Nano metal-complexes of theophylline derivative: synthesis, characterization, molecular structure studies, and antibacterial activity

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Abstract. The new divalent nano metal-complexes of Zn(II), Mn(II), and Fe(II) with new ligand derivate from theophylline were synthesis by ultrasonic sonication method. This method was applied to produce smaller, narrow distributed nanoparticles and without any aggregations. The nanocomplexes and new ligand (L) were diagnosed by different Physico-chemical studies as Analysis of elements (C.H.N), Measuring conductivity, FT-IR spectra, 1H NMR spectra, UV-Vis spectra, flame atomic absorption (FAA), and their microbial activities. The spectroscopic data of the nanocomplexes suggest their 2:1 (L: M) complex structures of Mn(II), Fe(II), and 1:1 (L: M) complex structure of Zn(II). Also, the spectroscopic studies suggested the octahedral structure for Mn(II) and Fe(II) ions and tetrahedral structure for Zn(II) ion. All spectroscopic data propose that new ligand act as a bidentate ligand with its metal ions. The size and morphology of nanocomplexes measured by TEM were in range (14-18) nm. The bacterial activity was checked with the synthesized ligand and nanocomplexes. Activities show that the nano complexes are more promising than their new ligand for microbial activities.

Keywords: Nanocomplexes, new ligand, theophylline derivative, Ultrasonic Sonication, bacterial strains, TEM.

1- Introduction

Purines and their derivatives have been studied extensively in the metal complexes [1-5]. In many biochemical interactions, these complexes play a major role [6,7]. Transition metal complexes (TMCs) are cationic, neutral, or anionic forms by which the transition metal is coordinated with ligands [8]. TMCs can be designed to a specific function during
necessary adjustments of either the core metal atom or the ligand sphere [9]. Transition metals appear in various oxidation states and can react with several negatively charged molecules [10]. Investigating the use of transition metal complexes as drugs for many human diseases has shown important advances., where transition metal complexes are the most utilized chemotherapeutic agents and make a big contribution to medicinal therapeutics [11]. Research into nanotechnology permits the development of nanomaterials below 100 nm in a nanoscale, The specific physical and chemical characteristics of nanomaterials are common in all fields [12-19]. The studies show that nanocomplexes have characteristic physical, chemical, and biological applications [20-22]. Theophylline functions as a monodentate ligand in a neutral environment and coordinates metal ions through N7 atoms (Scheme 1), theophylline derivative complexes indicate that bindings of metal ions at the purine through N7 and O6 sites. [23-27]. In some cases, it acts as a bidentate N7/O6 chelating ligand [28,29]. In the present paper, a description is given of the preparation of some metal nano complexes, Zn(II), Mn(II), and Fe(II) with theophylline derivative and evaluation of its antibacterial activity.

![Scheme 1. Structural Theophylline](image)

**2- Experimental**

**2.1 Materials and methods**

All chemical products and solvents applied were purchased from Sigma-Aldrich. Through the experimental steps, deionized water was used.
2.2 Synthesis of the new ligand (theophylline derivative)

The new ligand was obtained by adding (0.01 mole) of theophylline in 40 ml of dioxane. Then, benzoyl chloride (0.01 mole) was mixed with the obtained solution as shown in (Scheme 2). The mixture was stirred and refluxed for 5 hr at 105 °C. The solution was cooled for 30 minutes in an ice bath. Brawn participated was isolated and washed several times with hot water. Finally, it was dried in the oven at 60 °C for 2 h.

![Scheme 2. Syntheses of theophylline-9-benzoyl ligand (L).](image)

2.3 Synthesis of the metal nanocomplexes

Three nanocomplexes of the Mn(II), Fe(II), and Zn(II) with ligand were synthesized using the ultrasonic sonication method [30].

a. Mn (II) and Fe (II) complexes

Both Mn (II) and Fe (II) nanocomplexes were prepared according to (Scheme 3). by dissolve (0.002 mole) of a new ligand in 25 mL of ethanol, then metals (II) chloride of Mn(II) and Fe(II) (0.001 mol in 25 ml ethanol) were added to obtain a solution as a ratio (1:2) metal to a ligand. The reaction mixture kept under ultrasonic with reflux-heated for three hours at 50 °C. Appropriate crystals of good quality emerged at room temperature incubation after night. The nanocomplexes were isolated, washed with ethanol, and dried on air.
b. Zn (II) complex

