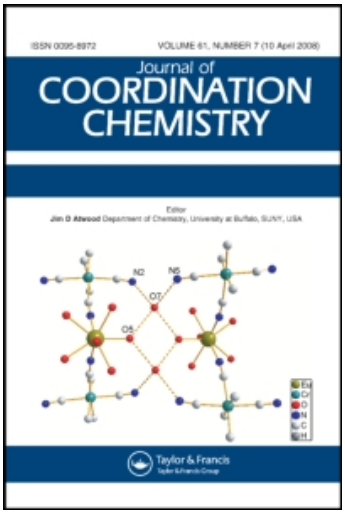

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHALEXIN METAL COMPLEXES

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The interactions of cephalexin (Hcepha) with transition and d^{10} metal ions have been investigated. The complexes [M(cepha)Cl]nH_2O [M = Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II)] were characterized by physicochemical and spectroscopic methods. The IR and ^1H NMR spectra of the complexes suggest that cephalexin behaves as a monoanionic tridentate ligand. *In vitro* antibacterial activities of Hcepha and the complexes were tested.

Keywords: Cephalexin; Antibiotic; Metal complexes; Antibacterial activity

INTRODUCTION

The antibiotic cephalexin is a first-generation cephalosporin and has good activity against gram-positive bacteria and relatively modest activity against gram-negative microorganisms. Many gram-positive microorganisms release relatively large amounts of β-lactamase into the surrounding medium which can destroy the β-lactamic antibiotics by hydrolysis of the β-lactam ring; this is the most prevalent mechanism of resistance [1–3]. The structure of cephalexin is shown in Fig. 1.

Many drugs possess modified toxicological and pharmacological properties when in the form of metal complexes. The most widely studied metal in this respect is copper(II) which has proved beneficial in diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers [4–7]. These results encouraged us to investigate the coordination chemistry of antibiotics and Schiff-base compounds with transition and d^{10} metal ions, in an attempt to examine the modes of binding in the solid state and to study biological activities [8–11]. As a continuation of our work on metal interactions with β-lactamic derivatives we report here the synthesis and characterization of metal complexes of cephalexin.

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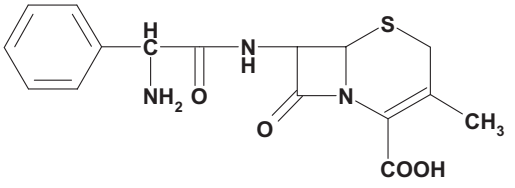

FIGURE 1 The structure of cephalexin.

EXPERIMENTAL

Physical Methods

Fourier transform infrared (FTIR) spectra of the ligand and its metal complexes as KBr pellets were recorded in the range 4000–400 cm^{-1} with a Perkin-Elmer Series 2000 spectrophotometer. FTIR spectra from polyethylene pellets were recorded between 450 and 120 cm^{-1} using a Brucker IFS 66 V spectrophotometer. UV-vis spectra were recorded using a Perkin-Elmer recording spectrometer. C, H, N and S were determined on a LECO CHNS 932 instrument. Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using $HgCo(NCS)_4$ as calibrant. EPR spectra were recorded on a Brucker ECS 106 spectrometer by the X-band method. 500 MHz ^{1}H NMR spectroscopic measurements were performed on a Bruker AM-500 NMR spectrometer, using TMS as an internal reference and deuterated dimethylsulfoxide as solvent. Thermogravimetric analyses were performed with a Cahn RG electromicrobalance in air at a heating rate of 4°C min^{-1} up to 400°C.

Antibacterial Activity Test

The *in vitro* antibacterial activities of cephalexin and its complexes were tested using the paper disc diffusion method [12]. The chosen strains were G(+) *Staphylococcus aureus* ATCC 25923 and G(−) *Proteus mirabilis* ATCC 35659, *Klebsiella pneumoniae* ATCC 556, *Salmonella enteriditis* 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 15 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36°C in an incubator. Mueller 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 in DMSO solutions were added dropwise to a 10 mm diameter filter paper disc placed at the centre 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 were made for each treatment.

Materials and Methods

All chemicals were of reagent grade, where possible, purchased from Aldrich and used without further purification. Solvents were redistilled by standard techniques before use. The complexes were prepared by mixing cephalexin (2 mmol) and metal(II) salts: $MnCl_2{\cdot}4H_2O$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$ or $HgCl_2$ (1 mmol) in MeOH (40 cm^3), then the pH of the solution was adjusted to 8.0 with 0.5 M NaOH. The

reaction mixture was stirred at room temperature for *ca.* 8 h and left to stand overnight. The volume was then reduced by rotary evaporation. The precipitated complexes were filtered off, washed with H_2O, MeOH and ether and dried under reduced pressure at room temperature. All the syntheses were carried out under a nitrogen atmosphere. Alternatively the same complexes have been obtained by mixing sodium cephalexinate with the metal salts in redistilled water as solvent.

