Journal of Siberian Federal University. Chemistry 2023 16(1): 5–15
~ ~ ~

EDN: YPNCXI

УДК 54–386:615.33

Complex of Ceftriaxone with Mg(II): Synthesis, Structure, Spectral and Antibacterial Properties

Galina V. Novikova[a*], Darya I. Tsyplenkova[a], Alexander A. Kuzubov[a, b], Oksana A. Kolenchukova[a, c], Alexander S. Samoilo[a] and Sergey A. Vorobyev[d]

[a]Siberian Federal University, Krasnoyarsk, Russian Federation
[b]L.V. Kirensky Institute of Physics SB RAS Federal Research Center "Krasnoyarsk Science Center SB RAS" Krasnoyarsk, Russian Federation
[c]Scientific Research Institute of Medical Problems of the North Federal Research Center "Krasnoyarsk Scientific Center of the SB RAS" Krasnoyarsk, Russian Federation
[d]Institute of Chemistry and Chemical Technology SB RAS Federal Research Center "Krasnoyarsk Scientific Center of the SB RAS" Krasnoyarsk, Russian Federation

Received 25.07.2022, received in revised form 06.10.2022, accepted 06.01.2023

Abstract. Magnesium complex of ceftriaxone was obtained and characterized by atomic-emission and elemental analysis, TGA, FTIR and Raman spectroscopy, X-ray diffraction and density functional theory calculations. Ceftriaxone was coordinated to the magnesium ion by the oxygen of the triazine cycle in the 6th position, the nitrogen of the amine group of the thiazole ring, and oxygen atoms of the lactam carbonyl and carboxylate groups. The disodium salt of ceftriaxone and magnesium complex were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Keywords: cephalosporin antibiotic, ceftriaxone, magnesium, density functional theory, molecular spectroscopy, antibacterial screening.

* Corresponding author E-mail address: galina-n@mail.ru

Acknowledgements. The research was funded by RFBR, Krasnoyarsk Territory and Krasnoyarsk Regional Fund of Science, project number 20–43–240007.
Authors thank the Centre for Equipment Joint User of School of Petroleum and Natural Gas Engineering of Siberian Federal University, Institute of Chemistry and Chemical Technology SB RAS for their technical support.

Citation: Novikova, G.V., Tsyplenkova, D.I., Kuzubov, A.A., Kolenchukova, O.A., Samoilo, A.S., Vorobyev, S.A. Complex of ceftriaxone with Mg(II): synthesis, structure, spectral and antibacterial properties. J. Sib. Fed. Univ. Chem., 2023, 16(1), 5–15. EDN: YPNCXI

Комплекс цефтриаксона с Mg(II): синтез, структура, спектральные и антибактериальные свойства

Г. В. Новикова[а], Д. И. Цыпленкова[а], А. А. Кузубов[а, б], О. А. Коленчукова[а, в], А. С. Самойло[а], С. А. Воробьев[г]
[а]Сибирский федеральный университет
Российская Федерация, Красноярск
[б]Институт физики им.Л.В. Киренского СО РАН,
ФИЦ «Красноярский научный центр СО РАН»
Российская Федерация, Красноярск
[в]Научно-исследовательский институт
медицинских проблем Севера
Федеральный исследовательский центр КНЦ СО РАН
(ФИЦ КНЦ СО РАН НИИ МПС)
Российская Федерация, Красноярск
[г]Институт химии и химической технологии СО РАН
ФИЦ «Красноярский научный центр СО РАН»
Российская Федерация, Красноярск

Аннотация. Получен и охарактеризован магниевый комплекс цефтриаксона методами атомно-эмиссионного и элементного анализов, ТГА, ИК- и КР-спектроскопии, РФА и расчетов теории функционала плотности. Цефтриаксон координируется к иону магния через кислород триазинового цикла в шестом положении, азот аминогруппы тиазольного цикла и атомы кислорода карбоксильной и лактамной групп. Динатриевая соль цефтриаксона и комплекс магния были исследованы на антибактериальную активность в отношении *Staphylococcus aureus*, *Escherichia coli* и *Pseudomonas aeruginosa*.

Ключевые слова: цефалоспориновые антибиотики, цефтриаксон, магний, теория функционала плотности, молекулярная спектроскопия, антибактериальный скрининг.

