REVIEW

Department of Pharmaceutics and Industrial Pharmacy[1], Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt, Institute of Applied Dermatopharmacy[2], Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany, Department of Pharmaceutics and Industrial Pharmacy[3], Faculty of Pharmacy, Al-Azhar University, Nassr City, Cairo, Egypt, Institute of Pharmacy[4], Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

Characterization and activity of cephalosporin metal complexes

S. H. Auda[1], Y. Mrestani[2], M. I. Fetouh[3], R. H. H. Neubert[4]

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Prof. Dr. Reinhard H. H. Neubert, Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, D-06120 Halle (Saale), Germany
reinhard.neubert@pharmazie.uni-halle.de

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Semi-synthetic cephalosporin antibiotics have structures similar to that of penicillins, and both groups of compounds are characterized by similar properties and determined by the same methods. Most antibiotics, including cephalosporins and their decomposition products, contain electron donor groups that can bind naturally occurring metal ions *in vivo*. Cephalosporin antibiotics exhibit a change in their toxicological properties and biological performance when they were tested as metal complexes. The proposed reason for such a behavior is the capability of chelate binding of the cephalosporins to the metals. In an attempt to understand the coordination mode of metals with cephalosporins, different spectroscopic techniques such as IR, UV-visible, NMR spectroscopy and voltammetric measurements were carried out to elucidate the structure of the metal-cephalosporin complexes. Synthesis, characterization and biological screening of the cephalosporins and of the cephalosporin-metal complexes are discussed in this review. However, little information is available on the influence of the metal ions on the pharmacokinetics of the cephalosporin derivatives.

1. Introduction

The cephalosporins, or cephem antibiotics, are semisynthetic antibacterials derived from cephalosporin C, a natural antibiotic produced by the mould *Cephalosporium acremonium* (Parfitt 2002). Cephalosporin antibiotics are very closely related to penicillins, and mechanism of action, mechanism of resistance and some other properties are identical to those of penicillins (Anacona and Rodriguez 2005). Both cephalosporins and penicillins belong to the β-lactam antibiotics (Bergan 1987). Cephalosporins are classified into 4 generations according to their spectrum of activity (Parfitt 2002). The first generation cephalosporins are very active against gram-positive cocci. They have limited activity against gram-negative bacteria (Kalman and Barriere 1990). The second generation cephalosporins exhibit an increased activity against gram negative microorganisms but are much less active than the third and fourth generation agents (Anacona and Rodriguez 2005; Anacona and Gil 2005).

Beta-lactam antibiotics interact with metal ions and this interaction is of a complex nature (Van Krempin et al. 1988). Generally speaking, many drugs possess modified toxicological and pharmacological properties when they are in the form of metal complexes. Due to the spread of resistance to chloroquine (antimalarial drug), there is a need for new effective antiparasitic agents. For this purpose gold and ruthenium complexes of chloroquine and clotrimazole have been studied. It was reported that some chloroquine complexes being useful even in chloroquine-resistant cases (Navarro et al. 1997, 2001; Sanchez-Delgado et al. 1993, 1996). The therapeutic effects of oxovanadium complexes of thiourea and vanadium substituted polyoxotungstates were recently studied (Cruz et al. 2003; Shigeta et al. 2003). The results showed that these complexes have potent anti-HIV properties toward infected immortalized T-cells.

On the other hand, many complex forming metals are toxicologically and pharmacologically relevant. The most widely investigated metal in this respect is copper (II) that is used for the treatment of diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers (Williams 1971; Sorenson 1976; Brown et al. 1980; Sorenson and Nraign 1981). Cisplatin is widely used in cancer therapy (Prestayko et al. 1980). The success of the clinical administration of this platinum complex has stimulated considerable interest in new metal complexes as modern therapeutics, diagnostic and radiopharmaceutical agents, for example, silver(I) complexes commonly used as anti-microbial agents, bismuth(III) complexes for anti-ulcer treatment, gold(I) complexes as anti-arthritic agents, gadolinium(III), manganese(II) and iron(III) complexes as magnetic resonance imaging (MRI) contrast agents, tech-

netium (99Tc) and scandium (47Sc) as radiopharmaceutical agents (Raymond et al. 2007). The effect of metal ions on drug activity was confirmed in several other studies (Fazakerley and Jackson 1975; Jackson et al. 1981; Sorenson 1982; Nagar and Mohan 1991; Taniguchi et al. 1991). Many cephalosporin antibiotics have been studied as metal complexes such as ceftriaxone (Anacona and Rodriguez 2005; Williams 1971), cefoxitine (Anacona and Gil 2005), cefixime (Anacona and Gil 2006), cephradine (Anacona and Acosta 2005), cephalothine (Anacona and Jose 2003; Chohan and Supuran 2005), cefalaxin (Iqbal et al. 1999; Abo El-Maali 2005; Aly et al. 2005; Anaconaa and Rodriguez 2004), cefotaxime (Abo El-Maali et al. 2005), cephamandole and cephapirin (Abo El-Maali et al. 2005), ceftazidime and ceforuxime (Abo El-Maali et al. 2005; Anaconaa and Rodriguez 2004), cefoperazone (Abdel Gaber et al. 2000), cefadroxil and cefaloridine (Abo El-Maali et al. 2005; Abdel Gaber et al. 2000; Chakrawarti et al. 2000), cefazolin (Anacona and Alvarez 2002), cefdinir (Shuhei et al. 1994; Kato et al. 2002; Marie et al. 1995), cefaclor (Chohan 1991; Dimitrovska et al. 1996).