RESULTS AND DISCUSSION

The elemental analyses agree well with the proposed formulae of the complexes. The manganese(II), zinc(II) and cadmium(II) complexes are light yellow while the nickel(II), mercury(II) and copper(II) complexes are green. All are air-stable solids, soluble in DMSO and DMF, slightly soluble in MeCN and insoluble in MeOH and water. The conductivity values measured in DMSO at room temperature fall in the range of non-electrolytes [13], suggesting that the chloride ion is coordinated to the metal ion. Thermogravimetric analyses of the hydrated metal complexes show a mass loss equivalent to water molecules in the 90–110°C range, suggesting that these molecules are not coordinated to the metal ion. Attempts to form complexes of a well-defined stoichiometry, in the above mentioned conditions, with silver(I), tin(II) and lead(II) were unsuccessful. Analytical data of the compounds are given in Table I.

IR Spectra

The IR spectra of cephalexin and its complexes are similar; the main vibrational frequencies are listed in Table II. The lactam (C═O) band appears at 1750 cm^{-1} in

TABLE I Elemental analyses for the complexes

	Found (*Calcd.*) (%)			
Compound	*C*	*H*	*N*	*S*
[Mn(cepha)Cl]·3H_2O	39.3 (39.1)	4.1 (4.5)	8.3 (8.6)	6.3 (6.5)
[Co(cepha)Cl]·3H_2O	38.5 (38.8)	4.5 (4.4)	8.2 (8.5)	6.8 (6.5)
[Ni(cepha)Cl]·4H_2O	37.8 (37.5)	4.9 (4.7)	8.5 (8.2)	5.9 (6.2)
[Cu(cepha)Cl]·3H_2O	38.8 (38.5)	4.8 (4.4)	8.4 (8.4)	6.6 (6.4)
[Zn(cepha)Cl]·3H_2O	38.2 (38.3)	4.2 (4.4)	8.3 (8.4)	6.3 (6.4)
[Cd(cepha)Cl]	39.1 (38.9)	3.1 (3.2)	8.1 (8.5)	6.1 (6.5)
[Hg(cepha)Cl]	33.4 (33.0)	2.7 (2.8)	7.4 (7.2)	5.4 (5.5)

TABLE II Main vibrational frequencies (cm^{-1})

Compound	*ν(CO)lact*	*ν(CO)amide*	*ν(M–N)*	*ν(COO)asym*	*ν(COO)sym*	*Δν*
Cephalexin	1750	1680		1580	1390	190
[Mn(cepha)Cl]·3H_2O	1720	1670	445	1615	1380	235
[Co(cepha)Cl]·3H_2O	1715	1670	440	1610	1390	220
[Ni(cepha)Cl]·4H_2O	1750	1640	450	1610	1380	230
[Cu(cepha)Cl]·3H_2O	1750	1650	460	1610	1360	250
[Zn(cepha)Cl]·3H_2O	1750	1640	475	1620	1380	240
[Cd(cepha)Cl]	1750	1640	440	1580	1380	200
[Hg(cepha)Cl]	1750	1640	455	1580	1390	190

the spectrum of cephalexin while the amide (C=O) band appears at 1680 cm^{-1}; the complexes show these bands at *ca.* 1750 and 1640 cm^{-1}, respectively, suggesting that ligand coordination occurs through the oxygen atom from the amide carbonyl group rather than the lactam carbonyl group. The amide carbonyl bands were shifted toward lower frequencies (30–40 cm^{-1}) relative to the value of the uncomplexed cephalexin while the lactam carbonyl bands were not shifted. The exceptions were the manganese(II) and cobalt(II) complexes, the spectra of which suggest that coordination of the ligand occurs through the lactamic carbonyl group.

A carboxylate ligand can bind to the metal atom as either a monodentate or a bidentate ligand, giving changes in the relative positions of the antisymmetric and symmetric stretching vibrations [14]. The IR spectra of the complexes give a separation value of >200 cm^{-1}, suggesting monodentate bonding for the carboxylate group. The presence of M–N stretching vibrations in the 440–475 cm^{-1} range for the metal complexes (absent in the free ligand) suggests coordination by the ligand as a tridentate monoanionic chelating agent [15]. The coordination of the NH_2 group to the metal ion is not the only explanation of these absorption bands; alternatively, the NH group could coordinate to the metal ions in solid complexes, however steric constraints prevent coordination of this N atom along with the COO and lactamic CO groups. The water-containing complexes present a broad diffuse band of medium intensity in the 3450–3400 cm^{-1} region which may be assigned to the OH stretching vibration for the lattice water.