Благодарности. Исследование финансировалось РФФИ, Красноярским краем и Красноярским краевым фондом науки, проект № 20–43–240007. Авторы благодарят Центр совместного использования оборудования Школы нефтяной и газовой инженерии Сибирского федерального университета, Институт химии и химической технологии СО РАН за их техническую поддержку.

Цитирование: Новикова, Г.В., Цыпленкова, Д.И., Кузубов, А.А., Коленчукова, О.А., Самойло, А.С., Воробьев, С. А. Комплекс цефтриаксона с Mg(II): синтез, структура, спектральные и антибактериальные свойства. Журн. Сиб. федер. ун-та. Химия, 2023, 16(1). С. 5–15. EDN: YPNCXI

Introduction

The World Health Organization is concerned about the overuse of antibiotics [1]. During the SARS-CoV-2 pandemic, the prescription of antibiotics was also at a high level [2, 3]. Ceftriaxone was one of the antibiotics recommended in the treatment of SARS-CoV-2, as well as in the treatment of its complications [4]. The ceftriaxone (H_2CefTria) (Fig. 1) is cephalosporin antibiotic the III generation antibiotic of a wide action range against a number of gram positive and gram negative bacteria [5]. Ceftriaxone's bactericidal activity is caused by its inhibition of the synthesis of the bacterial cell wall [6]. Frequent prescription of ceftriaxone resulted in the fact that the antibiotic no longer worked, which may be associated with new mechanisms of bacterial action. One of the ways to solve this problem is the development of new derivatives of ceftriaxone, for example, with metals.

Nowadays, different metal complexes were synthesized with ceftriaxone. Complexes of ceftriaxone were obtained with Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), Pd(II), Sn(II), Pb(II), Ca(II), Au(III) and Fe(III) [7–13]. The type of coordination of ceftriaxone to metal ion in the complexes is different. Compound of Au(III) has anticancer activity against colon carcinoma cells and hepatocellular carcinoma cells [12]. Many metal complexes of this antibiotic have antibacterial properties [7,10,11] but the problem is that some of them lose their antibacterial properties *in vivo* when they interact with protein or human plasma.

Magnesium is biogenic metal. It is an important element in enzymatic reactions. It is necessary for the conversion of phosphate creatine into ATP – nucleotide, which is the supplier of energy in living cells [14].

Mg(II) compounds were studied for several cephalosporins. The complex of Mg(II) with cephradine was synthesized by N. Sultana et al. Cephradine was coordinated to metal ions through both carboxylate at C-3 and nitrogen of lactam groups [15]. Magnesium complex of cefazoline was obtained in a crystalline form and their cell parameters were determined [16]. Cefazolin was bound with magnesium ion by the oxygen atoms of the lactam and carboxylate groups as a bidentate ligand. Authors [13] obtained magnesium complex with ceftriaxone. However, this complex has a different structure from our compound and its antibacterial properties were not study.

A systematic study of metal ion complex formation with antibiotics is crucial for better comprehension of metal–ceftriaxone binding mechanisms in human organisms. The synthesis of such metal–antibiotic systems is an important area of pharmacology and medical chemistry.

We used disodium ceftriaxone (Fig. 1) as a reactant in our work to produce magnesium complex. It has a systematic IUPAC name: Disodium (6*R*,7*R*)-7-[[(2Z)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]

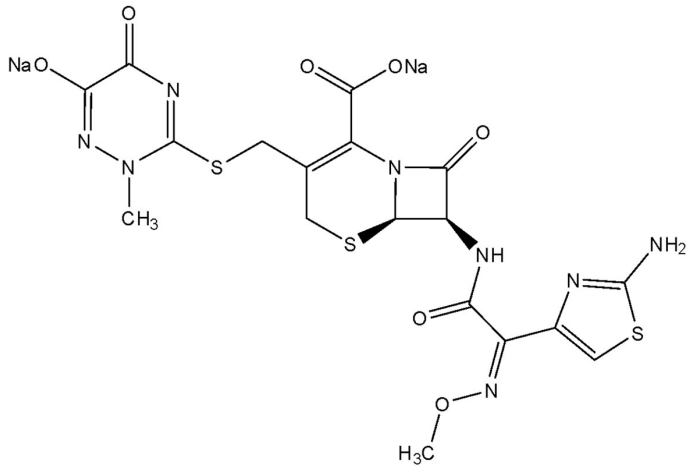

Fig. 1. Structure of disodium salt of ceftriaxone

amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 3.5 hydrate.

