

Research Article

Preparation, Diagnosis, Biological Activity, and Theoretical Studies of Some Mixed Drug Complexes

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This paper includes synthesis and characterization of mixed ligand complexes derived from mefenamic acid and metformin using transition metal ions such as Co(II) and Cu(II). These complexes have been characterized by magnetic susceptibility, molar conductance, TG analyses, and spectral techniques such as FTIR and UV spectra. The theoretical study of the ligands and their complexes using semiempirical (PM6) method was used to measure IR and UV spectroscopy, HOMO-LUMO categories of the ligands. These synthesized complexes are also studied for their biological activities. The studies made on these complexes proposed a six octahedral geometry.

1. Introduction

Mefenamic acid [L₁] 2-[(2,3-dimethylphenyl)amino]benzoic acid, an anthranilic acid derivative, which is widely used to relieve mild to moderate pain [1], demonstrates nonsteroidal anti-inflammatory, antipyretic, and analgesic activity [2, 3]. Metformin [L₂] has two amino groups that have an excellent ability to coordinate with many transitional chain elements, thus giving highly colored clonal complexes, especially copper (II), nickel (II), and cobalt (II), because these metal ion complexes can be measured spectrally [4–6]. Metformin is a low-cost antioxidant. Metformin tablets also help reduce body weight or at least weight stability. Most tablets are used to treat high blood sugar that causes increased body weight [7]. Metformin hydrochloride is freely soluble in water and practically soluble in acetone, ether, and chloroform, and pH of a 1% water solution of metformin hydrochloride is 6.68 [8]. All the synthesized mixed ligand complexes (0.5 mg/mL) were screened for their antibacterial activities against five bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus sp.*, *Candida albicans*, and *Pseudomonas aeruginosa*) by agar well diffusion assay method [9] using DMSO as control. The standard Tetracycline (0.05 mg/mL) antibiotic was used as an antibacterial agent. The inoculated plates were incubated at 30°C and 37°C temperature for 48 hours, and inhibition zone was measured in mm. Theoretically the PM6 semiempirical method was used to carry out the

calculations for the ligands and their complexes after being constructed and was used to analyze the structural and electronic parameters; the structures were fully optimized and vibrational analysis was carried out to verify that the optimized geometries corresponded to minimum global energy [10].

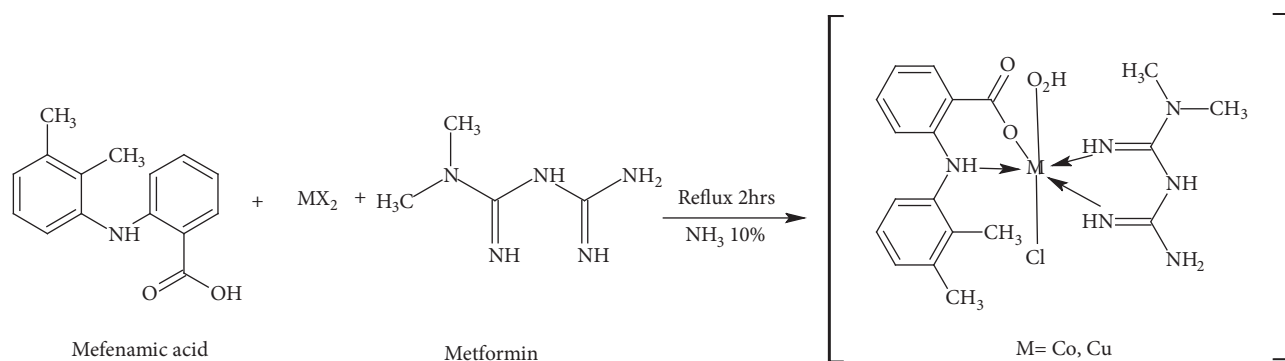
2. Materials and Methods

2.1. Materials. All the chemicals were supplied from Samarra Laboratories for Drugs Industry (IRAQ), BDH, and Fluka; these materials were used without further purification.

2.2. Instruments. Atomic absorption using Shimadzu model 6809, FTIR-8300 Shimadzu spectrophotometer, in the frequency range of 4000–400 cm⁻¹, UV-visible spectrophotometer using Varian model, and conductivity meter using Philips. The melting points were recorded in Coslab melting point apparatus. The magnetic susceptibility of the solid complexes was obtained at room temperature using Magnetic Susceptibility Balance Johnson Matthey. Elemental analysis (EA) was carried out using elemental analyzer.

2.3. Methods

2.3.1. Synthesis of Mixed Drug Complexes. Synthesis of mixed drug complexes was carried out using template method. A



SCHEME 1: Synthesis of mixed drug complexes.

TABLE 1: Physicochemical properties of ligands and synthesized complexes.