^{1}H NMR Studies

In the ^{1}H NMR spectrum of cephalexin single peaks attributed to methyl and COOH groups appeared at 1.95 and 10.1 ppm respectively. Three groups of double peaks given by CO–CH and N–CH on the β-lactam ring and NH appeared at 4.90, 5.45 and 9.01 ppm, respectively. One group of four resonance signals consistent with an AB system attributed to S–CH_2 on the dihydrothiazine ring was observed in the 3.15–3.45 ppm region with coupling constant 17.2 Hz for J_{AB}. Furthermore, coupling between NH_2 and the adjacent CH could not be distinguished and a broad single signal due to NH_2 protons was observed at 4.97 ppm. A multiplet in the range 6.72–8.42 ppm due to phenyl protons was also present. Comparison of the ^{1}H NMR spectrum of cephalexin with those of the diamagnetic complexes, shows that there is a downfield shift in the frequency of amino protons, confirming coordination of this group to the metal ions. The absence of the signal assigned to the COOH proton of cephalexin confirms deprotonation and suggests the formation of a metal–COO bond. Owing to their extremely low solubility it was not possible to record satisfactory ^{13}C NMR spectra for the diamagnetic complexes.

Electronic Spectra

The UV-vis spectra of cephalexin and its complexes in DMSO have three absorption maxima at 260, 280 and 300 nm which have their origin in the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions within the organic ligand. The manganese(II), nickel(II), copper(II), cadmium(II) and mercury(II) complexes show a very strong absorption in the 355–370 nm range, presumably due to charge-transfer transitions. Furthermore, the visible spectrum of the cobalt(II) complex shows two bands at 390 and 530 nm which are assigned to the spin-forbidden and $^4A_2 \rightarrow {}^4T_1(P)$ transitions, respectively, for the Co(II) ion in

tetrahedral complexes [16–18]; however, the presence of a six-coordinate structure cannot be ruled out. The nickel(II) complex also has one absorption band at 630 nm attributable to a d–d transition. The copper(II) complex exhibits two absorption bands at 420 and 670 nm; the low energy band at 670 nm corresponds to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition in distorted tetrahedral or square-planar geometry [16–18], although octahedral or tetragonal geometry cannot be ruled out. The high-energy band at 420 nm is assigned to a charge-transfer transition, metal→ligand or vice versa.

Magnetic Measurements

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants. The manganese(II) complex has a magnetic moment of 5.6 BM as predicted for a high-spin d^5 system with five unpaired electrons. The magnetic moment of the cobalt(II) complex is 4.1 BM while the expected value for tetrahedral complexes is 4.4–4.8 BM. The low value of the magnetic moment of the complex would point to a not purely tetrahedral geometry of Co(II) and a tendency to be distorted [19]. The magnetic moment of the nickel(II) complex is 3.16 BM, which confirms the presence of two unpaired electrons per Ni(II) ion, and indicates a tetrahedral structure for the complex [20]. The copper(II) complex has a magnetic moment of 2.2 BM at room temperature, which falls in the range associated with d^9 systems with one unpaired electron. The rather high value of the magnetic moment for the freshly prepared copper(II) complex could be explained, in part, by the fact that spin–orbital coupling in the ion can mix the ground state representing no orbital momentum with higher levels of identical multiplicity, resulting in a small orbital contribution [21]; however, the presence of impurities cannot be discounted.

The room temperature EPR spectrum of [Mn(cepha)Cl]·$3H_2O$ showed a single broad signal with poor resolution of the hyperfine structure. The calculated g_{av} value of 1.98 agrees well with the results obtained previously for high-spin manganese(II) complexes. The powder EPR spectrum of [Cu(cepha)Cl]$3H_2O$, displays two g values, $g_{\parallel} = 2.18$ and $g_{\perp} = 2.05$ with no hyperfine structure due to nuclear spins. The trend $g_{\parallel} > g_{\perp} > g_e$ (free ion value, 2.0023) and the axial symmetry value, $G = 3.6$ indicate that the unpaired electron is present in the $d_{x^3-y^3}$ orbital having $^2B_{1g}$ as a ground-state term [22].

