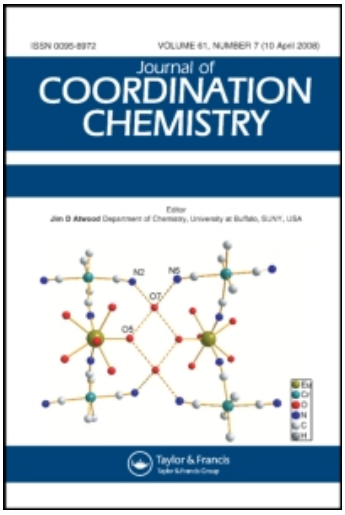

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Metalloantibiotics: synthesis and antibacterial activity of ceftazidime metal complexes

J. R. Anacona[a]; Candy Patiño[a]
[a] Departamento de Química, Universidad de Oriente, Cumaná, Venezuela

First published on: 01 October 2010

To cite this Article Anacona, J. R. and Patiño, Candy(2009) 'Metalloantibiotics: synthesis and antibacterial activity of ceftazidime metal complexes', Journal of Coordination Chemistry, 62: 4, 613 — 621, First published on: 01 October 2010 (iFirst)
To link to this Article: DOI: 10.1080/00958970802291480
URL: http://dx.doi.org/10.1080/00958970802291480

Journal of Coordination Chemistry
Vol. 62, No. 4, 20 February 2009, 613–621

Taylor & Francis
Taylor & Francis Group

Metalloantibiotics: synthesis and antibacterial activity of ceftazidime metal complexes

J. R. ANACONA* and CANDY PATIÑO

Departamento de Química, Universidad de Oriente, Apartado Postal 208, Cumaná, Venezuela

(*Received 31 December 2007; in final form 30 April 2008*)

Ceftazidime (Hceftaz) interacts with transition metal(II) ions to give octahedral [M(ceftaz)(H_2O)Cl] complexes [M = Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Cd(II)] which were characterized by physicochemical and spectroscopic methods. The spectra indicated that the ligand is a multidentate chelating agent. The complexes are insoluble in water and common organic solvents and probably have polymeric structures. The antibacterial activity of the metal complexes was found to be lower than that of free ceftazidime.

Keywords: Ceftazidime; Metalloantibiotics; Metal complexes

1. Introduction

Cephalosporin antibiotics are comprised of several different classes of compounds with dissimilar spectrums of activity and pharmacokinetic profiles [1]. All ''true'' cephalosporins are derived from cephalosporin C which is produced from *Cephalosporium acremonium* [2]. Cephalosporins are usually bactericidal against susceptible bacteria and act by inhibiting mucopeptide synthesis in the cell wall resulting in a defective barrier and an osmotically unstable spheroplast [3]. The mechanism for this effect has not been definitively determined, but beta-lactam antibiotics have been shown to bind to several enzymes (carboxypeptidases, transpeptidases, endopeptidases) within the bacterial cytoplasmic membrane that are involved with cell wall synthesis [4–7]. The different affinities that various beta-lactam antibiotics have for these enzymes (also known as penicillin-binding proteins) help explain the differences in spectrum of activity of these drugs that are not explained by the influence of beta-lactamases [8]. Like other beta-lactam antibiotics, cephalosporins are generally considered to be more effective against actively growing bacteria [9]. The cephalosporin class of antibiotics is usually divided into three generations. Ceftazidime belongs to the third generation cephalosporins, retaining the gram positive

*Corresponding author. Email: juananacona@hotmail.com

Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online © 2009 Taylor & Francis
DOI: 10.1080/00958970802291480

activity of the first and second generation agents, but have expanded gram negative activity [10, 11]. In continuation of our work on metalloantibiotics [12–18] we report here the synthesis, characterization and bactericidal activity of ceftazidime metal complexes. The chemical structure of ceftazidime is shown in figure 1.

