

# Synthesis, spectroscopic and thermal characterization of some transition metal complexes of folic acid

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## Abstract

Compounds having general formula:  $[M(FO)(Cl)_x(H_2O)_y] \cdot zH_2O$ , where (M = Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), FO = folate anion,  $x = 2$  or  $4$ ,  $y = 2$  or  $4$  and  $z = 0, 1, 2, 3, 5$  or  $15$ ) were prepared. The obtained compounds were characterized by elemental analysis, infrared as well as electronic spectra, thermogravimetric analysis and the conductivity measurements. The results suggested that all folate complexes were formed by 2:1 molar ratio (metal:folic acid) as a bidentate through both of the two carboxylic groups. The molar conductance measurements proved that the folate complexes are electrolytes. The kinetic thermodynamic parameters such as:  $E^*$ ,  $\Delta H^*$ ,  $\Delta S^*$  and  $\Delta G^*$  were estimated from the DTG curves. The antibacterial evaluation of the folic acid and their complexes was also done against some Gram positive/negative bacteria as well as fungi.

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**Keywords:** Folic acid; Infrared spectra; Thermal analysis; Thermodynamic parameters; Conductivity; Antibacterial activity

## 1. Introduction

Folic acid is structurally composed of pteric acid and glutamic acid connected via an amide linkage (Fig. 1). Several studies describe the derivatization of folic acid at the *c*-carboxyl group of the glutamate moiety [1]. To date folate conjugates of chemotherapeutic agents [2,3], antisense oligonucleotides and ribozymes [4,5], immunotherapeutic agents [6,7] have been synthesized and successfully tested in cancer cells overexpressing the folate receptor (FR) on their surface.

The structural diversity encountered in metal–folate complexes could be attributed to the versatile ligational behavior of the carboxylate group which can function like a bidentate ligand binding to a single metal or alternatively as a bridging bidentate ligand coordinating to two metals or as a monodentate ligand [8,9]. The three different coordination modes were reported as shown in Fig. 2 [10].

The aim of the present work is to show the interaction between some transition metal ions and folic acid. The composition and structure of the complexes were identified using: elemental anal-

ysis FTIR and UV–vis spectra, molar conductance and TGA techniques. On the other hand, folic acid and their synthesized complexes were selected to determine its inhibitory effect on some bacteria as well as fungi.

## 2. Experimental

### 2.1. Materials and instrumentation

All chemicals used were of the purest laboratory grade (Merck) and folic acid was presented from Egyptian International Pharmaceutical Industrial Company (EIPICO). Carbon and hydrogen contents were determined using a Perkin-Elmer CHN 2400. The metal content was found gravimetrically by converting the compounds into their corresponding carbides.

IR spectra were recorded on Bruker FTIR spectrophotometer (4000–400  $\text{cm}^{-1}$ ) in KBr pellets. The UV–vis spectra were studied in the DMSO solvent with concentration ( $1.0 \times 10^{-3}$  M) for the folic acid and their complexes by help of Jenway 6405 spectrophotometer with 1 cm quartz cell, in the range 800–200 nm.

Molar conductivities of freshly prepared  $1.0 \times 10^{-3}$   $\text{mol dm}^{-3}$  DMSO solutions were measured using Jenway

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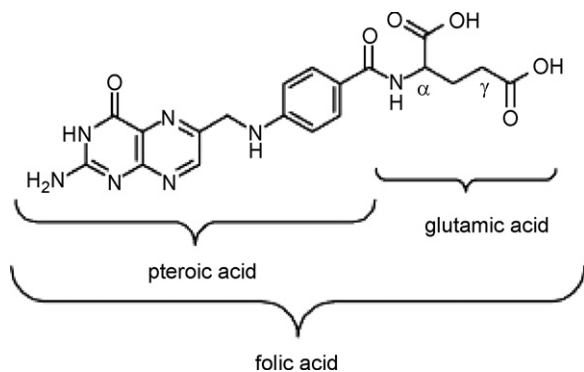


Fig. 1. Structure of folic acid.

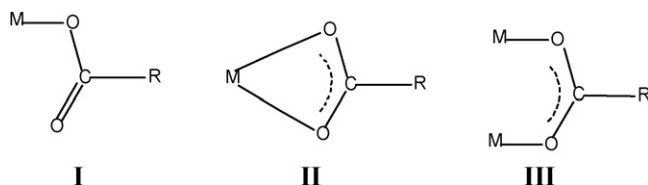


Fig. 2. Coordination modes of the carboxylate group.

4010 conductivity meter. Magnetic measurements were carried out on a Sherwood scientific magnetic balance using Gouy method.