The Zn (II) nanocomplex was synthesized by the same method and in (M: L) ratio (1:1) as shown in (Scheme 3), where a quantity of (0.002 mole) of ZnCl₂ in ethanol (25 mL) was slowly added to (0.002 mole in 25 ml ethanol) of ligand (L). The reaction mixture kept under ultrasonic with reflux-heated for three hours at 50 °C. Appropriate crystals of good quality emerged at room temperature incubation after night. The nano complex was isolated, washed with ethanol, and dried on air.

Scheme 3 Nanoscale complexes synthesized [M= Mn(II), Fe(II)]
2.4 Antibacterial activity

Several bacterial strains were used for antibacterial ligand and the obtained metal nanocomplexes, such as *Klebsiella pneumonia*, *Escherichia Coli*, *Staphylococcus aureus*, *Bacillus subtilis*. The procedure of diffusion of the agar used to determine these antibacterial activities. The antibacterial activity results are based on the inhibition zone diameter that can be read at 37 °C for 24 hours after incubation. Dimethyl sulphoxide (DMSO) was used as a solvent to dissolve a ligand and nanocomplexes in a concentration of 10^{-3} M. As a standard for antibacterial tests, McFarland was used.

3. Results and discussion

The new ligand and nanocomplexes of Mn(II), Fe(II), and Zn(II) were synthesized and characterization by some devices, FT-IR, UV-Vis, ^1^HNMR, elemental analysis, molar conductivity, and solubility. The results of elemental analysis for all metal nanocomplexes and ligand as shown in (Table 1) and have the suggested formulae in good agreement, where the molar proportion of the reactions were 2:1 of Mn(II) and Fe(II) while 1:1 of Zn(II).
Table 1 Elements analysis and atomic absorption spectroscopy for ligand and nanocomplexes

| Prepared Nano complexes | Molecular formula | M.wt g.mol$^{-1}$ | Metal analyses % Found (calc.) |
|------------------------|-------------------|-------------------|------------------------------|
|                        |                   |                   | C | H | N | M |
| L                      | C$_{14}$H$_{12}$N$_4$O$_3$ | 284              | 59.155 (59.12) | 4.225 (4.35) | 19.718 (18.55) | ------ |
| [Mn(L)$_2$(Cl)$_2$]     | C$_{14}$H$_{12}$N$_4$O$_3$MnCl$_2$ | 693.838          | 48.43 (48.33) | 3.46 (3.32) | 16.14 (15.45) | 7.92 (8.25) |
| [Fe(L)$_2$(Cl)$_2$]     | C$_{14}$H$_{12}$N$_4$O$_3$FeCl$_2$ | 694.747          | 48.36 (47.33) | 3.45 (3.42) | 16.12 (16.15) | 8.04 (8.1) |
| [Zn(L) (Cl)$_2$]       | C$_{14}$H$_{12}$N$_4$O$_3$ZnCl$_2$ | 420.27           | 39.97 (39.80) | 2.86 (2.83) | 13.35 (12.75) | 15.55 (15.51) |

The molar conductivity values for synthesized nanocomplex solutions (10$^{-3}$ M) in DMSO indicated that all chlorine ions involved in coordination (Table 2).

Table 2 Molar conductance of the prepared metal nano complexes

| Prepared Nano complexes | Color       | Molar Cond. $\Omega^{-1}\cdot cm^2\cdot mole^{-1}$ |
|------------------------|-------------|-----------------------------------------------|
| [Mn (R)$_2$(Cl)$_2$]   | Light brown | 14.22                                         |
| [Fe (R)$_2$(Cl)$_2$]   | Brown       | 13.57                                         |
| [Zn (R) (Cl)$_2$]     | White       | 14.36                                         |

Different solvents were used to examine the solubility of ligand and its metal nano complexes as illustrative in Table 3.
Table 3. The solubility of the new ligand and nanocomplexes

| Nano complexes | DMSO | Cold water | Hot water (above 40°C) | Acetone | EtOH | CHCl3 | MeOH |
|----------------|------|------------|------------------------|---------|------|-------|------|
| [Mn (L)₂(Cl)₂] ++ | + | + | - | - | + | ++ | |
| [Fe (L)₂(Cl)₂] ++ | - | + | + | - | - | + | |
| [Zn (L)(Cl)] ++ | - | - | - | - | - | - | |
| L ++ | + | ++ | + | ++ | + | ++ | |

Where: (++ High solubility), (+ Partial solubility), (- insoluble).