2. Experimental

2.1. *Physical methods*

IR spectra of the ligand and its metal complexes were recorded as KBr pellets in the 4000–400 cm^{-1} range with a Perkin-Elmer Series 2000 spectrophotometer. FTIR spectra as polyethylene pellets were recorded between 450–120 cm^{-1} using a Bruker IFS 66V spectrophotometer. UV–Vis spectra were recorded using a Perkin-Elmer spectrometer. C, H, N and S were analyzed on a LECO CHNS 932 model microanalytical instrument. Metal contents were estimated spectrophotometrically on an atomic absorption spectrometer. Thermogravimetric analyses were performed with a Cahn RG electromicrobalance in air at a heating rate of 4°C min^{-1} up to 200°C. Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using mercury(II) tetrathiocyanato-cobaltate(II) as calibrant. EPR spectra were recorded on a Bruker ECS 106 spectrometer by the X-band.

2.2. *Antibacterial activity test*

In vitro antibacterial activities of ceftazidime and its complexes were tested using the paper disc diffusion method [19]. The chosen strains were G(+) *Staphylococcus aureus* ATCC 25923 and G(−), *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella enteritidis* ATCC 497, *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 35939. The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 121°C and at 15 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36°C in an incubator. Muller Hinton broth was used for preparing basal media for the bioassay of the organisms. Nutrient agar was poured onto a plate and allowed to solidify. The test compounds (Nujol mulls) were added dropwise to a 10 mm diameter filter paper disc placed at the center of each agar plate. The plates were then kept at 5°C for 1 h then transferred to an incubator maintained at 36°C. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicates

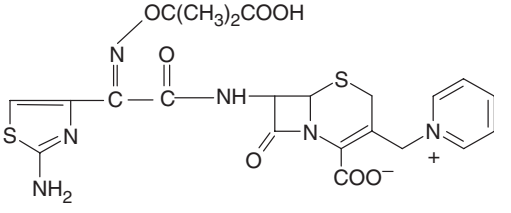

Figure 1. The structure of ceftazidime.

were made for each treatment. Growth inhibition was compared with that of ceftazidime. In order to clarify any participating role of DMSO and the metal(II) chlorides salts in the biological screening, separate studies were carried out with the solutions alone of DMSO and free metal salts and they showed no significant activity against any bacterial strains.

2.3. *Materials and methods*

All chemicals were commercially obtained in their purest form and were used without purification. Solvents were redistilled by standard techniques before use [20]. The complexes were prepared by mixing ceftazidime sodium salt (1 mmol) and metal salts: $MnCl_2 \cdot 4H_2O$, $FeCl_2$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$ or $CdCl_2$ (1 mmol) in MeOH (40 cm^3). The reaction mixture was then stirred at room temperature for *ca.* 10 h, and a colored precipitate formed. The precipitated complexes were filtered off, washed with water, MeOH and ether and dried under reduced pressure at room temperature. All syntheses were carried out under nitrogen.

3. Results and discussion

Ceftazidime has three ionizable groups; two carboxylates and NH_3^+ (pKa = 1.8, 2.7 and 4.1) [21]; it thus exists predominantly as monoanionic at a physiological pH. The elemental analyses (table 1) agree with a 1 : 1 metal to ligand stoichiometry for all the complexes. The manganese(II) and cobalt(II) complexes are beige and dark red, respectively, while the iron(II), nickel(II) and copper(II) complexes are green. Cadmium(II) complex is white, are air-stable solids, insoluble in H_2O and other common organic solvents such as EtOH, benzene, acetone, acetonitrile, ether, DMF and DMSO. The general formulas [M(ceftaz)(H_2O)Cl] have been assigned to the complexes. The insolubility and high melting of the complexes (>300°C) suggest that they are polymeric [22]. Therefore, solution studies like molar conductance and NMR are not possible. Thermograms of the hydrated metal complexes indicate endothermic decompositions in the 150–160°C range due to the loss of coordinated water and also reveal that the complexes are stable and have no hydration water or solvent. Attempts to form complexes of a well-defined stoichiometry,

Table 1. Elemental analyses for the complexes.