Thermogravimetric analysis (TGA and DTG) was carried out in dynamic nitrogen atmosphere (30 ml/min) with a heating rate of 10 °C/min using a Shimadzu TGA-50H thermal analyzer.

## 2.2. Synthesis of metal complexes

### 2.2.1. $[Mn_2(FO)(H_2O)_2Cl_2] \cdot H_2O$ (I, $C_{19}H_{23}N_7O_9Cl_2Mn_2$ ) complex

Folic acid (0.441 g, 1.0 mmol) was added to 30 ml methanol and titrated against aqueous sodium hydroxide (0.1 M) to adjust pH at 7.0, then 10 ml methanolic solution of (0.198 g, 1.0 mmol) of  $MnCl_2 \cdot 4H_2O$  was added with continuously stirring, after that the mixture was warmed at about ~60 °C and then neutralized. Immediately, the brown precipitate was settled down and filtered off, washed several times by minimum amounts of hot methanol and dried under *vacuo* over anhydrous  $CaCl_2$ .

### 2.2.2. $[Fe_2(FO)(Cl)_4(H_2O)_4] \cdot 7H_2O$ (II, $C_{19}H_{29}N_7O_{17}Cl_4Fe_2$ ) complex

A similar procedure as that described for complex (I) was carried out, by mixing (1.0 mmol) of folic acid with  $FeCl_3 \cdot 2H_2O$  (0.162 g and 1.0 mmol).

### 2.2.3. $[Co_2(FO)(H_2O)_2(Cl)_2] \cdot 3H_2O$ (III, $C_{19}H_{27}N_7O_{11}Cl_2Co_2$ ) complex

A pale yellow complex,  $[Co_2(FO)(H_2O)_2(Cl)_2] \cdot 3H_2O$  was prepared during the reaction of (1.0 mmol) folic acid with 0.257 g and 1.0 mmol of  $CoCl_2 \cdot 6H_2O$  by a method similar to that described above.

### 2.2.4. $[Ni_2(FO)(Cl)_2(H_2O)_2] \cdot 15H_2O$ (IV, $C_{19}H_{51}N_7O_{23}Cl_2Ni_2$ ) complex

Like the above procedure of the preparation of complexes, a methanolic solution of  $NiCl_2 \cdot 6H_2O$  (0.238 g and 1.0 mmol) was mixed with an equal volume of folic acid solution (1.0 mmol) in methanol. The mixture was allowed to stay at room temperature for about 1 h with constant stirring and then heated on a water bath at ~60 °C for 30 min. The pale yellow complex was filtered off, washed several times with hot methanol, dried under *vacuo* over anhydrous  $CaCl_2$ .

### 2.2.5. $[Cu_2(FO)(H_2O)_2(Cl)_2] \cdot 2H_2O$ (V, $C_{19}H_{25}N_7O_{10}Cl_2Cu_2$ ) complex

The copper(II) folate complex was prepared by the same method used for preparation of the Mn(II) and Co(II) complexes. The weight of  $CuCl_2 \cdot H_2O$  was (0.152 g and 1.0 mmol) mixed with folic acid by (1:1) molar ratio in methanol as solvent.

### 2.2.6. $[Zn_2(FO)(Cl)_2(H_2O)_2]$ (VI, $C_{19}H_{21}N_7O_8Cl_2Zn_2$ ) complex

The complex,  $[Zn(FO)(Cl)_2(H_2O)_2]$  was prepared by mixing equal volumes (30 ml) of folic acid (1.0 mmol) with  $ZnCl_2$  (0.136 g and 1.0 mmol). The mixture was neutralized by titration with NaOH to adjust pH at 7.0 and then heated on a water bath at 60 °C with constant stirring for about 45 min. A white solid complex was precipitated and its amount increased with increasing time of heating. The obtained precipitate was separated, washed several times with hot methanol and then dried in *vacuo* over anhydrous  $CaCl_2$  and recrystallization occurs using a mixture of water and methanol (1:1).

### 2.2.7. $[Cd_2(FO)(Cl)_2(H_2O)_2] \cdot 3H_2O$ (VII, $C_{19}H_{27}N_7O_{11}Cl_2Cd_2$ ) and $[Hg_2(FO)(Cl)_2(H_2O)_2]$ (VIII, $C_{19}H_{21}N_7O_8Cl_2Hg_2$ ) complexes

Preparation of these two complexes followed essentially by the same procedure as preparation of (I), but the weight of  $CdCl_2$  and  $HgCl_2$  were 0.201 g, 1.0 mmol and 0.271 g, 1.0 mmol, respectively. The pH was adjusted at 7.0.