Comp.	M.Wt	Color	Melting Point (°C)	Yield(%)	Molar Conductance (ohm ⁻¹ .mol ⁻¹ .cm ²)	B.M.(μ)
C15H15NO2 [L ₁]	241	White	230-231	-----	-----	----
C4H11N5 [L ₂]	129	White	221-222	-----	-----	----
[Co(L ₁)(L ₂) H ₂ OCl]	481	Violet	>300	71	18	4.7
[Cu(L ₁)(L ₂) H ₂ OCl]	486	Pink	>300	83	11	1.65

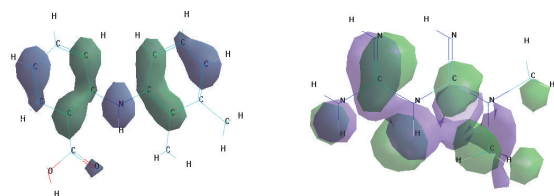


FIGURE 1: HOMO-LUMO categories of the ligands (mefenamic acid and metformin).

hot ethanolic solution (10 ml) of respective transition metal salts (1 mmol) was mixed with a hot solution (1 mmol) of mefenamic acid and metformin (1 mmol). Few drops of dil. NH₃ 10% were added to the mixture. The resulting mixture was then left under reflux for 2 hours. After refluxing for 2hrs appropriate complexes were precipitated out on cooling the reaction mixture. Those were then filtered, washed with ethanol, and washed using cold water [11, 12]. The synthesized complexes were obtained in 65-72% yield. Scheme 1 shows the mechanism for synthesis of the mixed drug complexes.

3. Results and Discussion

3.1. Physicochemical Properties of Ligands and Complexes. All the synthesized complexes were thermally stable and colored. The magnetic moments of the solid complexes were measured by Farady's method at 300 K and the values observed for copper(II) complex were 1.69-1.78 BM which fall in one of the expected electrons for d⁹ configurations [13]. However, the increased magnetic susceptibility of cobalt(II) complexes in the 4.75-4.85 BM region supports the octahedral geometry around Co(II) ion due to orbital contribution. Table 1

shows the physicochemical properties of these synthesized complexes.

Figure 1 shows the HOMO-LUMO categories for the ligands according to semiempirical method (PM6).

3.2. IR Spectra of Ligands and Complexes. The ligand (metformin) shows three sharp absorption bundles at regions 3373cm⁻¹ and 3159-3298 cm⁻¹ assigned to groups NH and NH₂, respectively; peak C = N appears at 1583- 1626cm⁻¹ and 1276-1419cm⁻¹ assigned to C=N; and N-N appears at 937cm⁻¹. Mefenamic acid has N-H band appearing at 3340cm⁻¹. The vibrational spectrum of the synthesized complexes showed the difference in the severity of the packs C = N and NH, respectively, having clearer deviations than in the free ligand. This is evidence of the participation of the nitrogen atom in the uniformity of the metal ion. This is shown by resonance and the change in the intensity of the group C = N towards the red displacement in the complex was recorded in the free ligand (1583-1626cm⁻¹) (1612-1629cm⁻¹) in the metal complex due to consistency and the formation of the motor stabilized system [10]. The presence of 812cm⁻¹ refers to the frequency of M-N=C group and the new band in the range of (486-449)cm⁻¹ in the spectra of products was assigned to M-O group [14] as in Figures 2 and 3. Table 2 shows the IR spectra of ligands and their complexes experimentally and theoretically using semiempirical (PM6) method.

3.3. UV-Visible Spectra. The electronic spectra of the ligands L₁, L₂ and their metal complexes were measured in ethanol and DMSO solutions. The mefenamic acid and metformin base ligand L₁, L₂ displayed absorption around and in the 220 nm region assigned to the π → π* transition that is unaffected in the formation of complexes. The peaks around

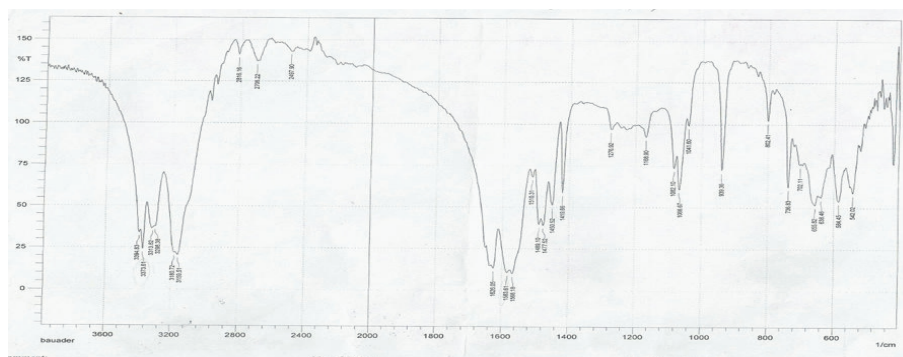


TABLE 2: Experimental and theoretical IR spectrum bands of ligands and their complexes.