	Found (Calcd) %				
Compound	C	H	N	S	Metal
[Mn(ceftaz)(H_2O)Cl]	40.7 (40.3)	3.6 (3.8)	12.8 (12.8)	9.5 (9.8)	8.3 (8.4)
[Fe(ceftaz)(H_2O)Cl]	40.5 (40.2)	3.4 (3.8)	12.9 (12.8)	9.4 (9.8)	8.7 (8.5)
[Co(ceftaz)(H_2O)Cl]	39.8 (40.0)	3.6 (3.8)	12.3 (12.7)	9.6 (9.7)	8.5 (8.9)
[Ni(ceftaz)(H_2O)Cl]	40.4 (40.1)	3.8 (3.8)	12.8 (12.7)	9.3 (9.7)	8.4 (8.9)
[Cu(ceftaz)(H_2O)Cl]	39.6 (39.8)	3.4 (3.8)	12.3 (12.6)	9.9 (9.6)	9.3 (9.6)
[Cd(ceftaz)(H_2O)Cl]	37.4 (37.0)	3.8 (3.5)	11.6 (11.8)	8.5 (9.0)	

under the above-mentioned conditions, with zinc(II) and mercury(II) ions were unsuccessful.

3.1. *IR spectra*

IR spectra of ceftazidime and its complexes are similar and assigned mainly to those specific wavenumbers directly involved in complex formation. The main IR wavenumbers are recorded in table 2. Generally the ring carbonyl absorption frequency is shifted to higher wavenumbers as the ring becomes more strained. The lactam (C=O) band appears at 1750 cm^{-1} in the spectrum of ceftazidime, while the complexes show this band at 1660–1700 cm^{-1}. The amide carbonyl ν(C=O)–NH in free ceftazidime appears at 1630 cm^{-1} while the complexes show this band at 1610–1640 cm^{-1}, suggesting that ligand coordination with these metal ions occurs through the oxygen atom from the lactam carbonyl group rather than the amide carbonyl group, where the shifting was not significant.

The band at 1570 cm^{-1}, corresponding to the carboxylate asymmetric stretch, is shifted to higher wavenumbers (1580–1610 cm^{-1}) after complexation with the metal(II), indicating coordination through carboxylate [23, 24]. The remaining carboxylate bands, ν_{sym}(COO), γ(COO), ω(COO) and ρ(COO), formerly at 1400, 785, 610 and 530 cm^{-1}, respectively, also change as a result of coordination. A carboxylate can bind to the metal either monodentate or bidentate, giving changes in the relative positions of the antisymmetric and symmetric stretching vibrations [25]. The IR spectra of the complexes give a separation value of >200 cm^{-1} suggesting monodentate carboxylate group.

The presence of ν(M–N) in the 450–490 cm^{-1} range for the metal complexes (absent in the free ligand) provide evidence that NH_2 is bonded to the metal through nitrogen. Coordination of the NH_2-thiazole to the metal is not the only explanation of these absorption bands, alternatively the N of the CONH group could coordinate to the metal in solid complexes, however steric constraints prevent coordination of N along with the COO and lactam C=O groups. Furthermore, the C–N–C stretching and the N–H stretching vibrations of CONH observed in free ceftazidime at 1180 and 3240 cm^{-1}, respectively, either do not shift or show a slight shift in all metal complexes indicating that these nitrogens are not involved in coordination.

These IR spectra suggest coordination of the ligand as a multidentate chelating agent. The bands in the 350–400 cm^{-1} region observed in the complexes, and absent in free ceftazidime, are tentatively assigned to ν(M–O).

Table 2. Main vibrational wavenumbers (cm^{-1}).

