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Synthesis, spectral, thermal and antimicrobial studies of some new tri metallic biologically active ceftriaxone complexes

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ABSTRACT

Iron, cobalt, nickel and copper complexes of ceftriaxone were prepared in 1:3 ligand:metal ratio to examine the ligating properties of the different moieties of the drug. The complexes were found to have high percentages of coordinated water molecules. The modes of bonding were discussed depending on the infrared spectral absorption peaks of the different allowed vibrations. The Nujol mull electronic absorption spectra and the magnetic moment values indicated the Oh geometry of the metal ions in the complexes. The ESR spectra of the iron, cobalt, and copper complexes were determined and discussed. The thermal behaviors of the complexes were studied by TG and DTA techniques. The antimicrobial activities of the complexes were examined and compared to that of the ceftriaxone itself.

1. Introduction

Ceftriaxone is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive and Gram-negative bacteria. Ceftriaxone sodium is marketed by Hoffman–La Roche under the trade name Rocephin, as well as under various other names in several countries. Ceftriaxone (Rocephin) is widely used in the treatment of many conditions including acute otitis media, gonorrhea, meningitis, and infections of the skin and lower respiratory tract. It is indicated for use in both adults and children. In July, 2007 the FDA along with the manufacturer issued a safety alert addressing new information regarding the concomitant use of intravenous (I.V.) ceftriaxone and calcium containing products in neonates [1–3]. A small number of post-marketing reports had identified cases of fatal reactions in neonates caused by the precipitation of calcium–ceftriaxone complexes in the lungs and kidneys of both term and premature neonates. The patients had received I.V. doses of ceftriaxone and calcium solutions (sometimes even in different lines). More recently, a September 2007 MedWatch alert expanded the warning to include patients of all ages. In addition, it recommended avoiding the administration of ceftriaxone and calcium containing products within 48 h of each other, even in different infusion lines or at different sites. There is currently no data on the risk of combining intramuscular (I.M.) ceftriaxone with I.V. or oral calcium salts. It has long been established that ceftriaxone can produce biliary sludge or pseudolithiasis (most commonly in children). High concentrations of ceftriaxone are known to bind with calcium and form insoluble stones. This has occurred not only in the biliary system, but in the kidneys as well. Several cases of ceftriaxone-induced nephrolithiasis have appeared in the literature. In one study, 4 of 51 children receiving I.M. or I.V. ceftriaxone developed small, asymptomatic kidney stones that resolved spontaneously in three of the four patients. The study did not describe use of concomitant calcium solutions or products. In another trial, kidney stones were reported in 4 of 284 pediatric patients receiving I.V. ceftriaxone. Again, little data was presented regarding concomitant administration of calcium salts. Based on FDA reports and previous documentation of the formation of insoluble precipitates, ceftriaxone should probably not be administered within 48 h of calcium salts [1–3]. This gave me a strong push to study the ligating properties of the ceftriaxone drug as well as to start with the coordination ability of iron, cobalt, nickel and copper especially as a series of papers was published concerning with the synthesis and physicochemical studies of many complexes [4–17]. The literature survey about the chemistry of ceftriaxone complexes was not rich as expected from its structure [18–40] where it has many functional groups may form many coordination bonds with a central metal ion.

Ceftriaxone has a systematic IUPAC name of: (6R,7R)-7-{[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino}-3-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and it has molecular formula of: $C_{18}H_{18}N_8O_7S_3$ with a molecular weight of 554.58 amu. It can be represented by the following structure:

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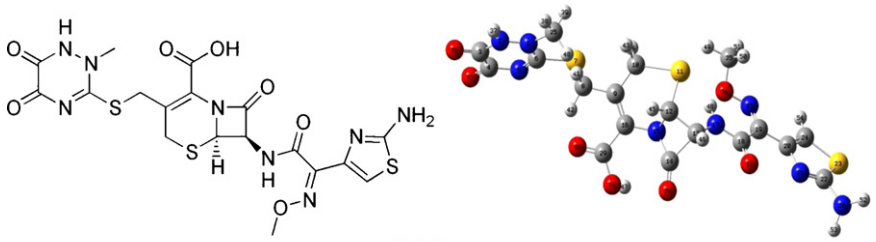
Table 1
Charge density of the atoms in the ceftriaxone.