## 2.3. Antibacterial and anti-fungal investigation

From these studies, according to Gupta et al. [11], the hole-well method was applied. The investigated isolates of bacteria were seeded in tubes with nutrient broth (NB). The seeded NB (1 cm<sup>3</sup>) was homogenized in the tubes with 9 cm<sup>3</sup> of melted (45 °C) nutrient agar (NA). The homogeneous suspensions were poured into Petri dishes. The holes (diameter 4 mm) were done in the cool medium. After cooling in these holes, 2 × 10<sup>-3</sup> dm<sup>3</sup> of the investigated compounds were applied using a micropipette. After incubation for 24 h in a thermostat at 25–27 °C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. An inhibition zone diameter over 7 mm indicates that the tested compound is active against the bacteria under investigation.

The antibacterial activities of the investigated compounds were tested against *Escherichia coli* (Gram –ve), *Bacillus sub-*

tilis (Gram +ve) and anti-fungal (*Trichoderma* and *Penicillium* activities).

### 3. Results and discussion

The results of the elemental analysis and some physical characteristics of the obtained compounds were discussed. The complexes are air-stable, with high-melting points, insoluble in H<sub>2</sub>O and most of organic solvents except for DMSO and DMF are slightly soluble. The elemental analysis data of the complexes indicate that the 2:1 (metal:ligand) stoichiometry with general formula: [M<sub>2</sub>(FO)(H<sub>2</sub>O)<sub>x</sub>(Cl)<sub>y</sub>].nH<sub>2</sub>O (where M=Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), and x=2 in the case of all complexes except for Fe(III) x=4, y=2.

#### 3.1. Molar conductivities of metal chelates

The molar conductivity values for the folic acid complexes in DMSO solvent (1.0 × 10<sup>-3</sup> mol) were in the range of 53.50–93.70 Ω<sup>-1</sup> cm<sup>-1</sup> mol<sup>-1</sup>, suggesting them to be electrolytes in nature. Conductivity measurements provide a method of testing the degree of ionization of the complexes, the molecular ions that a complex liberates in solution (in case of presence of anions outside the coordination sphere), the higher will be its molar conductivity and vice versa [12]. It is clear from the obtained data that the complexes present seem to be electrolytes. Also the molar conductance values indicate that the anions may be exhibits inside the coordination sphere. These results were

strongly supported with the elemental analysis data, where Cl<sup>-</sup> ions are detected by addition of AgNO<sub>3</sub> solution, inside the coordination sphere of the complexes by the degradation of the all complexes using nitric acid.

#### 3.2. Infrared spectra

The essential infrared data are summarized in Table 1. Folic acid exhibits a very strong absorption band at 1694 cm<sup>-1</sup> due to the stretching vibration of ν(C=O) of free ketonic of the carboxylic group. This group is shifted or disappeared in the spectra of its complexes. Interestingly, there are two bands which appeared at the range of 1514–1564 cm<sup>-1</sup> and corresponded to ν<sub>as</sub>(COO<sup>-</sup>) and the other band is exhibited within the range of 1333–1352 cm<sup>-1</sup> assigned to ν<sub>s</sub>(COO<sup>-</sup>). Accordingly, the antisymmetric and symmetric stretching vibration modes (ν<sub>as</sub>(COO<sup>-</sup>), and ν<sub>s</sub>(COO<sup>-</sup>)) of the COO<sup>-</sup> group, the structure of our complexes could be elucidated [13]. The direction of the frequency shift of the ν<sub>as</sub>(COO<sup>-</sup>) and the ν<sub>s</sub>(COO<sup>-</sup>) bands with respect to those of the free ion depends on the coordination mode of the COO<sup>-</sup> group with the metal ion.

Nakamoto and McCarthy [14] have established that if the coordination is monodentate (structure I) the ν<sub>as</sub>(COO<sup>-</sup>) and ν<sub>s</sub>(COO<sup>-</sup>) will be shifted to higher and lower frequencies, respectively. Whereas, if the coordination is chelating bidentate (structure II) or bridging bidentate (structure III) both ν<sub>as</sub>(COO<sup>-</sup>) and ν<sub>s</sub>(COO<sup>-</sup>) frequencies will change in the same direction because the bond orders of both C=O bonds would change by the same amount. Based on these facts and

Table 1  
IR frequencies (cm<sup>-1</sup>) of folic acid (FO) and its metal complexes