Compound	ν(C=O) lactam	ν(C=O) amide	ν(COO) asym	ν(COO) symm	$\Delta\nu$(COO)
Ceftazidime	1750	1630	1570	1370	200
[Mn(ceftaz)(H_2O)Cl]	1660	1610	1580	1380	200
[Fe(ceftaz)(H_2O)Cl]	1700	1640	1610	1390	220
[Co(ceftaz)(H_2O)Cl]	1670	1610	1590	1380	210
[Ni(ceftaz)(H_2O)Cl]	1660	1610	1580	1380	200
[Cu(ceftaz)(H_2O)Cl]	1690	1640	1600	1390	210
[Cd(ceftaz)(H_2O)Cl]	1760	1650	1600	1390	210

3.2. *Electronic spectra*

The UV-Vis spectra of ceftazidime in DMSO and its complexes in nujol mulls present absorption maxima at 235–250 nm assigned to a $\pi \rightarrow \pi^*$ transition due to orbitals originating in the N–C–S moiety [26, 27]. An intraligand band at 260–270 nm is related to the $\pi \rightarrow \pi^*$ transitions within the thiazole moiety. The band in the 310–330 nm region is ascribed to an intraligand transition of the $n \rightarrow \pi^*$ type in accordance with the literature data for transitions due to sulfur [26, 28]. The fact that the bands due to sulfur atoms are not shifted suggests that these atoms are not involved in coordination to metal.

The local symmetry around metal(II) ions belong to lower symmetry than O_h, therefore, accurate band assignment is not possible. The manganese(II) complex showed very weak absorption bands probably due to spin-orbit forbidden transitions. The iron(II) complex showed two weak bands at 420 and 600 nm. The cobalt(II) complex presents one absorption maxima at 480 nm presumably due to intraligand excitation (figure 2). Because of the unsaturation of ceftazidime, the intense UV absorption has a tail in the visible region hampering assignment of the relatively weak d–d transitions of cobalt(II) and iron(II). The nickel(II) complex showed a broad absorption band at 580–650 nm attributable to a d–d transition. The copper(II) complex exhibits a d–d transition as a broad band centered at 680 nm falling in the range of those usually reported for five or six-coordinate copper(II) [29].

3.3. *Magnetic measurements*

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants. The magnitudes of the magnetic moments fall within the ranges associated with high spin ions in octahedral fields and are unlikely to be of value in discriminating between the metal ions in six or five coordinate geometries [30]. The manganese(II) complex has a magnetic moment of 5.72 B.M. consistent with high spin d^5 systems with five unpaired electrons and an $S = 5/2$ ground state. The iron(II) complex has a magnetic moment of 4.65 B.M. corresponding to a high spin d^6 system with four unpaired electrons and an $S = 2$ ground state [31]. Since the experimental value obtained for the magnetic moment of cobalt(II) in the cobalt(II)

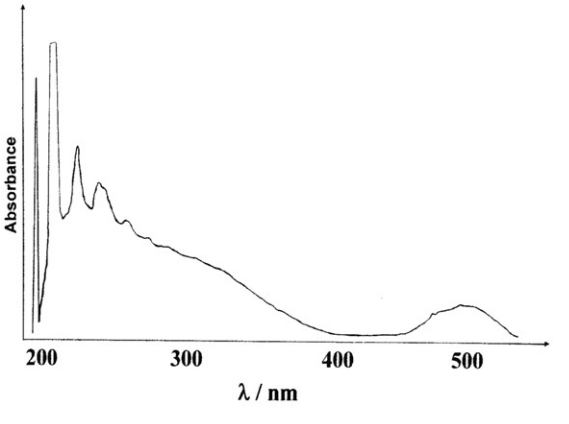

Figure 2. Electronic absorption spectrum of [Co(ceftaz)(H_2O)Cl].

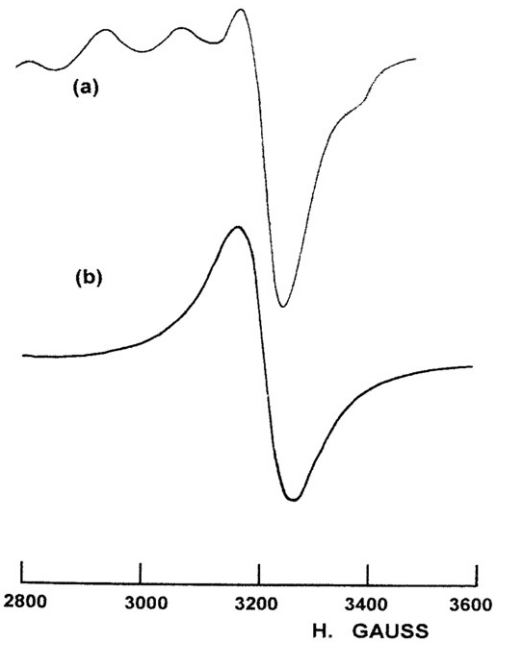

Figure 3. EPR spectra of (a) [Cu(ceftaz)(H_2O)Cl]; (b) [Mn(ceftaz)(H_2O)Cl].