1 N	2 N	3 C	4 C	5 N	6 C
−0.205756	−0.281232	0.265300	0.245134	−0.274100	0.105259
7 S	8 C	9 C	10 C	11 S	12 C
0.199610	−0.205848	0.010566	−0.204885	0.179253	−0.042478
13 C	14 C	15 N	16 C	17 N	18 C
0.039544	0.303562	−0.299345	0.043015	−0.354810	0.305432
19 C	20 C	21 N	22 C	23 S	24 C
0.085796	0.063187	−0.286146	0.099844	0.268430	−0.178707
25 C	26 O	27 O	28 O	29 C	30 O
−0.085943	−0.213849	−0.183944	−0.260361	0.303805	−0.267239
31 O	32 O	33 N	34 O	35 C	36 N
−0.331601	−0.234356	−0.126622	−0.183639	−0.057299	−0.384391
37 H	38 H	39 H	40 H	41 H	42 H
0.225192	0.089464	0.065385	0.076366	0.073228	0.118877
43 H	44 H	45 H	46 H	47 H	48 H
0.072861	0.076147	0.075753	0.110331	0.252488	0.205483
49 H	50 H	51 H	52 H	53 H	54 H
0.082199	0.076480	0.078875	0.176681	0.193866	0.09513



Ceftriaxone

The charge density of the ceftriaxone is calculated using HF with STO-3G basis set by Hyper chemistry program and gathered in Table 1.

2. Experimental

The metal–ceftriaxone complexes were prepared by mixing the molar amount of the metal salts ($FeCl_3$, $CoCl_2$, $NiCl_2$ and $CuCl_2$) dissolved in 10 ml water with the calculated amount of the ceftriaxone dissolved in water to reach the 3:1 metal:ligand ratios. The mixture was refluxed for about 5 min. The complexes were precipitated and were filtered, then washed several times with water and dried in a desiccator over anhydrous $CaCl_2$. The metal ion contents were determined by normal complexmetric titration procedures [41]. The halogen content was determined by titration with standard $Hg(NO_3)_2$ solution using diphenyl carbazone indicator [42]. Elemental analyses were performed on a Perkin Elmer 2400 CHNS elemental analyzer. The analytical data of the prepared complexes are collected in Table 2.

Infra red spectra were recorded as KBr disk using Perkin Elmer spectrophotometer model 1430 covering range of 200–4000 cm^{-1}. The electronic absorption spectral data were recorded by Perkin Elmer spectrophotometer model 4B covering the range of 190–900 nm. The spectrum was measured in Nujol mull, following the method described by Lee et al. [43]. Room-temperature magnetic susceptibility of well ground solid sample was measured using an Evan balance. The measurements were calibrated against a $Hg[Co(SCN)_4]$ standard [44]. The ESR spectra were recorded at 100 kMz modulation and 10G modulation amplitude on Varian E-9 spectrometer. Incident power of 10 mV was used and resonance conditions were at ca. 9.45 GHz (X-band) at room temperature. The field was calibrated with powder sample of 2,2-diphenylpyridylhydrazone (DPPH) $g = 2.0037$ [45]. Thermogravimetric measurements and differential thermal analysis were performed on a Du Pont 9900 computerized thermal analyzer. The heating rate was 10 °C min^{-1}. A 60 mg sample was placed in a platinum crucible. Dry nitrogen was flowed over the sample at a rate 10 mL min^{-1} and a chamber cooling water flow rate was 10 L h^{-1}. The speed was 5 mm min^{-1}. The antimicrobial activities of the complexes were examined in Pharco Pharmaceuticals, Kilo 31, Alexandria/Cairo Desert Road, Amriya, Alexandria, Egypt.