Assignments	Compound								
	FO	I	II	III	IV	V	VI	VII	VIII
ν(OH); H <sub>2</sub> O	3544	3344	3381	3385	3379	3377	3353	3338	3419
	3415								
ν(NH)	3322	3180	3175	3190	3200	3200	3170	3170	3200
ν <sub>as</sub> (CH)	3106	3083	3090	2920	2940	3030	3008	3000	3050
	2926	2927	2930			2930	2915	2910	2927
ν <sub>s</sub> (CH)	2840	2865	2870	2820	2850	2860	2860	2867	2863
		2723	2770	2750	2805	2805	2810		2810
ν(COOH)	1694	–	–	–	–	–	–	–	–
ν <sub>as</sub> (COO <sup>-</sup> )	1565	1564	1543	1544	1548	1522	1514	1545	1563
	1544								
δ(CH)	1483	1448	1445	1444	1448	1446	1453	1451	1456
	1453	1409	1407	1404	1406	1401	1402	1403	1401
	1413								
ν <sub>s</sub> (COO <sup>-</sup> )	1338	1339	1337	1345	1352	1333	1339	1342	1335
ν <sub>as</sub> (CC)	1294	1301	1303	1300	1277	1295	1188	1184	1295
ν(CN)	1230	1187	1188	1185	1187	1184	1127	1102	1180
	1191	1108	1127	1103	1102	1108	1105		1121
	1160								
	1134								
	1106								
ν <sub>s</sub> (CC)	971	952	950	968	945	955	963	993	951
δ(CC)	764	786	768	767	768	769	786	786	768
		759		669	666	669			
ν(M–O)	–	585	582	579	582	585	580	578	576
		519	520	416	466	521	453	457	516

Table 2  
Asymmetric and symmetric stretching vibrations of the carboxylate group

Compound	$\nu_{as}(\text{COO}^-)$	$\nu_s(\text{COO}^-)$	$\Delta\nu = \nu_{as}(\text{COO}^-) - \nu_s(\text{COO}^-)$	Bonding mode
FO	1565	1453	112	Bidentate
$[\text{Mn}_2(\text{FO})(\text{H}_2\text{O})_2\text{Cl}_2] \cdot \text{H}_2\text{O}$ (I, $\text{C}_{19}\text{H}_{23}\text{N}_7\text{O}_9\text{Cl}_2\text{Mn}_2$ )	1564	1339	225	Bidentate
$[\text{Fe}_2(\text{FO})(\text{Cl})_4(\text{H}_2\text{O})_4] \cdot 7\text{H}_2\text{O}$ (II, $\text{C}_{19}\text{H}_{29}\text{N}_7\text{O}_{17}\text{Cl}_4\text{Fe}_2$ )	1543	1337	206	Bidentate
$[\text{Co}_2(\text{FO})(\text{H}_2\text{O})_2(\text{Cl})_2] \cdot 3\text{H}_2\text{O}$ (III, $\text{C}_{19}\text{H}_{27}\text{N}_7\text{O}_{11}\text{Cl}_2\text{Co}_2$ )	1544	1345	199	Bidentate
$[\text{Ni}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2] \cdot 15\text{H}_2\text{O}$ (IV, $\text{C}_{19}\text{H}_{51}\text{N}_7\text{O}_{23}\text{Cl}_2\text{Ni}_2$ )	1548	1352	196	Bidentate
$[\text{Cu}_2(\text{FO})(\text{H}_2\text{O})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$ (V, $\text{C}_{19}\text{H}_{25}\text{N}_7\text{O}_{10}\text{Cl}_2\text{Cu}_2$ )	1522	1333	189	Bidentate
$[\text{Zn}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2]$ (VI, $\text{C}_{19}\text{H}_{21}\text{N}_7\text{O}_8\text{Cl}_2\text{Zn}_2$ )	1514	1339	175	Bidentate
$[\text{Cd}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2] \cdot 3\text{H}_2\text{O}$ (VII, $\text{C}_{19}\text{H}_{27}\text{N}_7\text{O}_{11}\text{Cl}_2\text{Cd}_2$ )	1545	1342	203	Bidentate
$[\text{Hg}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2]$ (VIII, $\text{C}_{19}\text{H}_{21}\text{N}_7\text{O}_8\text{Cl}_2\text{Hg}_2$ )	1563	1335	228	Bidentate

comparison the  $\nu_{as}(\text{COO}^-)$  and  $\nu_s(\text{COO}^-)$  frequencies of the folate complexes by the  $\nu_{as}(\text{COO}^-)$  and  $\nu_s(\text{COO}^-)$  frequencies of sodium carboxylate [15], as shown in Table 2, man can say that all the prepared complexes are chelating bidentate structure.