This paper describes the synthesis and antibacterial properties of Mg(II) complex with ceftriaxone. The structure of magnesium complex is characterized by FT-IR spectroscopy, TGA measurements, X-ray diffraction. DFT studies of molecular structure and vibrational properties are carried out to obtain more information.

Experimental

Measurements

The elemental analysis for C, N, H, S was carried out by Chromatographic analyzer HCNS-O EA1112 (Flash, USA. The content of sodium and magnesium ions was confirmed by capillary electrophoresis instrument "KAPEL – 104T" with UV photometric detector. Thermogravimetric analysis (TGA) was performed by simultaneously using SDT-Q600 TA Instruments thermal analyzer and IR attachment Nikolet 380 (USA) in the argon atmosphere within 307–577 K at the scan rate of 10 K/min. The IR and Raman spectra of ceftriaxone disodium salt and magnesium complex were obtained from a KBr pellet within 4000–400 cm^{-1} with a Nicolet 6700 spectrometer. Spectra were processed in the Omnic program. The CuK-edge X-ray adsorption spectra were collected with a ''X'Pert Pro'' (PANanalytical) diffractometer.

Synthesis of magnesium complex

All chemicals were obtained in pure form, no further purification was performed: $MgSO_4 \cdot 7H_2O$ (Aldrich), ceftriaxone disodium salt (hemi)heptahydrate (Qilu Antibiotics Pharmaceutical Co., Ltd).

The ceftriaxone disodium salt (hemi)heptahydrate (0.50 g, $7.6 \cdot 10^{-4}$ mole) was dissolved in 8 mL water-ethanol medium (1:1) and consequently mixed with metal salt $MgSO_4 \cdot 7H_2O$ (0.09 g, $3.8 \cdot 10^{-4}$ mole). The complex was obtained in the ratio of M: L=1:2, pH=6.5. The milky precipitate was formed in 1h. The compound of magnesium was obtained by sparging N_2 through the solution. Then, the reaction mixture of magnesium complex was filtered, washed with H_2O, Et_2O and dried in a sealed vessel with

granulated $CaCl_2$. The paper presents the optimal synthesis conditions under which a precipitate of a given composition is formed (salt type, M: L, synthesis time, pH solutions during synthesis, without adding additional substances). Elemental Anal. Calcd for $C_{18}H_{22}N_8O_{10}S_3Mg$ (%): C, 34.3; H, 3.5; N, 17.8; S, 15.2; Mg, 3.8. Found: C, 34.3; H, 3.7; N, 17.5; S, 14.8; Mg, 3.8. IR ($C_{18}H_{22}N_8O_{10}S_3Mg$): 3371 (b), 3203 (b), 2936 (vw), 1767 (vs), 1632 (w), 1559 (vs), 1398 (s), 1358 (s), 1286 (w), 1214 (w), 1177 (w), 1105 (w), 1036 (s), 887 (w), 812 (w), 713 (w), 654 (w), 552 (w), 469 (w).

Computational methods

The geometry optimization and harmonic vibrational frequency calculations of the most stable conformers were performed with B 3LYP [17] density functional in combination with SBKJC(p, d) basis set [18,19] augmented with s diffuse functions, as implemented in the GAMESS suite of electronic structure programs [20,21]. The relativistic effective core potential (ECP) was used for Mg atom. The applicability of this basis set and ECP to such complex was demonstrated earlier [22,23]. The Grimme's D 3 dispersion correction of ceftriaxone with Mg(II) was used in all DFT calculations [24]. The partial atomic charges were obtained from Mulliken population analysis. All molecular structures were visualized by the Chemcraft program.