3. Results and discussion

The literature pK values of protonated ceftriaxone [46], which was determined using pH-potentiometric titration at $I = 0.1$ M NaCl at $t = 25$ °C, reflected the complicated situation where three overlapping processes coexist with pK_1 2.37 (COOH), pK_2 3.03 (aminothiazole) and pK_3 4.21(hydroxytriazinone). Protolysis of the amide group happened in the alkaline medium and was found to be completely separated process from those in the acidic medium [46]. The acidity constant of ceftriaxone which corresponded to the amide group was pK_4 10.74. According to the above pK values we can symbolize the protonated ceftriaxone as H_4A^+, the α-pH plot, Fig. 1, gave that at pH of 6.5 the species HA^{-2} predominates so we expect that ceftriaxone reacted with the metal ions at pH of 7 was in the form of HA^{-2}.

The dissociation reactions can be summarized in the following equations:

$$H_4A^+ \rightleftharpoons H^+ + H_3A \quad pK_1 = 2.37(\text{COOH})$$

$$H_3A \rightleftharpoons H^+ + H_2A^- \quad pK_2 = 3.03(\text{protonatedaminothiazole})$$

$$H_2A^- \rightleftharpoons H^+ + HA^{2-} \quad pK_3 = 4.21(\text{hydroxytriazinone})$$

$$HA^{2-} \rightleftharpoons H^+ + A^{3-} \quad pK_4 = 10.74(\text{protonatedamidegroup})$$

Neglecting the two pK values of the protonated function groups, we can obtain the diagram shown in Fig. 2. The diagram facilitates that at pH of 7, the negative tow species of the

Table 2
The analytical data of the prepared complexes.

Complexes	% C	% H	% N	% S	% Cl	% M	m.p./°C	Colour
Iron III	18.83	3.42	9.76	8.38	18.55	16.11	206.3	Pale brown
Cobalt II	18.66	4.44	9.66	8.30	9.19	15.26	265.1	Pink
Nickel II	20.59	3.74	10.67	9.16	10.14	16.77	170.3	Green
Copper II	19.97	3.82	10.35	8.88	9.84	17.61	175.2	Green

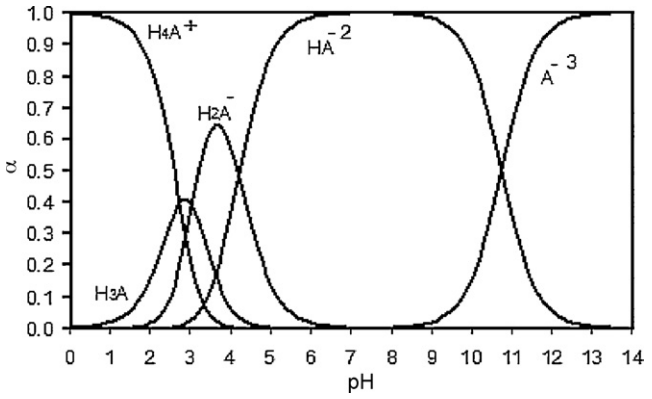


Fig. 1. The species of the protonated ceftriaxone according to the published p*K* values [46].

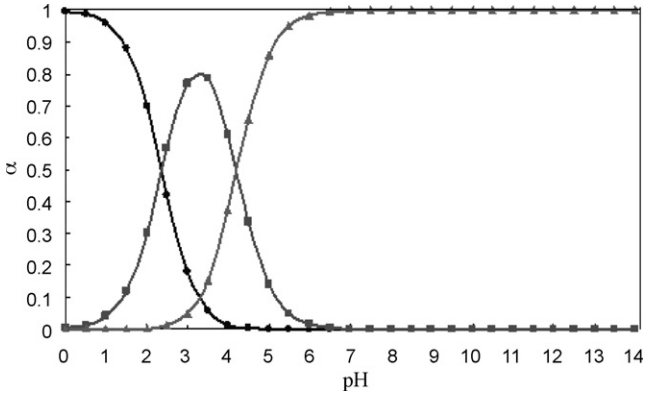


Fig. 2. The species of the ceftriaxone according to the published p*K* values [46] and neglecting p*K* values due to the protonated function groups.

ceftriaxone predominates. Thus, we can expect that the ceftriaxone react with the metal ions at pH = 7 as doubly negative ligand.