The stretching broadband vibration of  $\text{OH}^-$  group  $\nu(\text{O}-\text{H})$  occurred as expected [16] at the range  $\sim 3400 \text{ cm}^{-1}$ . The angular deformation motions of the coordinated water in the hydrated folate complexes can be classified into four types of vibrations:  $\delta_b$  (bend),  $\delta_r$  (rock),  $\delta_t$  (twist) and  $\delta_w$  (wag). The assignments of these motions in all complexes are as follows: The bending motion,  $\delta_b(\text{H}_2\text{O})$ , is assigned to its characteristic band around at  $\sim 1600 \text{ cm}^{-1}$  (from medium to very strong band). The rocking motions,  $\delta_r(\text{H}_2\text{O})$ , is assigned at  $\sim 760 \text{ cm}^{-1}$  and the wagging motion,  $\delta_w(\text{H}_2\text{O})$ , is observed at  $\sim 600 \text{ cm}^{-1}$ . The twisting motion,  $\delta_t(\text{H}_2\text{O})$ , is observed at above  $600 \text{ cm}^{-1}$ . It should be mentioned here that these assignments for both the bond stretches and angular deformation of the coordinated water molecules fall in the frequency regions reported for related complexes [14].

### 3.3. Magnetic measurements

Magnetic measurements were carried out according to the Gauy method. The calculations were evaluated by applying the equations:

$$\chi_g = \frac{cI(R - R_0)}{10^9 M}, \quad \chi_m = \chi_g MWt, \quad \mu_{\text{eff}} = 2.828 \sqrt{\chi_m T}$$

where  $\chi$  is mass susceptibility per g of sample;  $c$  is the calibration constant;  $R$  is the balance reading for the sample and tube;  $R_0$  is the balance reading for the empty tube;  $M$  is the weight of the sample in g.

The magnetic moments of Ni and Cu complexes at  $T = 300 \text{ K}$  and their corresponding hybrid orbitals are given in Table 3. The observed values of the effective magnetic moments  $\mu_{\text{eff}}$  are

convenient with experimental values [17] obtained for square planner complexes.

### 3.4. Electronic absorption spectra

The spectra of free folic acid and their complexes in DMSO are followed. There are two detected absorption bands at around (225 and 240 nm) and (256, 290 and 355 nm) assigned to  $\pi-\pi^*$  and  $n-\pi^*$  intraligand transitions, respectively. The first bands are probably due to a  $\pi-\pi^*$  of the alkyl group as well as aromatic rings, but the other bands are due to presence of  $\text{COOH}$ ,  $-\text{NH}$ ,  $\text{NH}_2$  and  $\text{C}=\text{O}$  groups [18]. After complexation, they are shifted to *ca.* 20–30 nm and  $\sim 25 \text{ nm}$ , for  $\pi-\pi^*$  and  $n-\pi^*$ , respectively, confirming the complexation of metal ions via carboxylic group. Some complexes have a band at range  $\sim 360 \text{ nm}$  may be assigned to the ligand to metal charge transfer [19,20].

### 3.5. Thermogravimetric analysis

Thermal analysis curves (TG/DTG) of the folic acid and their transition metal complexes are studied and interpreted as follows

The folic acid ligand melts at 493 K with simultaneous decomposition. The first mass loss was observed at 368 K. From the TG curve, it appears that the sample decomposes in three stages over the temperature range 368–1073 K. The first step occurs at 298–368 K with a mass loss of (obs. = 8.04% and calc. = 7.70%). The second step starts at 523 K and ends at 994 K with a mass loss (obs. = 32.90% and calc. = 33.10%). The last one within the range 994–1027 K was accompanied by mass loss (obs. = 57% and calc. = 56.4%). From the corresponding DTG curve, three endothermic peaks are noted. The first maximum is at 368 K and the second, third at 523 and 994 K, respectively.

The thermal decomposition of Mn complex occurs also at three steps. The first degradation step take place in the range of 298–319.5 K and it is corresponds to the elimination of 3 $\text{H}_2\text{O}$  molecules beside 0.5 $\text{N}_2$  molecule due to weight

Table 3  
Magnetic moment of the Ni(II) and Cu(II) complexes

Complex	$\mu_{\text{eff}} (\mu_B)$		Hybrid orbitals	Stereochemistry
	Found	Calculated		
$[\text{Ni}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2] \cdot 15\text{H}_2\text{O}$	0.00	0.00	$\text{dsp}^2$	Square planner
$[\text{Cu}_2(\text{FO})(\text{H}_2\text{O})_2(\text{Cl})_2] \cdot 2\text{H}_2\text{O}$	1.85	1.50	$\text{dsp}^2$	Square planner

loss (obs. = 9.84% and calc. = 10.09%). The second step falls in the range of 319.5–620 K which is assigned to loss of  $C_6H_{12}O_2$  (organic rest) with a weight loss (obs. = 17.29% and calc. = 17.2%). The last decomposition step within the range 620–1073 K was accompanied by mass loss (obs. = 39.63% and calc. = 38.43%) which is assigned to loss of  $C_3H_5N_6O_4Cl_2$  (organic rest). The  $(2MnCl_2 + 6C)$  is the final product remains stable till 1073 K.