Antibacterial activity

The complex was screened *in vitro* for antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* 25923 and Gram-negative bacteria *Escherichia coli* 25922 and *Pseudomonas aeruginosa* 13883. The effects of disodium ceftriaxone and [MgCefTria]·$3H_2O$ on the bacteria were investigated using the paper disk diffusion method [25]. The method includes the following steps: (1) preparation of the Mueller–Hinton growth medium; (2) preparation of the micro-organism suspensions of a 0.5 McFarland standard (final concentration 1×10^8 CFU mL^{-1}); (3) inoculation; (4) pouring the nutrient agar onto a plate and its solidification; (5) dropwise addition of the test substance to a 5 mm diameter filter paper disk placed at the center of each agar plate with following incubation; and (6) measuring the diameters of the inhibition zones. The bacteria were cultured in an incubator for 18–24 h at 36 °C. Standard disks were impregnated with the solutions of the compounds in phosphate buffer (pH=6).

Results and discussion

The chemical and elemental analysis results showed that the ratio of M: L=1:1. The chemical analysis did not give any evidence of sodium ion presence in the complex. Thus, the novel compound has the chemical composition [MgCefTria]·$3H_2O$. Complex of Mg(II) is soluble in water and insoluble in EtOH and acetone. According to XRD analysis data the [MgCefTria]·$3H_2O$ was proven being X-ray amorphous (Fig. S 1, Supplementary File). A single crystal of this compound couldn't be grown due to ceftriaxone's natural features, such as its destruction in an aqueous solution after 8 hours and at heating above 35 °C.

The thermal behavior of [MgCefTria]·$3H_2O$ was studied by differential scanning calorimetry coupled with FT-IR in a temperature range of 307–577 K under an inert atmosphere. The thermal analysis of the complex [MgCefTria]·$3H_2O$ showed that the mass of the compound decreased by 8.6 % (Calc. 8.6 %) from 307 to 407K, which was equivalent to three molecules of crystallization water (Fig. 2). A considerable

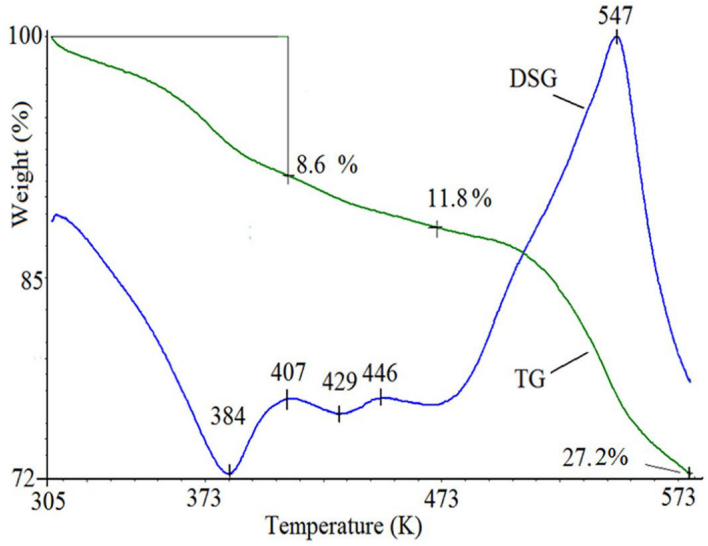

Fig. 2. DSC and thermogravimetric analysis of [MgCefTria]·3H_2O

loss of mass exceeding 407 K was caused by ligand decomposition. Thermal decomposition evolved by emission of NH_3, CO_2, CS_2 and HNCO. The weight loss at 407K, 446K, 547K was followed by exoeffect and at 384K, 429K – by endoeffect.