The infrared spectra of ceftriaxone gave the major absorption transitions, Table 3. The band at 1610 cm^{-1} is shifted to a higher wave number in the complexes 1620–1630 cm^{-1} indicating that the COO group is involved in the complexation. The presence of absorption peaks in the range of 450–475 cm^{-1} and 350–400 cm^{-1} in the spectrum of the complexes indicated the M–N and M–O modes of vibration, respectively. The band at 1530 cm^{-1} in the free ligand appears at 1540–1546 cm^{-1} in the metal complexes in agreement with the N, O atoms of the triazole moiety bonded to the metal ions. On the other hand, it is found that the bands due to amide group are not affected by complexation and this provides evidence that the amide group was not involved in the complexation.

Table 3
The infrared spectral modes of ceftriaxone and its assignments.

ν cm^{-1}	Assignment
3530–3570	N–H stretching vibration of the hydrogen bonded NH_2 group.
2948	C–H stretching vibration in the β-lactam ring.
1744	β-lactam C=O stretching vibration.
1670	Amide C=O stretching vibration.
1610	Asymmetric COO stretching vibration.
1592	C=N stretching vibration.
1530	C–O stretching vibration of the triazolic ring.
1460	CH_3 deformation of the methoxy group.
1096	C–O stretching vibration of the methoxy group.
1060, 1025	C–O and N–O stretching vibration of the carbamate and oxime moieties.

The Nujol mull electronic absorption spectra of the iron complex gave four bands at 193.2. 221.6, 301.6 and 434.2 nm. The bands were of great variation in the widths. All of the features of the spectrum are easily understood in terms of ligand field theory. At first, the weakness of the bands was due to the pale brown colour of the complex. The reason for the weakness is very simple, where the ground state of d^6 orbital is ^6S which is not split by ligand field. This however, is the only sextuplet state possible for every conceivable alternation of the electron distribution t_{2g}^2 e_g^2, which all spin parallel, results in pairing of two or four spins making quartet or doublet states. Hence all excited states of the d^5 system have different spin multiplicity from the ground state, and transitions to them are spin forbidden. Because of weak spin orbit interactions, such transitions are not totally absent, but they give rise to only a very weak absorption bands. The magnetic moment value, 5.9 BM/Fe^{3+}, facilitates the octahedral geometry of the complex with three Fe atoms in it. The Nujol mull electronic absorption spectra of the cobalt complex gave two bands at 530.2 and 480 nm corresponding to the transitions of the ground state $^4T_1(F)$ to the states $^4T_{1g}(P)$ and $^4A_{2g}$, respectively. The magnetic moment value of this complex, 4.95 BM/Co^{2+}, indicated a high spin octahedral geometry with high orbital contribution since the spin only moment for the three unpaired electrons is only 3.89 BM. The Nujol mull electronic absorption spectra of the nickel complex gave two bands at 308.8, 438.2 and 896.0 nm. This spectrum can be readily interpreted by referring to the energy level diagram of d^8. Thus the peak at 308.8 nm was corresponding to $^3A_{2g} \rightarrow {}^3T_{1g}(P)$, the peak located at 438.2 nm was due to $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and the last peak at 896.0 nm was due to $^3A_{2g} \rightarrow {}^3T_{2g}$ transitions, respectively. These transitions with the magnetic moment value, 3.1 BM/Ni^{2+}, indicated the octahedral geometry of the complex. The Nujol mull electronic absorption spectra of the copper complex gave only one broad maximum band at 725 nm due to the $^2T_{2g} \rightarrow {}^2E_g$ transition. This indicated the octahedral geometry of the complex. The magnetic moment value, 1.72 BM/Cu^{2+}, indicated that the copper ions are separated in the complex. Thus, from the above data one can suggest the structure of the complexes as reported in Fig. 3.

The ESR spectra of the free ligand were reported at room temperature [47], Fig. 4. The spectrum of the drug reflects the low number of free radicals generated due to the storage or exposing to gamma irradiation.