Structure of the Complexes

The coordination chemistry of some β-lactamic antibiotics with transition and d^{10} metal ions has been reported [8–11,23]. The cephalexinate ion has several potential donor atoms but, due to steric constraints, the ligand can provide a maximum of three donor atoms to any one metal center. From the IR and NMR data it appears that there are three types of complexes, (a) for [Mn(cepha)Cl] [M = Ni(II), Cu(II), Zn(II)] the cephalexin coordination occurs through the carboxylate, amide carbonyl and NH_2 groups, (b) for [M(cepha)Cl] [M = Mn(II), Co(II)] coordination occurs through the carboxylate, the lactam carbonyl and NH_2 groups along with a weak interaction from the amide C=O, and (c) in the Cd(II) and Hg(II) complexes, where the COO group clearly does not bond to the metal because there is no shift in the IR frequency, the S may act as a binding site in addition to the amide C=O and NH_2 groups. It is feasible that the ions are tetra coordinate with one molecule of cephalexin

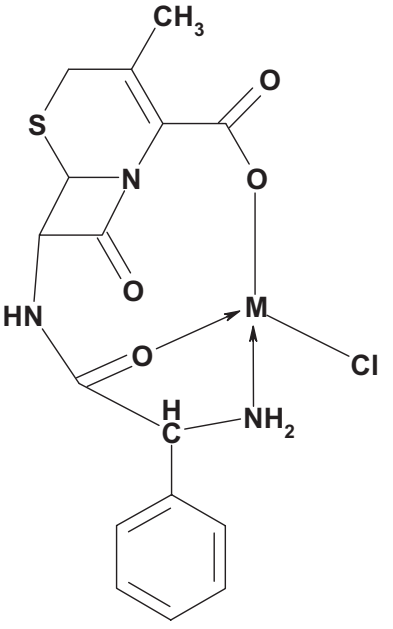

FIGURE 2 Tentative structure of the cephalexin metal complexes [M(cepha)Cl], [M = Ni(II), Cu(II), Zn(II)].

and chloride anion at the vertices of a tetrahedron. Despite the crystalline nature of the products none proved suitable for X-ray structure determination.

When the energy was minimized by using MM2 calculations, reasonable M–O and M–N bond lengths were obtained, suggesting that coordination of COO, amide or lactam C═O and NH_2 moieties to the metal is possible giving nearly tetrahedral bond angles around the metal. The suggested structure for some cephalexin metal complexes, obtained with Chemdraw and Gaussview molecular modelling programs are shown in Fig. 2.

Biological Activity Test

The susceptibility of certain strains of bacterium towards cephalexin and its metal complexes was judged by measuring the inhibition diameter. As assessed by color, the complexes remain intact during biological testing. The antibiotic and the complexes presented bactericide diameters larger than 13 mm, intermediately sensitive [24,25]. The results are shown in Table III.

The cobalt(II) and nickel(II) complexes show no activity at all against the bacteria. Among the paramagnetic complexes the copper(II) complex was found to have higher activity than that of cephalexin against the bacteria strains studied under the test conditions, showing that it has good activity as a bactericide. No compound showed activity against *P. aeruginosa* except the mercury(II) complex, which also showed the highest bactericidal activity. The zinc(II), cadmium(II) and copper(II) complexes showed no activity against *K. pneumoniae*.

Unfortunately there is only one pair of complexes with lactamic CO coordination [Mn(cepha)Cl] and [Co(cepha)Cl], so it is hard to draw conclusions about the

TABLE III Antibacterial activity of the cephalexin metal complexes

	Zone of inhibition (mm)				
Compound	*S.A.*	*E.C.*	*K.P.*	*S.E.*	*P.M.*
Cephalexin	22	10	12	10	13
[Mn(cepha)Cl]·$3H_2O$	14	0	0	0	0
[Co(cepha)Cl]·$3H_2O$	0	0	0	0	0
[Ni(cepha)Cl]·$4H_2O$	0	0	0	0	0
[Cu(cepha)Cl]·$3H_2O$	24	13	0	12	17
[Zn(cepha)Cl]·$3H_2O$	25	15	0	12	8
[Cd(cepha)Cl]	22	12	0	13	13
[Hg(cepha)Cl]	38	29	25	28	22

[a]S.A. *Staphylococcus aureus* ATCC 25923; E.C. *Escherichia coli* 35939; K.P. *Klebsiella pneumoniae* 556; S.E. *Salmonella enteriditis* ATCC 497; P.M. *Proteus mirabilis* ATCC 35659. All doses were 400 µg/disc. Estimated error ± 1 mm.

importance of structural differences. Nevertheless, the comparison of biological activities (Table III) does not support the idea that the presence of lactamic CO bonding may enhance the antimicrobial activity of the cephalexin metal complexes.

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