FTIR and Raman spectroscopy

The FTIR spectra of disodium ceftriaxone and [MgCefTria]·3H_2O were analyzed to establish the type of coordination of ceftriaxone to magnesium ion. A ceftriaxone has several donor atoms: a nitrogen atom of amino group, oxygen atoms of carboxylate, lactam, and amide carbonyl group and oxygen of thiazole cycle. Symmetric and asymmetric stretching vibrations of COO^- group belong to the bands in the 1300–1700 cm^{-1} spectral region with C=O absorption bands observed in the 1600–1700 cm^{-1} cycle (Na_2CefTria·3.5H_2O: $\nu_{as}(COO^-)$=1602 cm^{-1} and $\nu_s(COO^-)$=1395 cm^{-1}) [10]. In the experimental FTIR spectrum of the complex, $\nu(COO^-)$ is shifted relative to a free ligand ($\nu_{as}(COO^-)$=1559 cm^{-1} and $\nu_s(COO^-)$=1358 cm^{-1}). In the spectrum of [MgCefTria]·3H_2O, the difference between symmetric and asymmetric vibrations of COO^- groups is more than 200 cm^{-1} (Δ = 201 cm^{-1}), suggesting the monodentate coordination for carboxylate group [7,11]. In the FTIR spectrum of the complex ν(C=O-lactam)=1767 cm^{-1} vibration is shifted in the spectrum of the complex relative to spectrum of disodium ceftriaxone ν(C=O-lactam)=1738 cm^{-1} (Table 1, Fig. S 2 and Fig. S 3, Supplementary File). This shows that the oxygen of the lactam group is bound to the metal ion. The shift of the ν(C=O)-lactam group vibrational wavenumbers leads to the formation of chelate complex. The FTIR spectra shows that the wavenumbers of the ν(C=O)-triazine modes is shifted after ceftriaxone coordination to metal ion (ν(C=O)-triazine=1632 cm^{-1} for [MgCefTria]·3H_2O and ν(C=O)-triazine=1648 cm^{-1} for Na_2CefTria·3.5H_2O). The broad banding of the complex spectrum from 1700 to 1600 cm^{-1} has high intensity and low resolution due to the overlap of several vibrational modes, including ν(C=N) aminothiazole, $\nu(COO^-)$. These shifts indicate that the carboxylate group (COO^-), the lactam carbonyl group (C=O) and the oxo group of the triazine cycle are involved in the

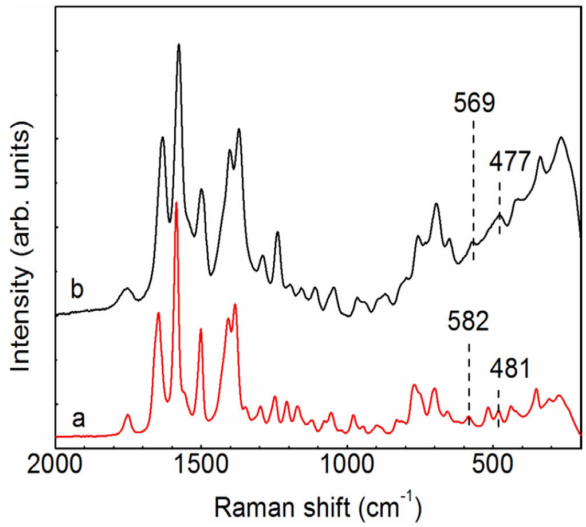

Fig. 3. Raman spectra of $Na_2CefTria{\cdot}3.5H_2O$ (a) and $[MgCefTria]{\cdot}3H_2O$ (b)

Table 1. Experimental FTIR frequencies and calculated B3LYP vibrational frequencies of Mg(II) with ceftriaxone, cm^{-1}

Mg(II)		
Exp. IR freq.	Calc. IR freq.	Functional group
1767	1771	ν(C=O) oxo group + ν(C=O) lactam + δ(NH)
1632 1559	1655 1556	ν(C=N) aminothiazole + ν(C=N) ν(C-O)-triazine + ν(C=N) triazine + ν(C=N) aminothiazole + +δ(CH_3) triazine + τ(NH_2) aminothiazole
1398	1383	ν_{as}(C=N) cephem + δ(CH) lactam + δ(C=O) lactam + δ(CH_2) + ν(COO^-)
1358	1366	ν(C=N) triazine + δ(CH_3) triazine + ν(COO^-)
1286	1286	ν(C=N) aminothiazole + τ(NH_2) aminothiazole + ω(CH_2) azabicycle+ + δ(CH) lactam
1214	1219	ω(CH_2) + r(CH) lactam + ω(CH_3) + r(CH) aminothiazole
1177	1176	ω(CH_3) + δ(NH) + δ(CH) aminothiazole
1105	1101	ν(C=N) lactam + r(CH_2)
1036	1036	ν(C=N) triazine + ν(N-N) triazine + ω(CH_3) triazine

formation of a bond with metal ions. In the Raman spectra (Fig. 3) of the complex there are bands of stretching vibrations which characterize the vibration of the bonds M-O (569 cm^{-1}) and M-N (477 cm^{-1}). These analyses are in agreement with previous studies where ceftriaxone is described as a polydentate ligand [7–13].