3.1. Infrared spectra (FT-IR) of the Ligand and nanocomplexes

The ligand behaves as a bidentate, as its structure describes. The FT-IR indicated ligand coordination behavior with metal ions well [Mn(II), Fe(II), and Zn(II)]. Compared with free ligand sites contributing to coordination, FTIR complex spectra were thus expected to change the position of some bands on the free ligand spectrum, (Figs. 1-4). FT-IR of new ligand (Fig. 1) showed The absorbing bands at 1681.98 cm⁻¹ and 1600.97 cm⁻¹ referring to (υC=O) and (υC=N), these bands were shifted to lower wavenumber in all complexes (figs. 2-4), this means that The ligand is therefore coordinated by oxygen (O6) and nitrogen atoms (N7) with the metal ions.
Fig. 1 FT-IR spectrum of new ligand (L).

Fig. 2 FT-IR spectrum of the [Mn (L)₂(Cl)₂] nanocomplex.
Fig. 3 FT-IR spectrum of the [Fe (L)\(_2\)(Cl)\(_2\)] nanocomplex.

Fig. 4 FT-IR spectrum of the [Zn (L) (Cl)\(_2\)] nanocomplex.
3.1.1 UV–visible spectra of the ligand and its metal nano complexes

Ligand (L) and Nanocomplexes absorption spectrums were recorded with DMSO as a solvent between 200 and 800 nm. Ligand (L) and Nanocomplexes electronic spectrums (Figs 5-8) were illustrated.

The spectrum U.V-Visbel of the free ligand (Fig. 5) shows an absorption band at 274 nm (36496 cm\(^{-1}\)) in (DMSO) solvent, this band is attributed to (\(\pi - \pi^*\)). The [Mn (L)\(_2\)(Cl)\(_2\)] and [Zn (L)(Cl)\(_2\)] complexes (Figs. 6,7) Display no d-d transitions bands as anticipated in the visible area for a half- and fully-filled metal complex electronic configuration. The spectrum U.V-Visbel of [Fe (L)\(_2\)(Cl)\(_2\)] complex (Fig. 8), exhibited a weak absorption band at 598 nm (16722 cm\(^{-1}\)) which refers to \(^5T_{2g} \rightarrow ^5E_g\) transition. Also, according to the above-mentioned results (C.H.N), (FAA), (FT-IR), and (Uv.Vib), we can suggest the Tetrahedral Geometry of Zn(II) ion and an Octahedral Geometry of both Mn(II) and Fe(II) ions [31,32].

![Fig.5 The UV-Vis spectra of Ligand](image-url)
Fig. 6. A) The UV-Vis spectra of [Mn (L)2 (Cl)2] complex at low concentration and B) only Visible spectra at high concentration

Fig. 7 A) The UV-Vis spectra of [Zn (L) (Cl)2] complex at low concentration and B) only Visible spectra at high concentration
Fig. 8. A) The UV-Vis spectra of [Fe (L)2 (Cl)2] complex at low concentration and B) only Visible spectra at high concentration

3.1.2 $^1$HNMR spectra of the ligand

The $^1$HNMR ligand spectrum was measured using the Bruker Advance 400 NMR spectrometer with dimethyl sulfoxide (DMSOd6). Fig. 9 show the ligand chemical shifts ($\delta$). The ligand spectrum is characterized by peaks 3.87 ppm and 4.48 ppm that match the two imidazole proton groups of CH3 (N1-CH3 and N3-CH3). We have observed that proton (CH = N) is the signal at 9.33 ppm. We also noticed the multiples (7.46–8.51) ppm of the aromatic proton that shows the aromatic ring hydrogen.
3.2 TEM Measurement