complex is 4.12 B.M., while the calculated value for a d^7 high spin electronic distribution is 3.87 B.M., we conclude that cobalt(II) in [Co(ceftaz)(H_2O)Cl] is in a five coordinate or octahedral geometry with a high spin configuration. The experimental value obtained for the magnetic moment for nickel(II) in [Ni(ceftaz)(H_2O)Cl] is 3.17 B.M., which is close to the expected value for a five-coordinate geometry (3.20–3.40 B.M.) [32, 33]. For [Cu(ceftaz)(H_2O)Cl] the experimental magnetic moment is equal to 2.22 B.M. while the calculated one for a d^9 configuration is 1.73 B.M., suggesting the presence of impurities in the complex. Although lowered moments can be accounted for by antiferromagnetic interactions between the ions, higher moments would require ferromagnetic interactions which are significantly rarer [34].

The EPR spectrum at liquid nitrogen temperature of the powder sample of the copper(II) complex showed four lines (^{63}Cu, $I = 3/2$) and is anisotropic at higher magnetic field (figure 3). The three peaks of low intensity in the weaker field region are considered to originate from the $g_\parallel$ component. The calculated g values, $g_\parallel = 2.12$ and $g_\perp = 2.07$ and $A_\parallel = 120 \times 10^{-4}\,cm^{-1}$, indicate that the unpaired electron resides in the $d_{x^2-y^2}$ orbital having $^2B_{1g}$ as a ground state term [35, 36]. EPR spectra of a powdered sample of the manganese(II) complex at room temperature was obtained, but there was simply a single broad band centered at 3200 G (figure 2) with no evidence of fine structure due to ^{55}Mn (100% natural abundance, $I = 5/2$).

3.4. *Structure of complexes*

Coordination of some beta-lactam antibiotics with transition and d^{10} metal ions has been reported [12–16, 37]. In our case, the ceftazidime anion has several potential donor

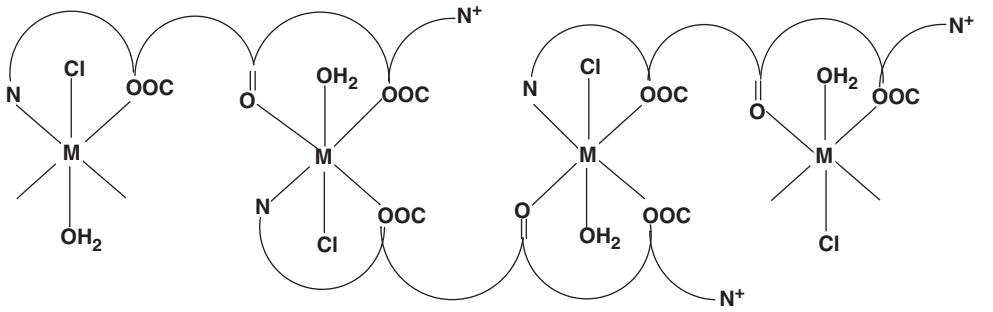

Figure 4. Suggested structure of [M(ceftaz)(H_2O)Cl] complexes.

Table 3. Antibacterial activity of the ceftazidime metal complexes.