Computational studies

As a single crystal of this compound couldn't be grown, thus quantum chemical calculations were performed for structure establishing. Full conformation analysis was carried out earlier [11] using CONFLEX 6.0 program with MMFF94s molecular mechanics force field and Newton–Raphson

method for geometry optimization [26, 27]. The results showed two $CefTria^{2-}$ dianions in the most stable conformations. This investigation indicated that the s-cys–s-cys conformer was more energetically favorable than the s-trans–s-cys conformer [26]. The more energetically favorable s-cys–s-cys conformer geometry was used as a ceftriaxone dianion involved in the complex formation. The geometry of the $CefTria^{2-}$ dianion in this conformation was optimized with B 3LYP density functional theory as in an earlier study [11].

Ceftriaxone complex with Mg(II)

According to the B 3LYP calculations, the coordination of **I** is 7.7 $kcalM^{-1}$ lower in energy than the coordination of **II** for Mg(II) compound. This correspondence indicates that complex has **I** coordination in view of more favorable energy values (Fig. 4).

Table 1 demonstrates the comparison of experimental and calculated vibrational frequencies of the compound of magnesium complex. The average deviations of the B 3LYP frequencies from the experimental values are 6.3. The maximum absolute deviations are 23.3. It was found that all calculated vibrational frequencies were in good agreement with the experimental IR frequencies.

Microbiological screening

The cephalosporins are the antibiotics of broad-spectrum coverage. Antibacterial properties of complex salts can be increased or decreased in relation to antibiotic [10,16]. The biological activities of magnesium complex and disodium ceftriaxone were studied against Gram-positive and Gram--negative bacteria in the concentrations of 0.4, 0.6 mg mL^{-1}. The effects of compounds on the growth of such bacterial strains as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are summarized in Table 2. The increase of antibacterial activity of $[MgCefTria]\cdot 3H_2O$ (44–50 %) relative to $Na_2CefTria$ against *Staphylococcus aureus* may be explained by releasing of the lactam group due to solvating. The antibacterial activity of ceftriaxone results from inhibition of cell wall synthesis through ceftriaxone binding to penicillin-binding proteins and eventually to cell lysis [29, 30]. The biological activity of metal complex of ceftriaxone slightly changed relative to the biological activity of $Na_2CefTria$ against *Escherichia coli* in the concentrations of 0.4 and 0.6 mL^{-1}. Table 2 shows that

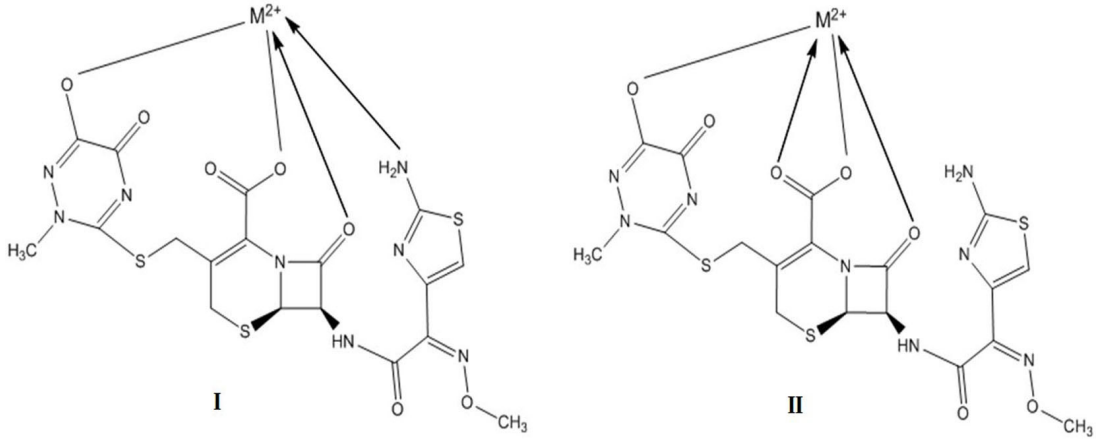

Fig. 4. Possible structures of ceftriaxone complex with Mg(II)

Table 2. Antibacterial activity of ceftriaxone disodium salt and complex

Compound	Concentration, mg mL^{-1}	Zone of inhibition (mm)		
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
$[MgCefTria]\cdot 3H_2O$	0.4	35	43	growth
	0.6	40	46	growth
$Na_2CefTria$	0.4	20	46	38
	0.6	there is no growth	46	42

the complex $[MgCefTria]\cdot 3H_2O$ did not have antibacterial activity against *Pseudomonas aeruginosa*, and we observed the growth of bacteria.