Transmission electron microscope (TEM) was used to measure the prepared metal nanocomplexes, which gave a clear picture of the prepared nano with a different approximation scale (Figs. 10-12). The prepared nanocomplexes show empty from any conglomerate. The dimensions in all synthesized complexes are within the nanoscale (less than 100 nm) which indicates that the kind of nano is particles and zero dimension which is more preferred in preparing nanoscales [30]. The average size was calculated randomly from the figures as shown in Table (4).
Fig. 10 TEM of \([\text{Fe} (\text{L})_2 (\text{Cl})_2]\) nanocomplex

Fig. 11 TEM of \([\text{Mn} (\text{L})_2 (\text{Cl})_2]\) nanocomplex
Table 4 Average size nano complexes investigated by TEM

| Complexes formula | ave. Particles size (nm) |
|-------------------|--------------------------|
| [Fe (L)₂(Cl)₂]    | 18                       |
| [Mn (L)₂(Cl)₂]    | 14                       |
| [Zn (L) (Cl)₂]    | 15                       |

3.3 Antibacterial activity

The antibacterial activity of the ligand and nanocomplexes were tested against four types of bacteria: *Staphylococcus aureus*, *Bacillus subtilis* for Gram-positive (+ve) and *Klebsiella pneumonia*, *Escherichia Coli* for Gram-negative (-ve). As an antibacterial control, McFarland was used as a standard. DMSO was used as a solvent to dissolving the recently synthesized compounds, then dumping them on agar media at 37 °C for 24 hours, prepared the solution for the concentration ( 10⁻³ M). The tested solution diffused and microorganism growth was affected during this time. The zone of inhibition has been calculated on the plate. Free ligand
and nanocomplexes have been described in action against bacteria shown in Table 5. The Results show approximately high activity in the direction of metal nanocomplexes towards strains of bacteria than free ligand (Fig. 13).

![Fig. 13](image)

**Fig. 13** The activity of the synthesized ligand and metal nanocomplexes in inhibiting bacteria
Table 5 The effect of ligand and metal nanocomplexes is represented by inhibition zone (mm) against different bacterial species

| Complexes formula (Nano scales) | Staphylococcus aureus | Escherichia Coli | Bacillus subtilis | Klebsiella pneumonia |
|-------------------------------|----------------------|-----------------|------------------|-------------------|
| [Zn (L) (Cl)₂] (4)            | 2                    | 3               | 14               | 6                 |
| [Mn (L)₂(Cl)₂] (5)            | 4                    | 5               | 12               | 8                 |
| [Fe (L)₂(Cl)₂] (6)            | 2                    | 4               | 9                | 3                 |
| Ligand (S)                    | -                    | -               | 5                | 2                 |
| DMSO                          | -                    | -               | -                | -                 |

4. Conclusions

In our research on transitional metal chemistry, Theophylline derivative was structured for us to synthesize and characterize coordinated with Mn(II), Zn(II), and Fe(II) in nanoscale. Ultrasonic sonication method was used to synthesize complexes in nanoscale. Our method has a display that the nano complexes can easily be synthesized without aggregation. The complexes solution were non-electrolyte conductivity. The complexes were arranged to coordinate the theophylline derivative with metal ions during the N7 and O6 atoms. From the results given by (molar conductance, solubility, atomic flame absorption, (C.H.N) elemental analysis, FT-IR, UV - visible spectroscopy), the metal ions Mn(II), Fe(II) have an octahedral geometry while Zn(II) ion has a tetrahedral geometry. Also, the TEM calculation shows that the particle size of nanocomplexes was to be range (14-18) nm. Besides, Mn(II), Zn(II), and Fe(II) nanocomplexes appear antibacterial activity against Gram-positive bacteria and Gram-negative bacteria higher than theophylline derivative.
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References

1. Naskar S, Guha R, Müller J. Metal- Modified Nucleic Acids: Metal- Mediated Base Pairs, Triples, and Tetrads. Angewandte Chemie International Edition. 2020 Jan 20;59(4):1397-406.