The room temperature polycrystalline X-band ESR spectra of iron–, cobalt–, and copper–ceftriaxone complexes, were performed, Fig. 5. The transition metal complexes have played a seminal rule in many aspects of ESR including the development of

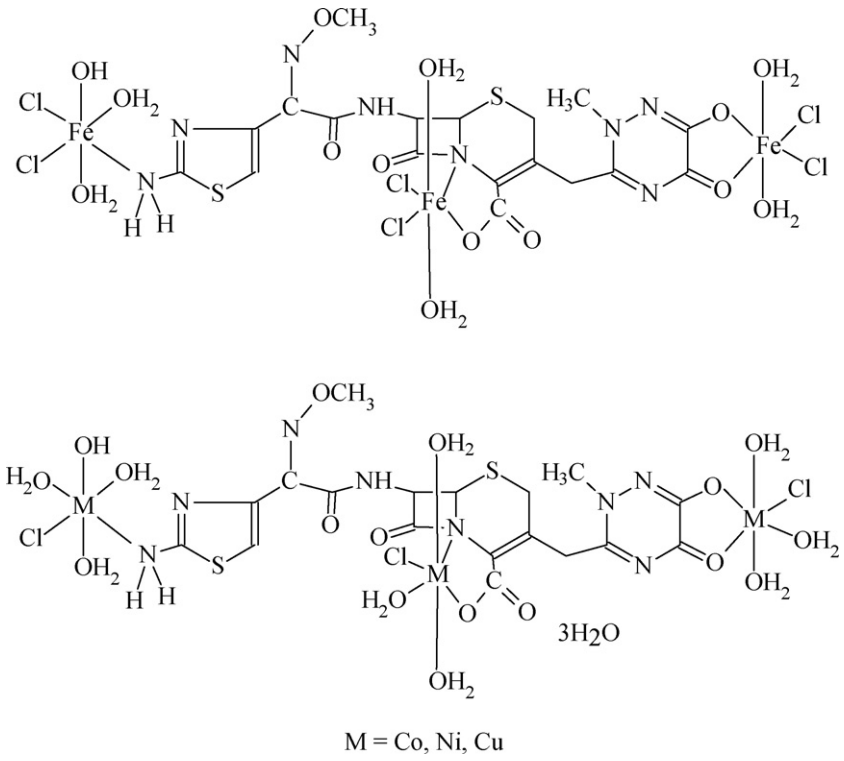


Fig. 3. The structure of the prepared ceftriaxone complexes.

spin Hamiltonian concept. Their importance was based on: availability of various numbers of unpaired electrons per species (total spin $S = 0 \rightarrow 5/2$), availability of species with simple local symmetry and well characterized neighbors to the central metal ion, ease of preparation and stability and yet with the variety of possible oxidation states and the availability of reasonably applicable and adequate electronic theory, for example crystal field model [48]. The ESR spectrum of the iron ceftriaxone complex contained a broad band with $g = 2.015$ indicating a high spin Oh nature. The complex has ^6S ground state and there are no other sextet states. The $^4T_1$ is the closest other term second order spin–orbit coupling effects are needed to mix in this configuration. So, the contributions are small. Thus the electron spin life time is long and the ESR spectra are easily detected at room temperature. Furthermore with odd number of electrons, Kramers' degeneracy exists even when there is large zero field splitting. The ground state for an Oh cobalt ceftriaxone complex is $T_{1g}(F)$. With the extensive spin–orbit coupling ESR measurements are possible only at low temperatures. With $S = 3/2$ and three orbital components in T, a total of 12 low-lying spin states results. At the low temperatures needed the low-lying doublet is populated giving a single peak from an effective $S = 1/2$. This is not the case in the iron ceftriaxone complex and the obtained spectrum which resembles the spectrum of the ceftriaxone, may be due to the ceftriaxone itself not the iron. The room temperature poly crystalline X-band ESR spectral pattern of the copper ceftriaxone complex proved the isotropic nature with $g = 2.15$ and A of $100 \times 10^{-4}\,cm^{-1}$. The absence of signals at $g = 4$ may assign that the copper ions are separated and no Cu–Cu interaction exist.

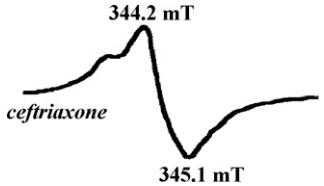


Fig. 4. Electron spin resonance of ceftriaxone [47].