The thermal decomposition of Fe complex occurs completely in three steps. The first step ranged at 298–369 K corresponding to the loss of  $11H_2O$  molecules and  $CH_2O$  organic molecule, representing weight loss (obs. = 25.63% and calc. = 25.59%). The second and third steps occurring at 369–736 and 763–1073 K corresponding to the loss of  $C_2H_5N_7O_5$  (organic part) and two chlorine molecules, representing weight loss (obs. = 39.02% and calc. = 40.30%). The  $2FeCl_2 + 12C$  is the final product remains stable till 1073 K.

The Co complex decomposed in three steps. The first step ranged at 298–324 K corresponding to the loss of  $5H_2O$  molecules representing weight loss (obs. = 12.96% and calc. = 12.53%). The second step occurring at 324–625 K corresponding to the loss of  $C_6H_{12}O_6$  organic molecule, the weight loss associated with this stage (obs. = 25.35% and calc. = 25.07%). The final step of decomposition occurs at a temperature range from 636 to 1073 K. The weight loss at this step is obs. = 25.24% and calc. = 25.77%. Associated with the loss of  $CH_5N_7$  (organic moiety) and chlorine molecule. The final residue at the end of this stage is  $2CoCl_2 + 8C$ .

To make sure about the proposed formula and structure for the new Ni complex, thermogravimetric (TG) and differential thermogravimetric analysis (DTG) was carried out for this complex under  $N_2$  flow. The thermal decomposition of the nickel(II) complex proceeds approximately with main three degradation steps. The first stage occurs at maximum temperature of 341 K. The weight loss associated with this stage (obs. = 11.94% and calc. = 11.56%) corresponding to the loss of  $6H_2O$ . The second step occurs at the maximum temperature 623 K. The weight loss at this step (obs. = 14.08% and calc. = 15.42%) associated with the loss of  $8H_2O$ . The final decomposition stage occurs at the maximum temperature 759 K. The weight loss at this step (obs. = 39.35% and calc. = 39.73%) associated with the loss of  $C_3H_{23}N_7O_9$  organic part and chlorine. The final residue at the end of this stage is  $2NiCl_2 + 12C$ .

The thermal degradation of the Cu complex occurs in mainly three degradation stages. The first stage of decomposition occurs at a temperature maximum of 565 K. The weight loss found associated with this step (obs. = 8.81% and calc. = 10.14%) and may be attributed to the loss of  $4H_2O$ . The second step of decomposition occurs at a temperature maximum of 839 K. The weight loss found at this step equals to (obs. = 17.73% and calc. = 17.61%) corresponds to the loss of  $C_2H_5O_6$  (organic moiety). Final step occurring at 636–1073 K is corresponding to the loss of  $C_7H_{12}N_7$  organic moiety and chlorine molecule representing weight loss (obs. = 37.81% and calc. = 37.21%). The final thermal products obtained at 1073 K are  $2CuCl_2 + 6C$ .

As mentioned above in the copper(II) complex, the zinc(II) folate complex, also has three decomposition steps. These steps located in the range between 298–328, 328–636 and 636–1073 K and the weight loss for the first step (obs. = 9.40% and calc. = 10.34%) due to the loss of  $2H_2O + 2NH_3$ . The second decomposition stage occurs at the maximum temperature 636 K. The weight loss at this step is (obs. = 18.7% and calc. = 18.17%) associated with the loss of  $C_4H_5N_5$  organic part. The weight loss of the final decomposition stage is (obs. = 36.69% and calc. = 36.05%) associated with the loss of  $C_6H_6O_6$  organic part and chlorine molecule. The final residue at the end of this stage is  $2ZnCl_2 + 5C$ .

The thermal decomposition of Cd complex proceeds with three main degradation steps. The first step of the degradation occurs at maximum temperature of 322 K in the range of 298–630 K is accompanied by weight loss (obs. = 9.30% and calc. = 8.72%) correspond to the loss of  $4H_2O$ . The second decomposition stage occurs at the maximum temperature of 630 K. The weight loss at this step (obs. = 9.90% and calc. = 9.69%) associated with the loss of  $C_2H_8O_3$  organic part. The final decomposition stage occurs at the maximum temperature of 1054 K. The weight loss at this step is (obs. = 29.33% and calc. = 29.46%) associated with the loss of  $NH_3 + 3N_2 + 4H_2O$  and chlorine molecule. The final residue at the end of this stage is  $2CdCl_2 + 13C$ .