2. Kumar D, Jain V, Rai B. Imidazole Derivatives as Corrosion Inhibitors for Copper: A DFT and Reactive Force Field Study. Corrosion Science. 2020 May 5:108724.

3. Müller J. Nucleic acid duplexes with metal-mediated base pairs and their structures. Coordination Chemistry Reviews. 2019 Aug 15;393:37-47

4. Blindauer CA, Holý A, Operschall BP, Sigel A, Song B, Sigel H. Metal Ion- Coordinating Properties in Aqueous Solutions of the Antivirally Active Nucleotide Analogue (S)- 9- [3-Hydroxy- 2- (phosphonomethoxy) propyl] adenine (HPMPA)–Quantification of Complex Isomeric Equilibria. European Journal of Inorganic Chemistry. 2019 Sep 22;2019(35):3892-903

5. Vakarov SA, Gruzdev DA, Levit GL, Krasnov VP, Charushin VN, Chupakhin ON. Synthesis of enantiomerically pure 2-aryloxy carboxylic acids and their derivatives. Russian Chemical Reviews. 2019;88(10):1063.

6. Castilho N, Gabriel P, Camargo TP, Neves A, Terenzi H. Targeting an artificial metal nuclease to DNA by a simple chemical modification and its drastic effect on catalysis. ACS Medicinal Chemistry Letters. 2019 Aug 12.
7. Kitamura Y, Nagai K, Furuzono T, Katsuda Y, Ihara T. Cooperative recognition of a repetitive sequence through consecutive formation of triplex and duplex structures. Nucleosides, Nucleotides & Nucleic Acids. 2020 Feb 20;39(1-3):97-108.

8. Li D, Ma P, Niu J, Wang J. Recent advances in transition-metal-containing Keggin-type polyoxometalate-based coordination polymers. Coordination Chemistry Reviews. 2019 Aug 1;392:49-80.

9. Li W, Xiong D, Gao X, Liu L. The oxygen evolution reaction enabled by transition metal phosphide and chalcogenide pre-catalysts with dynamic changes. Chemical Communications. 2019;55(60):8744-63

10. Ranjbar, Zohreh Rashidi, Marzieh Khatamifar, and Seyed Jamilaldine Fatemi. "Chelation therapy: Assessing the impact of deferasirox size on lead (II) release from biological systems." Main Group Chemistry 17.2 (2018): 181-189.

11. Babu E, Bhuvaneswari J, Mareeswaran PM, Thanasekaran P, Lee HM, Rajagopal S. Transition metal complexes based aptamers as optical diagnostic tools for disease proteins and biomolecules. Coordination Chemistry Reviews. 2019 Feb 1;380:519-49.

12. Rheima AM, Mohammed MA, Jaber SH, Hameed SA. Synthesis of Silver Nanoparticles Using the UV-Irradiation Technique in an Antibacterial Application. Journal of Southwest Jiaotong University. 2019;54(5).

13. Mohammed MA, Rheima AM, Jaber SH, Hameed SA. The removal of zinc ions from their aqueous solutions by Cr2O3 nanoparticles synthesized via the UV-irradiation method. Egyptian Journal of Chemistry. 2020 Feb 1;63(2):5-6.

14. Rheima AM, Mohammed MA, Jaber SH, Hasan MH. Inhibition effect of silver-calcium nanocomposite on alanine transaminase enzyme activity in human serum of Iraqi patients with chronic liver disease. Drug Invention Today. 2019 Nov 15;12(11).
15. Rheima, A.M., D.H. Hussain, and H.I. Abdulah, Silver nanoparticles: Synthesis, Characterization and their used as counter electrodes in novel Dye sensitizer solar cell. IOSR Journal of Applied Chemistry, 2016. 9(10): p. 6–9.

16. Hussain DH, Rheima AM, Jaber SH, Kadhim MM. Cadmium ions pollution treatments in aqueous solution using electrochemically synthesized gamma aluminum oxide nanoparticles with DFT study. Egyptian Journal of Chemistry. 2020 Feb 1;63(2):417-24

17. Rheima AM, Hussain DH, Almijbilee MM. Graphene-Silver Nanocomposite: Synthesis, and Adsorption Study of Cibacron Blue Dye from Their Aqueous Solution. Journal of Southwest Jiaotong University. 2019;54(6).