The increase of antibacterial activity of ceftriaxone with metal ions may play an important role in the inhibition of bacterial growth after the complex decomposition.

Conclusion

The novel compound $[MgCefTria]\cdot 3H_2O$ was synthesized by the reaction of ceftriaxone disodium salt (hemi)heptahydrate with metal salt in water–ethanol medium. The structure of the complex was studied using elemental, atomic-emission analysis, TGA, FTIR and Raman spectroscopy and DFT calculations. TGA indicated the existence of three crystallization water in the complex of Mg(II). Ceftriaxone was coordinated to magnesium ion by oxygen of the triazine cycle in the 6th position, the nitrogen of the amine group of the thiazole cycle, and the oxygen atoms of the lactam carbonyl and carboxylate groups. The complex $[MgCefTria]\cdot 3H_2O$ has antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

References

[1] World Health Organization. Antibiotic resistance. 2020. https://www.who.int/news-room/fact--sheets/detail/antibiotic-resistance (accessed 26 March 2021).

[2] Avdeev S., Rachina S., Belkova Yu., Kozlov R., Versporten A., Pauwels I., Goossens H., Bochanova E., Elokhina E., Portnjagina U., Reshetko O., Sychev I., Strelkova D. Antimicrobial Prescribing Patterns in Patients with COVID-19 in Russian Multi-Field Hospitals in 2021: Results of the Global-PPS Project. *Topical Medicine and Infectious Disease 2022.* 7(5), 2–12.

[3] Adebisi1 Y.A., Jimoh N.D., Ogunkola I.O., Uwizeyimana T., Olayemi A.H., Ukor N.A., Lucero--Prisno III D.E. The use of antibiotics in COVID-19 management: a rapid review of national treatment guidelines in 10 African countries. *Tropical Medicine and Health 2021.* 49(51), 1–5.

[4] Nestler M.J., Godbout E., Pharm K.L., Pharm J.K., Noda A.J., Taylor P., Pryor R., Markley J.D., Doll M., Bearman G., Stevens M.P. Impact of COVID-19 on pneumonia-focused antibiotic use at an academic medical center. *Infection Control & Hospital Epidemiology 2021.* 42(7), 915–916.

[5] European Pharmacopoeia 10th Edition. Strasbourg: Council of Europe, 2019. 4370.

[6] Richards D.M., Heel R.C., Brogden R.N., Speight T.M., Avery G.S. Ceftriaxone. A review of its antibacterial activity, pharmacological properties and therapeutic use. *Drugs 1984.* 27(6), 469–527.

[7] Anacona J.R., Rodriguez A.A. Synthesis and antibacterial activity of ceftriaxone metal complexes. *Transition Metal Chemistry 2005.* 897–901.

[8] Anacona J. R., Brito L., Peña W. Cephalosporin Tin(II) Complexes: Synthesis, Characterization, and Antibacterial Activity. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry 2012*. 42, 1278–1284.

[9] Alekseev V. G., Golubev M. V., Nikol'skii V. M. Experimental and theoretical study of iron(III) salts with penicillin and cephalosporin anions. *Russian Journal of Inorganic Chemistry 2013*. 58, 1536–1541.

[10] Novikova G. V., Tsyplenkova D. I., Kuzubov A. A., Kolenchukova O. A., Samoilo A. S., Vorobyev S. A. Complex of Ca(II) with ceftriaxone: synthesis, structure, spectral and antibacterial properties. *Journal of Siberian Federal University. Chemistry 2021*. 14(3), 290–301.

