ºK	465-499 ºK	502-530 ºK		
Ni complex	-7.295	4.2573	0.4112	-0.5561	
	308.72	-01784.5	-163.66	336.09	
	419-421 ºK	421-428 ºK	495-529 ºK	530-549 ºK	

Table 6. The slopes "α" and intercept "γ" for DSC curves of the complexes.

Complex	α_1	α_2	α_3	α_4	α_5
	$[\gamma_1]$	$[\gamma_2]$	$[\gamma_3]$	$[\gamma_4]$	$[\gamma_5]$
	-0.0675	0.1022	0.0152	$2.0*10^{-8}$	0.0328
Cu	[1.2461]	[-1.8965]	[-0.2915]	[0.0.005]	[0.9133]
complex	(420-428) ºK	(432-438) ºK	(479-496) ºK	$(542\text{-}636)^2$ ºK	(501-521) ºK
	0.1118	0.0106	-0.0321		
Co	[-2.1162]	[-0.1835]	[0.9057]		
complex	(432-440) ºK	(473-502) ºK	(507-525) ºK		
Ni complex	-0.2832	0.1469	0.0077	-0.011	
	[5.0606]	[-2.5982]	[-0.1124]	[0.4277]	
	(419-421) ºK	(422-428) ºK	(495-529) ºK	(536-550) ºK	

Table 7. Kinetic parameters of the prepared Complexes Broido method

Compound	Decomposition Temperature range (°C)	Regression value (R^2)	Activation energy in Kcal/mol
Cu Complex	192-271	0.9894	30.59
	317-475	0.9620	3.73
	565-774	0.8594	12.69
Co complex	250-279	0.9940	34.52
	301-369	0.9483	4.77
	398-503	0.8981	4.07
	549-725	0.9391	13.05
Ni Complex	219-258	1.0000	18.46
	283-308	0.9034	40.95
	308-403	0.9567	3036
	450-751	0.9757	7.15

3.4 Antimicrobial Activity

The well diffusion method was used to evaluate the antibacterial efficacy of florfenicol complexes made of copper (Cu), cobalt (Co), and nickel (Ni) against human infections that were resistant to multiple drugs. To start, 0.5 McFarland standard bacterial inoculum (1×108 CFU/ml) was produced and added to MHA agar plates., which was then evenly spread across the plates. Next, different concentrations (0.005, 0.01, and 0.02µg/ml) of florfenicol and Complexes of copper (Cu), cobalt (Co), and nickel (Ni) were made. Following that, 25–50 µl of these solutions were added to the plate wells. Following a 24-hour incubation period at 30°C, the diameter of the inhibitory halo was measured on the plates. From Table 8. Bacterial growth suppression was assessed in Petri dishes after 24 hours at 37°C, with the diameter of the inhibition zone varying from (33 to 42 mm) and (31 to 43 mm) in E. coli and Staphylococcus aureus, respectively. Nickel complex strongly inhibits the growth of Staphylococcus aureus among the Gram-positive bacteria that were tested at higher concentrations (43 mm). Additionally, Gram-negative bacteria like E. coli displayed a 41 mm zone of inhibition when subjected to 0.02 μg/mL of the Ni complex. This outcome could be a result of nickel ions being released from the Ni complex, causing an increase in membrane permeability and the generation of reactive oxygen species, or ROS, which eventually cause cell death.

Followed by Copper complex that demonstrated remarkable antibacterial activity The Cu complex exhibited effective antibacterial activity against both the Gram-positive bacterium Staphylococcus aureus (38 mm) and Gram-negative bacterium E. coli (35 mm), despite the slightly thicker cell wall of the Gram-positive bacteria. The observed antibacterial activity may be attributed to the release of Cu+ ions [16-17]. Numerous studies have validated the cobalt complex's antibacterial activity against the tested bacterial pathogens [18]. The cobalt complex's antibacterial action could be explained by the electrostatic interaction that breaks down the cell wall between the complex and S. aureus bacteria. Moreover, the complex's release of cobalt ions may cause cellular proteins to become inactive and inhibit DNA replication. In the end, cell death and the prevention of bacterial growth are the outcomes of this interaction with the cell wall and disruption of the membrane structure [19].

Table 8. Inhibition zone diameters were measured against E. coli and Staphylococcus aureus (mm) using 25 and 50μl of various concentrations of florfenicol and its complexes

	The diameter of the inhibition zone against E. coli (mm)			Diameter of inhibition zone against Staphylococcus aureus (mm)		
Compound	0.005 μg/mL	0.01 μg/mL	0.02 μg/mL	0.005 μg/mL	0.01 μg/mL	0.02 μg/mL
L.25	36	30	31	35	30	31
L.50	39	36	35	40	39	35
Cu.25	35	33	33	38	36	39
Cu.50	40	38	38	40	38	40
Co.25	36	33	35	33	36	36
Co.50	40	39	38	40	40	38
Ni.25	40	38	37	36	37	38
Ni.50	41	40	39	43	41	40

(L= florfenicol, Cu, Co, Ni = Cu, Co, Ni complexes with florfenicol)

4. Conclusion

From IR and NMR studies, it's found that florfenicol bonded with the metals through chlorine atoms. The formed complexes are thermally stable and start to decompose after 200^{o} C. Complexation with Cu, Co, and Ni does not affect florfenicol's antibacterial activity against Escherichia coli and Staphylococcus aureus; however, high concentrations of the complexes may increase the antibacterial activity against both Escherichia coli and Staphylococcus aureus due to the release of metal ions. In the future, the effect of more metals on FF should be studied.

5. Conflicts of interest:

There are no conflicts to declare.

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