18. Hussain DH, Abdulah HI, Rheima AM. Synthesis and Characterization of γ-Fe2O3 Nanoparticles Photo Anode by Novel Method for Dye Sensitized Solar Cell. International Journal of Scientific and Research Publications. 2016;6(10):26-31.

19. Jabber SH, Hussain DH, Rheima AM, Faraj M. Comparing study of CuO synthesized by biological and electrochemical methods for biological activity. Al-Mustansiriyah Journal of Science. 2019;30(1):94-8.

20. Ismail AH, Al-Bairmani HK, Abbas ZS, Rheima AM. Synthesis, characterization, spectroscopic, and biological activity studies of Nano scale Zn(II), Mn (II) and Fe (II) theophylline complexes. Journal of Xi'an University of Architecture & Technology. 2020; XII (II): 2775-2789.

21. Ali AA, Al-Hassani RM, Hussain DH, Rheima AM, Abd AN, Meteab HS. Fabrication of Solar Cells Using Novel Micro-and Nano-Complexes of Triazole Schiff Base Derivatives. Journal of Southwest Jiaotong University. 2019;54(6).

22. Ali AA, Al-Hassani RM, Hussain DH, Rheima AM, Meteab HS. Synthesis, spectroscopic, characterization, pharmacological evaluation, and cytotoxicity assays of novel nano and micro scale of copper (II) complexes against human breast cancer cells. Drug Invention Today. 2020; 14(1).
23. Forizs, Edit, et al. "Synthesis, structure and DFT calculations on complexes of palladium (II) with theophylline." Rev Roum Chim 55.10 (2010): 697-704.

24. Gacki M, Kafarska K, Pietrzak A, Korona-Glowniak I, Wolf WM. Double Palindrome Water Chain in Cu (II) Theophylline Complex. Synthesis, Characterization, Biological Activity of Cu (II), Zn (II) Complexes with Theophylline. Crystals. 2020 Feb;10(2):97

25. Forizs, Edit, et al. "IR and ESR studies on novel Cu (II) theophylline complexes containing mono-or bidentate ligands." Journal of molecular structure 482 (1999): 143-147.

26. Bouhdada M, Amane ME, Mohammed BB, Yamni K. Synthesis, spectroscopic studies, X-ray powder diffraction data and biological activity of mixed transition metal complexes with oxalato and theophylline ligands. Journal of Molecular Structure. 2019 Feb 5;1177:391-400

27. Gacki M, Kafarska K, Pietrzak A, Korona-Glowniak I, Wolf WM. Synthesis, characterisation, crystal structure and biological activity of metal (II) complexes with theophylline. Journal of Saudi Chemical Society. 2019 Mar 1;23(3):346-54.

28. Pascual-Colino J, Beobide G, Castillo O, Luque A, Pérez-Yáñez S. Theophylline alkaloid as glue of paddle-wheel copper (II)-adenine entities to afford a rhomboid chain. Inorganica Chimica Acta. 2019 Jan 1;484:437-42

29. El Hamdani H, El Amane M, Duhayon C. Crystal structure of tetraaquabis (1, 3-dimethyl-2, 6-dioxo-7H-purin-7-ido-κN7) cobalt (II). Acta Crystallographica Section E: Crystallographic Communications. 2017 Sep 1;73(9):1302-4

30. Ismail AH, Al-Bairmani HK, Abbas ZS, Rheima AM. Nanoscale Synthesis of Metal (II) Theophylline Complexes and Assessment of Their Biological Activity. Nano Biomed. Eng. 2020;12(2):139-47

31. El-Halim HF, Mohamed GG, Khalil EA. Synthesis, spectral, thermal and biological studies of mixed ligand complexes with newly prepared Schiff base and 1, 10-phenanthroline ligands. Journal of Molecular Structure. 2017 Oct 15;1146:153-63.
32. Mahmoud WH, Deghani RG, Mohamed GG. Preparation, geometric structure, molecular docking thermal and spectroscopic characterization of novel Schiff base ligand and its metal chelates. Journal of Thermal Analysis and Calorimetry. 2017 Mar 1;127(3):2149-71