The TG and DTA diagrams of the iron ceftriaxone complex, Fig. 6, showed five dissociation peaks. The first at 88.7 °C, which is of endothermic nature with ΔH of 529.420 J/g, due to the dehydration of the adsorbed water molecules. The rest four peaks were due to thermal dissociation steps of the complex leading to formation of Fe_2O_3 at 550 °C. Excluding the first peak, 7.04 mg of the complex leaves 1.08 mg as a final Fe_2O_3, and this leads to calculate the molecular weight of the complex to be 1040.88 g/mole. This agreed with the %Fe determined by atomic absorption spectroscopy, leading to the same formula of the complex, Fe_3(ceftriaxone)$OHCl_6 \cdot 6H_2O$. The $1000/T - \ln \Delta T$ relation and the thermal parameters of the dissociation steps are shown in Fig. 7 and Table 4, respectively. The collision number showed a direct correlation to the energy of activation for the thermal decomposition steps for the Fe–ceftriaxone complex, Fig. 8. By the same mode of discussion, the cobalt ceftriaxone complex gave four thermal peaks. The first was endothermic with ΔH of 351.663 J/g and it assigned the dehydration step of the adsorbed water molecules. The rest peaks were due to the dissociation steps of the complex ended by formation of Co_2O_3 as a final product at 567.4 °C. The calculated molecular weight of the complex was 1158.88 g/mol leading to the formula of Co_3(ceftriaxone)$OHCl_3 \cdot 17H_2O$. Similar trend is obtained for the Ni ceftriaxone complex but the peaks were only three ended by the formation of Ni_2O_3. The first was endothermic with ΔH of 465.890 J/g assigning the dehydration of the adsorbed water molecules. The calculated molecular weight was 1050.15 g/mol to the formula of Ni_3(ceftriaxone)$OHCl_3 \cdot 11H_2O$. For the copper ceftriaxone complex the dehydration step overlapped with the first dissociation step to give a new net broad exothermic peak of with a maximum at 130 °C. The final product was assumed to be CuO and the calculated molecular weight

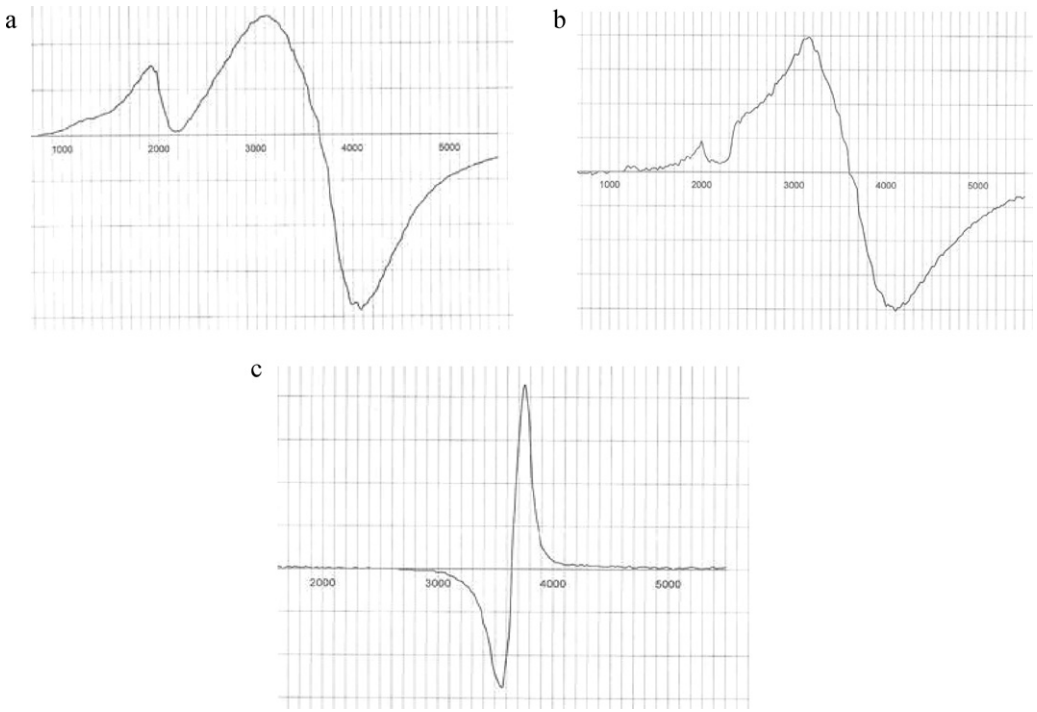


Fig. 5. ESR spectra of (a) Fe–, (b) Co– and (c) Cu–ceftriaxone complexes.