	Zone of inhibition (mm)					
Compound	S.A.	P.A.	E.C.	B.S.	K.P.	S.E.
Ceftazidime	23	35	40	23	35	37
[Mn(ceftaz)(H_2O)Cl]	0	8	19	8	19	18
[Fe(ceftaz)(H_2O)Cl]	0	11	22	6	18	18
[Co(ceftaz)(H_2O)Cl]	6	19	28	6	23	23
[Ni(ceftaz)(H_2O)Cl]	15	15	28	14	22	25
[Cu(ceftaz)(H_2O)Cl]	15	16	28	15	24	25
[Cd(ceftaz)(H_2O)Cl]	23	21	29	36	24	25

S.A. *Staphylococcus aureus* ATCC 25923, P.A. *Pseudomonas aeruginosa* ATCC 10145, E.C. *Escherichia coli* ATCC 35939, B.S. *Bacillus subtilis*, K.P. *Klebsiella pneumoniae*, S.E. *Salmonella enteritidis* ATCC 497. All doses were 400 µg/disc. Estimated error ±1 mm.

atoms that might be involved in coordination with the metal ions. The assumption that coordination of ceftazidime occurs through the carboxylates, lactam carbonyl moiety and NH_2-thiazole group seems likely from molecular models. It is feasible that the metal ions in [M(ceftaz)(H_2O)Cl] [where M = Mn(II), Fe(II), Co(II), Ni(II), Cu(II), and Cd(II)] containing one coordinated chloride and one water are six coordinate with octahedral geometries. Figure 4 shows a suggested structure of [M(ceftaz)(H_2O)Cl] complexes where the positive charges [a quaternary amine with one plus charge and the metal(II) cation] are balanced with the negative charges (two carboxylates and one chloride anion).

3.5. *Microbiological screening*

The susceptibility of certain strains of bacterium towards ceftazidime and its metal complexes was judged by measuring the size of inhibition diameter. As assessed by color, the complexes remain intact during biological testing. The antibiotic and the complexes presented bactericide diameters larger than 15 mm showing that they have a good activity as bactericides [38, 39]. The average results are shown in table 3.

The metal(II) complexes of ceftazidime are less active than free ceftazidime against all bacteria tested, probably due to poor solubility of the complexes. The highest

antibacterial activity between complexes was shown by the cadmium(II) complex which showed better activity than free ceftazidime against *B. subtilis*. In general, the iron(II) complex showed very poor activity against the tested microorganism in comparison with other complexes. Though some trends in metal-based bactericidal agents are noted, it does not seem to be possible to correlate the bactericidal activity with the metal complexes structure in any simple way.

Acknowledgements

Our sincere thanks to the Comision de Investigacion from the Universidad de Oriente for providing financial support. Authors are very grateful to Lic. Erasto Bastardo for the elemental analyses.