Comp.	$\nu(\text{N-H})$	$\nu(\text{C=O})$	$\nu(\text{COO})$	$\nu(\text{M-O})$
$\text{C}_{15}\text{H}_{15}\text{NO}_2$ [L_1]	3340 (3332) _P	1730 (1721) _P	1673-1422 (1676-1396) _P	-----
$\text{C}_4\text{H}_{11}\text{N}_5$ [L_2]	3373 (3388) _P	--	----	-----
$[\text{Co}(\text{L}_1)(\text{L}_2) \text{H}_2\text{OCl}]$	3327 (3315) _P	---	1678-1492 (1672-1494) _P	486 (490) _P
$[\text{Cu}(\text{L}_1)(\text{L}_2) \text{H}_2\text{OCl}]$	3336 (3334) _P	-----	1614-1462 (1615-1458) _P	491 (499) _P

*P: semiempirical (PM6) method.

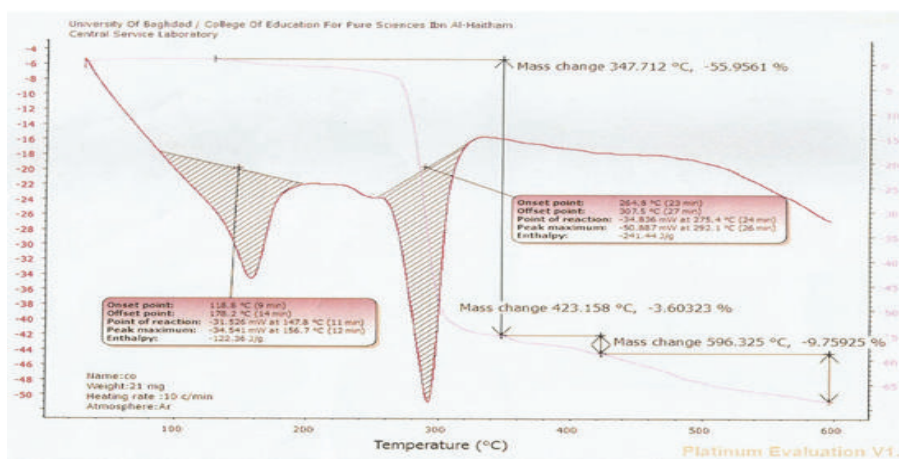


FIGURE 5: TG/DTG and DSC thermogram of $[\text{Co}(\text{L}^1)(\text{L}^2) \text{H}_2\text{OCl}]$ in argon atmosphere.

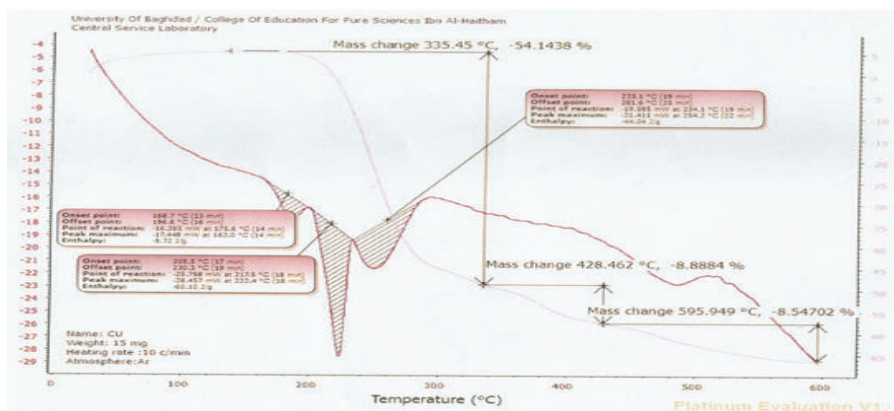


FIGURE 6: TG/DTG and DSC thermogram of $[\text{Cu}(\text{L}^1)(\text{L}^2) \text{H}_2\text{OCl}]$ in argon atmosphere biological activity.

aureus, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Candida albicans* (fungus). In this method pure isolate of 24hr growth was cultured in Muller-Hinton Agar plate (Hi Media, Mumbai, India) by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 8.0mm was used to bore four wells in each agar plates. A $10\mu\text{L}$ volume of each complex was applied by micropipette in the wells into Muller-Hinton Agar plate. Distilled water served as control. The plates were allowed to stand for 1h or more for diffusion

to take place and then incubated at 37°C for 24hrs. The zone of inhibition was recorded [19]. Several researches have shown that coordination of organic compounds to a metallic element causes significant changes in the biological activity of both the organic ligand and the metal. The ligands showed antimicrobial activity against both kinds of bacteria. The complex Co-ligand 1/ligand 2/DMSO has the highest inhibition zone among the others. The complex Cu-ligand 1/ligand 2/DMSO also showed significant antimicrobial activity effect, which does not have antibacterial activity against