[11] Lykhin A. O., Novikova G. V., Kuzubov A. A., Staloverova N. A., Sarmatova N. I., Varganov S. A., Krasnov P. O. A complex of ceftriaxone with Pb (II): synthesis, characterization, and antibacterial activity study. *Journal of Coordination Chemistry 2014*. 67, 2783–2794.

[12] Refat M. S., Altalhi T., Fetooh H., Alsuhaibani A. M., Hassan R. F. In neutralized medium five new Ca(II), Zn(II), Fe(III), Au(III) and Pd(II) complexity of ceftriaxone antibiotic drug: Synthesis, spectroscopic, morphological and anticancer studies. *Journal of Molecular Liquids. 2021*. 322, 114816.

[13] El-Megharbel S.M., Qahl S. H., Alaryani F. S., Hamza R. Z. Synthesis, Spectroscopic Studies for Five New Mg (II), Fe (III), Cu (II), Zn (II) and Se (IV) Ceftriaxone Antibiotic Drug Complexes and Their Possible Hepatoprotective and Antioxidant Capacities. *Antibiotics 2022*. 11, 547

[14] Al Alawi A. M., Majoni S. W., Falhammar H. Magnesium and Human Health: Perspectives and Research Directions. *International Journal of Endocrinology 2018*. 2018, Article ID 9041694, 17.

[15] Sultana N., Arayne M. S., Afzal M. Synthesis and antibacterial activity of cephradine metal complexes: part I complexes with magnesium, calcium, chromium and manganese. Pakistan Journal of Pharmaceutical Sciences *2003*, 16(1), 59–72

[16] Novikova G. V., Krasnov P. O., Samoilo A. S., Shidlovskiy I. P., Kondrasenko A. A., Andreeva E. A**.** Complex Formation of Cefazolin with Ca^{2+} and Mg^{2+}: Synthesis, DFT, Spectral and Antibacterial Determination. *ChemistrySelect 2021*. 6(34), 8974–8980.

[17] Becke A. D. Density-functional thermochemistry. *Journal of Chemical Physics 1993*. 98(7), 5648–5652.

[18] Binkley J. S., Pople J. A., Hehre W. J. Self-Consistent Molecular Orbital Methods. 21. Small Split-Valence Basis Sets for First-Row Elements. *Journal of the American Chemical Society 1980*. 102, 939–947.

[19] Stevens W. J., Basch H., Krauss M. Compact effective potentials and efficient shared-exponent basis sets for the first- and second-row atoms. *Journal of Chemical Physics 1984*. 81, 6026–6033.

[20] Schmidt M. W., Baldridge K. K., Boatz J. A., Elbert S. T., Gordon M. S., Jensen J. H., Koseki S., Matsunaga N., Nguyen K. A., Su S., Windus T. L., Dupuis M., Montgomery J. A., General atomic and molecular electronic structure system. *Journal of Computational Chemistry 1993*. 14, 1347–1363.

[21] Dykstra C. E., Frenking G., Kim K. S., Scuseria G. E., Theory and Applications of Computational Chemistry. Amsterdam: The First Forty Years, Elsevier, 2005. 1336.

[22] Petit L., Maldivi P., Adamo C. Predictions of optical excitations in transition-metal complexes with time dependent-density functional theory: influence of basis sets. *Journal of Chemical Theory and Computation 2005*. 1(5), 953–962.

[23] Yu L., Srinivas G. N., Schwartz M. Scale factors for C≡O vibrational frequencies in organometallic complexes. *Journal of Molecular Structure THEOCHEM 2003*. 625, 215–220.

[24] Grimme S., Antony J., Ehrlich S., Krieg H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *Journal of Chemical Physics 2010*. 132, 154104

[25] Balouiri M., Sadiki M., Ibnsouda S. K. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis 2016*. 6(2), 71–79.

[26] Goto H., Osawa E. Corner flapping: a simple and fast algorithm for exhaustive generation of ring conformations. *Journal of the American Chemical Society 1989*. 111(24), 8950–8951.

[27] Goto H., Osawa E. An efficient algorithm for searching low-energy conformers of cyclic and acyclic molecules. *Journal of the Chemical Society Perkin Transactions 2 1993*. 2, 187–19.

[28] Munita J. M., Arias C. A. Mechanisms of Antibiotic Resistance. *Microbiology Spectrum 2016*. 4(2). 1–24.