Table 4
The thermal parameters of the thermal dissociation steps of the Fe ceftriaxone complex.

$T_{max.}$ (K)	n	β (K s^{-1})	ΔE^* (J/mol)	Z	ΔS^* (kJ/mol)	Assignment
361.7	0.9997	1.999	45,280.82146	31.3935	−0.1199	Dehydration of the adsorbed water
499.28	0.828	1.585	364,034.1913	165.679	−0.1034	Dissociation steps and formation of Fe_2O_3 as a final product
525.56	0.6	0.667	63,921.95288	36.2941	−0.1156	
512.74	0.96	1.917	339,614.4755	178.660	−0.1026	

The calculations were done depending on Horowitz–Metzger equation [49], where n is the order of the thermal reaction, β is the heating rate, ΔE^* is the activation energy of the thermal decomposition steps, Z is the collision number, ΔS^* is the entropy of activation.

was 1082.72 g/mol to agree with the formula of Cu_3(ceftriaxone)(OH)$Cl_3 \cdot 12H_2O$.

In *vitro* antimicrobial activities of ceftriaxone and its complexes were tested using the reported methods [50,51]. The chosen strains covered the Gram positive and Gram negative bacteria as well as the antifungal activity, Table 5. The data showed that the complexes has less biological activity than that of the ceftriaxone except the copper complex that gave the same activity towards most of the tested bacteria strains. In addition Cu and Fe ceftriaxone complexes showed medium biological activity towards *E. coli* which is not affected bi ceftriaxone drug.

Table 5
The antimicrobial activity of ceftriaxone and its complexes.

	Ceftriaxone	Fe complex	Co complex	Ni complex	Cu complex	
Gram positive bacteria	*Bacillus subtilis*	+	–	+	++	+
	Streptococcus penumonia	++	+	–	+	++
	Staphylococcus aureas	+	+	++	++	+++
Gram negative bacteria	*E. coli*	–	++	–	–	+
	Pesudomonas sp.	+	–	++	+	–
Antifungal activity	*Candida*	+	+++	+	+	+++
	Asperigillus nigaer	+++	+	++	++	+++
	Penicillium sp.	+++	++	++	+++	+++

(–) No clearing zone; inactive.
(+) Small clearing zone; slightly active.
(++) Medium clearing zone; moderately active.
(+++) Large clearing zone; highly active.