The thermal decomposition data obtained support the proposed structure and indicate that the thermal decomposition of Hg complex proceeds with three main degradation steps. The first step occurs at a temperature maximum of 328 K. The weight loss found associated with this step (obs. = 10.80% and calc. = 9.92%) and may be attributed to the loss of the organic moiety ( $CH_3NO_2$ ). The second step of decomposition occurs at a temperature maximum of 626 K. The weight loss found at this step (obs. = 16.29% and calc. = 16.25%) corresponds to the loss of  $C_4H_{10}O_6$  (organic moiety). The final step of decomposition occurs at a temperature range from 1054–1073 K. The weight loss at this step is (obs. = 21.34% and calc. = 21.70%) associated with the loss of  $C_4H_4N_6$  (organic moiety) and chlorine molecule. The final residue at the end of this stage is  $2HgCl_2 + 6C$ .

### 3.6. Kinetic studies

In recent years, there has been increasing interest in determining the rate-dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves [21–27]. Most commonly used methods are the differential method of Freeman and Carroll [21] integral method of Coat and Redfern [22] and the approximation method of Horowitz and Metzger [25].

In the present investigation, the general thermal behaviors of the folate complexes in terms of stability ranges, peak temperatures and values of kinetic parameters, are discussed. The kinetic parameters have been evaluated using the Coats–Redfern equation:

$$\int_0^\alpha \frac{d\alpha}{(1-\alpha)^n} = \frac{A}{\varphi} \int_{T_1}^{T_2} \exp\left(-\frac{E^*}{RT}\right) dt \quad (1)$$

This equation on integration gives

$$\ln \left[ -\frac{\ln(1-\alpha)}{T^2} \right] = -\frac{E^*}{RT} + \ln \left[ \frac{AR}{\phi E^*} \right] \quad (2)$$

A plot of left-hand side (LHS) against  $1/T$  was drawn.  $E^*$  is the energy of activation ( $\text{J mol}^{-1}$ ) and calculated from the slop and  $A$  ( $\text{s}^{-1}$ ) from the intercept value. The entropy of activation  $\Delta S^*$  ( $\text{J K}^{-1} \text{mol}^{-1}$ ) was calculated by using the equation:

$$\Delta S^* = R \ln \left( \frac{Ah}{k_B T_s} \right) \quad (3)$$

where  $k_B$  is the Boltzmann constant,  $h$  is the Plank's constant and  $T_s$  is the DTG peak temperature [28].

The Horowitz–Metzger equation is an illustrative of the approximation methods.

$$\log \left[ \frac{1 - (1-\alpha)^{1-n}}{1-n} \right] = \frac{E^* \theta}{2.303 RT_s^2}, \quad \text{for } n \neq 1 \quad (4)$$

When  $n = 1$ , the LHS of Eq. (4) would be  $\log[-\log(1-\alpha)]$ . For a first-order kinetic process the Horowitz–Metzger equation may be written in the form:

$$\log \left[ \log \left( \frac{w_\alpha}{w_y} \right) \right] = \left( \frac{E^* \theta}{2.303 RT_s^2} \right) - \log 2.303$$

where  $\theta = T - T_s$ ,  $w_y = w_\alpha - w$ ,  $w_\alpha =$  mass loss at the completion of the reaction;  $w =$  mass loss up to time  $t$ . The plot of  $\log[\log(w_\alpha/w_y)]$  vs.  $\theta$  was drawn and found to be linear from the slope of which  $E^*$  was calculated. The pre-exponential factor,  $A$ , was calculated from the equation:

$$\frac{E^*}{RT_s^2} = \frac{A}{\phi \exp(-E^*/RT_s)}$$

The entropy of activation,  $\Delta S^*$ , was calculated from Eq. (3). The enthalpy activation,  $\Delta H^*$ , and Gibbs free energy,  $\Delta G^*$  were calculated from  $\Delta H^* = E^* - RT$  and  $\Delta G^* = \Delta H^* - T\Delta S^*$ , respectively.

$\Delta G$  is positive for reaction for which  $\Delta H$  is positive and  $\Delta S$  is negative. The reaction for which  $\Delta G$  is positive and  $\Delta S$  is negative considered as unfavorable or non-spontaneous reactions.

Reactions are classified as either exothermic ( $\Delta H < 0$ ) or endothermic ( $\Delta H > 0$ ) on the basis of whether they give off or absorb heat. Reactions can also be classified as exergonic ( $\Delta G < 0$ ) or endergonic ( $\Delta G > 0$ ) on the basis of whether the free energy of the system decreases or increases during the reaction.

The thermodynamic data obtained with the two methods are in harmony with each other. The activation energy of  $\text{Mn}^{2+}$  and  $\text{Hg}^{2+}$  complexes is expected to increase in relation with decrease in their radii [29]. The smaller size of the ions permits a closer approach of the ligand. Hence, the  $E$  value in the first stage for the  $\text{Mn}^{2+}$  complex is higher than that for the other  $\text{Hg}^{2+}$  complex.