References

[1] A. Novelli, S. Conti, M.I. Cassetta, S. Fallani. *Clin. Microbiol. Infect.*, **3**, 50 (2000).
[2] J.S. Lim, J.H. Kim, C. Kim, S.W. Kim. *Korea-Aust. Rheol. J.*, **14**, 11 (2002).
[3] S. Magnet, A. Arbeloa, J.L. Mainardi, J.E. Hugonnet, M. Forgeaud, L. Dubost, A. Marie, V. Delfosse, C. Mayer, L.B. Rice, M. Arthur. *J. Biol. Chem.*, **282**, 13151 (2007).
[4] T. Bergan. *Drugs*, **34**, 89 (1987).
[5] B. Cunha. *Clin. Ther.*, **14**, 616 (1982).
[6] H. Neu. *Drugs*, **34**, 135 (1987).
[7] D.R. Williams. *The Metals of Life*, Van Nostrand Reinhold, London (1971).
[8] J.L. Mainardi, J.E. Hugonnet, F. Rusconi, M. Forgeaud, L. Dubost, A.N. Moumi, V. Delfosse, C. Mater, L. Gutmann, L.B. Rice, M. Arthur. *J. Biol. Chem.*, **282**, 30414 (2007).
[9] K.A. Caprile. *J. Vet. Pharmacol. Ther.*, **11**, 1 (1988).
[10] J.R.J. Sorenson. *J. Med. Chem.*, **19**, 135 (1976).
[11] D.H. Brown, W.E. Smith, J.W. Teape. *J. Med. Chem.*, **23**, 729 (1980).
[12] J.R. Anacona, I. Rodriguez. *J. Coord. Chem.*, **57**, 1263 (2004).
[13] J.R. Anacona, C.C. Gil. *Transition Met. Chem.*, **30**, 605 (2005).
[14] J.R. Anacona, A. Rodriguez. *Transition Met. Chem.*, **30**, 897 (2005).
[15] J.R. Anacona, F. Acosta. *J. Coord. Chem.*, **59**, 621 (2006).
[16] J.R. Anacona, J. Estacio. *Transition Met. Chem.*, **31**, 227 (2006).
[17] A.H. Osman, A.M. Ali, N.A. El-Maali, G.A.A. Al-Hazmi. *Synth. React. Inorg. Met.-Org. Chem.*, **32**, 1289 (2002).
[18] Z.F. Chen, Y-Z. Tang, H. Liang, X-X. Zhon, Y. Li. *Inorg. Chem. Commun.*, **9**, 322 (2006).
[19] D. Liu, K. Kwasniewska. *Bull. Environ. Contam. Toxicol.*, **27**, 289 (1981).
[20] D.D. Perrin, W.F. Armerago. *Purification of Laboratory Chemicals*, Pergamon Press, Oxford (1988).
[21] A. Kar. *Pharmacognosy and Pharmacobiotechnology*, New Age International Publishers, New Delhi (2007).
[22] U.P. Singh, R. Ghose, A.K. Ghose. *Bull. Chem. Soc. Jpn*, **63**, 1226 (1990).
[23] D.K. Sau, N. Saha, R.J. Butcher, S. Chaudhuri. *Transition Met. Chem.*, **29**, 81 (2004).
[24] K. Nakamoto. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley Interscience, New York (1986).
[25] G. Socrates. *Infrared Characteristic Group Frequencies*, John Wiley & Sons, Ltd, Great Britain (1980).
[26] G.C. Franchini, A. Giusti, C. Preti, L. Tosi, P. Zannini. *Polyhedron*, **9**, 1553 (1985).
[27] C.C. Hadjikostas, G.A. Katsoulos, S.K. Shakhatreh. *Inorg. Chim. Acta*, **133**, 129 (1987).
[28] M. Castillo, J.J. Criado, B. Macias, M.V. Vaquero. *Inorg. Chim. Acta*, **124**, 127 (1986).
[29] B.J. Hathaway. In *Comprehensive Coordination Chemistry*, G. Wilkinson, R.D. Gillard, J.A. Cleverty (Eds), Pergamon Press, New York (1987).
[30] J.C. Bailar, H.J. Emeleus, R. Nyholm, A.F. Trotman-Dickenson (Eds.). *Comprehensive Inorg. Chem.*, Vol. 3, Pergamon Press, Oxford, (1975).
[31] F.A. Cotton, G. Wilkinson. *Advanced Inorganic Chemistry*, 3rd Edn, Interscience Publishers, New York (1972).

[32] K.A.R. Salib, A.A. Saleh, S.A. El-Wafa, H.F.O. El-Shafiy. *J. Coord. Chem.*, **56**, 283 (2003).
[33] A. Earnshaw. *Introduction to Magnetochemistry*, Academic Press, London (1968).
[34] B.N. Figgis, J. Lewis. In *Modern Coordination Chemistry*, J. Lewis, R.G. Wilkins (Eds), Interscience, New York (1960).
[35] B.A. Goodwin, J.B. Raynor. *Adv. Inorg. Chem. Radiochem.*, **13**, 136 (1970).
[36] M.C. Jain, A.K. Srivastava, P.C. Jain. *Inorg. Chim. Acta*, **23**, 199 (1977).
[37] J.R. Anacona. *J. Coord. Chem.*, **54**, 355 (2001).
[38] D.L. Shungu. *J. Clin. Microbiol.*, **18**, 888 (1988).
[39] D.L. Shungu. *Antimicrob. Agents Chemother.*, **23**, 256 (1983).