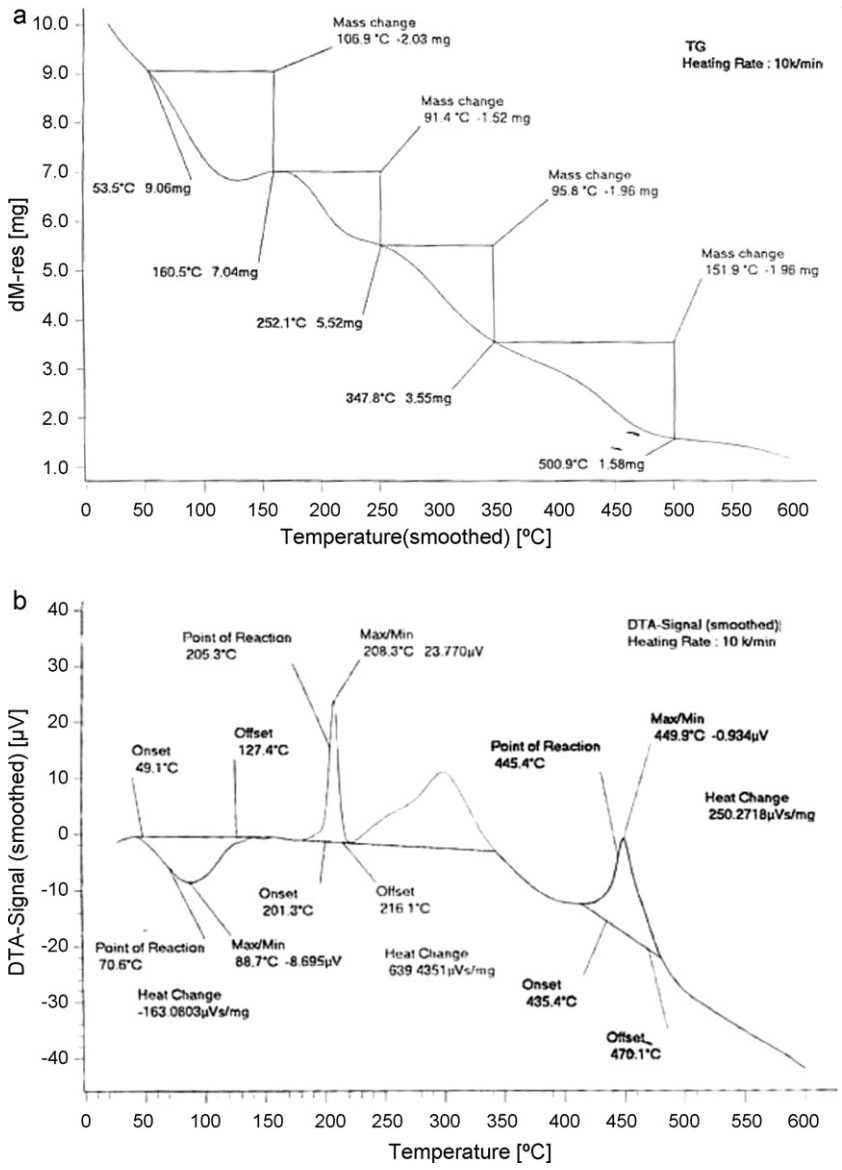


Fig. 6. (a) TG and (b) DTA curves of Fe–ceftriaxone complex as a representative example.

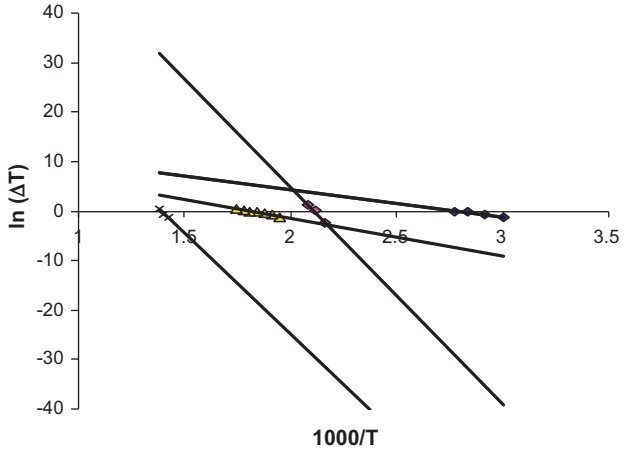


Fig. 7. The $1000/T - \ln(\Delta T)$ relation for the four thermal steps of iron complexes.

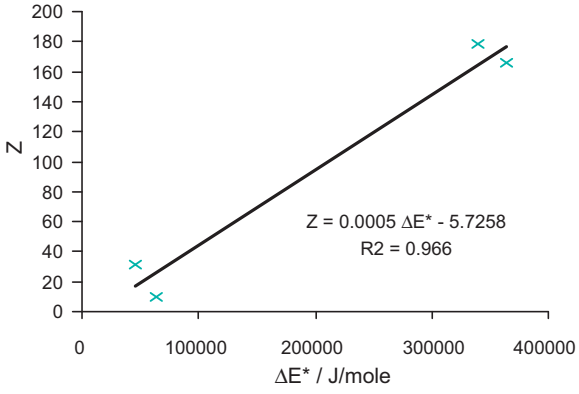


Fig. 8. The direct correlation between the collision number Z and the energy of activation of the thermal decomposition steps of Fe–ceftriaxone complex.

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