The correlation coefficients of the Arrhenius plots of the thermal decomposition steps were found to lie in the range of 0.9845–0.9999, showing a good fit with linear function. It is clear that the thermal decomposition process of all folate complexes is non-spontaneous, i.e., the complexes are thermally stable.

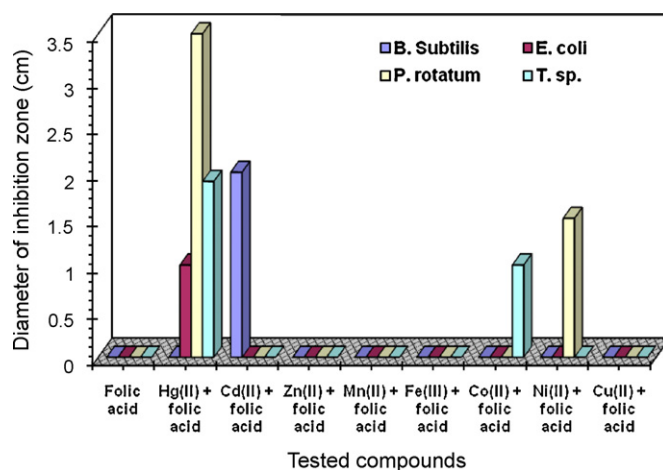
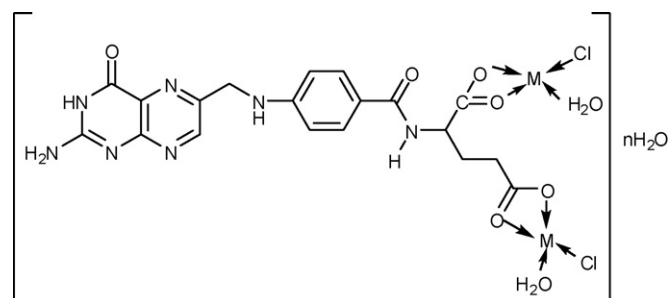


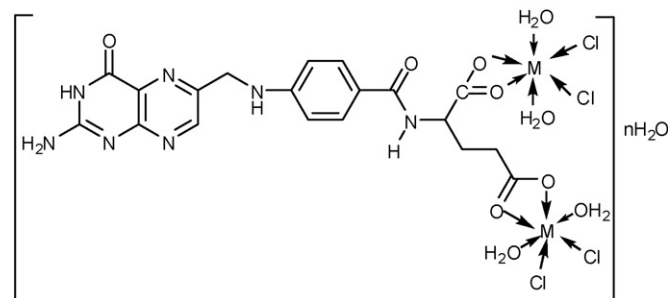
Fig. 3. Statistical representation for biological activity of folic acid and its complexes.

### 3.7. Antimicrobial activity

The results of antimicrobial activities (bacteria and fungi) in vitro of the folic acid ligand and their complexes (Fig. 3) show that, the  $[\text{Hg}_2(\text{FO})(\text{Cl})_2(\text{H}_2\text{O})_2]$  test complex have high activities against *Penicillium* > *Trichoderma* > *E. coli*. On the other hand, the cadmium(II), cobalt(II) and nickel(II) folate complexes have antimicrobial activities against *B. subtilis*, *Trichoderma* and *Penicillium*, respectively. These results clearly obviously that, some metal ions after complexation give the sensitive nature for the ligand against some bacteria and fungi.



$M = \text{Mn}^{+2}, \text{Co}^{+2}, \text{Ni}^{+2}, \text{Cu}^{+2}, \text{Zn}^{+2}, \text{Cd}^{+2}$  and  $\text{Hg}^{+2}$ .  
 $n = 1, 3, 15, 2, 0, 3$  and  $0$ , respectively



$M = \text{Fe}^{+3}$  and  $n = 7$

Fig. 4. The mode of chelation of the folate complexes.

### 3.8. Structure of the folate complexes

Finally on the basis of the above studies, the suggested structures of the folate complexes can be represented in Fig. 4.

## 4. Conclusion

The complexation between transition metal ions like (Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II)) with folic acid produced 2:1 molar ratio (metal:folic acid) as a bidentate via both two carboxylic groups and give general formula:  $[M(FO)(Cl)_x(H_2O)_y] \cdot zH_2O$ , where FO = folate anion;  $x = 2$  or  $4$ ,  $y = 2$  or  $4$  and  $z = 0, 1, 2, 3, 5$  or  $15$ . The resulted folate compounds were assigned by infrared and electronic spectra. Thermogravimetric analysis and kinetic thermodynamic parameters have proved the thermal stability feature of folate complexes. The antimicrobial activities of the transition metal complexes of folic acid recorded a significant effect against some bacteria and fungi.

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