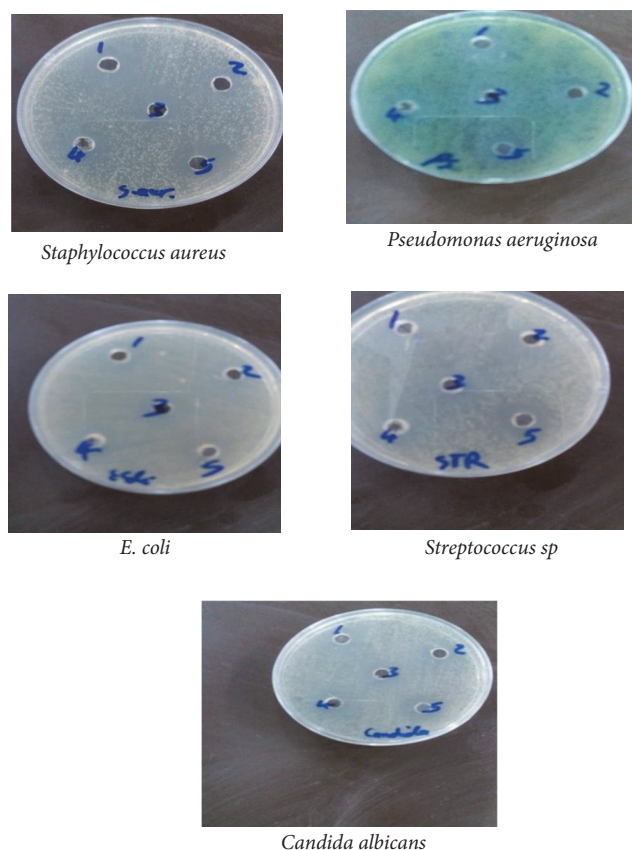


FIGURE 7: Effect of ligands and their complexes in the inhibition of different kinds of bacteria.

TABLE 3: Experimental and theoretical UV-Vis spectrum bands of ligands and their complexes.

Compounds	Bands (nm)	Geometry
$C_{15}H_{15}NO_2 [L_1]$	324 – 247 (323-237) _p	---
$C_4H_{11}N_5 [L_2]$	237 – 266 (222 – 265) _p	----
$[Co(L_1)(L_2) H_2OCl]$	610 – 489 – 341 (596 – 484 – 337) _p	Octahedral
$[Cu(L_1)(L_2) H_2OCl]$	580 – 370 – 290 (670 – 365 – 296) _p	Octahedral

*P: semiempirical (PM6) method.

Staphylococcus aureus and *Streptococcus mutans* shown in the higher inhibition zone of metal complexes than those of the ligands, which can be explained on the basis of Overton's concept and chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. The weak antibacterial activity for Cu-ligand/DMSO and Co-ligand/DMSO against gram negative bacteria was ascribed to the presence of an outer membrane, which poses hydrophilic polysaccharides chains as a barrier to these complexes. Cobalt is not generally considered to be a very toxic element [20, 21]. A large number of reports on the antibacterial properties of cobalt complexes have shown Co(II) complexes to be the most

studied probably due to their aqueous stability, accessibility, and ease of synthesis. However, only a small number of cobalt (III) complexes have biochemical roles. Vitamin B12 is a cobaloxime, a cobalt complex having a glyoxime ligand, and is one of the unusual examples of a naturally occurring organometallic complex, i.e., possessing a metal carbon bond [22]. Figure 7 and Table 4 show the effect of ligands and their complexes on different kinds of bacteria.

4. Conclusions

We concluded in this work that the synthesized complexes, prepared from mixed drugs (mefenamic acid and metformin), had octahedral geometric shapes; the biological

TABLE 4: Biological activities of the ligands and their complexes against different bacterial isolates.

Bacterial isolates	DMSO	Ligand 1\DMSO	Ligand 2/DMSO	Ligand 1+2/DMSO	Co ligand 1+2/DMSO	Cu-ligand 1+2/DMSO
<i>Staphylococcus aureus</i>	-	21	15	14	14	15
<i>Pseudomonas aeruginosa</i>	-	14	-	-	13	10
<i>E. coli</i>	-	15	-	-	15	12
<i>Streptococcus sp.</i>	-	15	-	12	15	11
<i>Candida albicans</i>	-	14	14	12	16	13

activity of these drugs against different kinds of bacteria increased after being mixed with Cu(II) and Co(II). We concluded that there is a great convergence between experimental and theoretical results using semiempirical (PM6) method.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was performed in the Laboratories of the Chemistry and Biology Departments in Al-Mustansiriyah University.

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