Although the influence of metal ions on the pharmacokinetics and the bioavailability of different antibiotics, especially tetracycline, is well known (Healy et al. 1997; Neuvonen 1976; Gugler and Allgayer 1990; Mapp and McCarthy 1976; Dearborn et al. 1957; Kaplan et al. 1957; Hubel et al. 1958), there is a little information concerning the influence of metal ions on the pharmacokinetics of cephalosporins. So that there is a need to study this influence in the future.

2. Complex preparation

The cephalosporin-metal complexes were usually prepared by mixing the cephalosporin and metal salts in a ratio of 1 : 1 mmol (Zayed and Abdallah 2004) or 2 : 1 mmol (Chohan 1991; Anacona and Gil 2006) dissolved in methanol (Anacona and Rodriguez 2005; Anacona and Gil 2005) or in distilled water (Iqbal et al. 1999; Yszczek 2004). The reaction mixture was then stirred at room temperature for 30 min (Iqbal et al. 1999), 1 h (Zayed and Abdallah 2004) or 8 h and then left to stand overnight (Anacona and Gil 2005). The precipitated complexes were filtered off, washed and dried under reduced pressure at room temperature. The synthesis procedures were carried out under a N_2 atmosphere (Anacona and Rodriguez 2005) or under the normal conditions (Iqbal et al. 1999). The complexes are colored and are insoluble in water and other common organic solvents such as ethanol, benzene, acetone, acetonitrile, diethylether, but they are soluble in *N*,*N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) (Anacona and Acosta 2005; Iqbal et al. 1999).

3. Characterization of the cephalosporin metal complexes

3.1. Analytical methods

3.1.1. Elemental analysis

C, H, N and S were analyzed using microanalytical instruments and the metal content was estimated spectrophotometrically on an atomic absorption spectrometer (Yszczek 2004; Anacona and Gil 2006). Metal contents of the complexes might be determined by titration against standard ethylenediaminetetraacetic acid (EDTA) after complete digestion in aqua regia in a Kjeldahl flask several times and adjusting the pH of the solution to a suitable one (Zayed and Abdallah 2004). The elemental and metal analyses indicate the formation of 1 : 1 metal-to-ligand stoichiometry (Yszczek 2004; Anacona and Acosta 2005). Other authors have pointed to the formation of 1 : 2 metal-to-ligand stoichiometry (Iqbal et al. 1999).

3.1.2. Voltammetric measurements

Both osteryoung square wave voltammetry and cyclic voltammetry have been used to investigate and confirm the possible complexation reaction that occurs between the various cephalosporin antibiotics and Cd(II), Cu(II) and Zn(II) (Abo El-Maali et al. 2005). The studied cephalosporins were cefalexin, cefapirin, cefamandole, cefuroxime, cefotaxime and ceftazidime. Voltammetric measurements clearly confirm complex formation between the cephalosporins and the used metal ions. Such phenomena could be used for the determination of either the antibiotic or the metal ion using adsorptive stripping voltammetry.

3.1.3. Thermo-gravimetric analyses

Thermo-gravimetric diagrams of the metal complexes indicate endothermic decompositions in the 80–110 °C range due to the loss of molecules of water of hydration, and also reveal that the complexes are stable with no coordinated water and solvent molecules (Anacona and Rodriguez 2005) or in the range of 120–300 °C indicating molecules of water of coordination (Zayed and Abdallah 2004). The fragments of the thermal decomposition were different according to the type of the ligand, the metal, and the used solvent.

3.1.4. IR spectroscopy

Evidence for complex formation was obtained by comparing the most characteristic infrared spectral bands of the free cephalosporins and their complexes. In general, the infrared spectra of cephalosporin antibiotics exhibit the characteristic band arising from stretching vibrations of the carbonyl group of the β-lactam ring. Another characteristic group from cephalosporin ligands is an amide carbonyl group from the side chain. Cephalosporins have a zwitterionic character, so, their spectra of free ligand show bands of antisymmetric (νas) and symmetric (νs) vibrations of the carboxylate group. Disappearance of one or more of such bands may indicate participation in metal coordination. New bands due to M–O or M–N vibration might be shown on complexation giving a confirmation for complex formation.

In a study of cobalt, nickel, copper and zinc complexes of cefadroxil (Yszczek 2004), free cefadroxil exhibited a band at 3505 cm^{-1} which attributed to the stretching vibrations of the 'free' OH group of hydroxyphenyl from cefadroxil. This band disappeared in the spectra of metal complexes, indicating the ionization of the hydroxyl group. The band of NH^{3+} group at 2600 cm^{-1} in cefadroxil spectrum disappeared in the spectra of metal complexes. A characteristic band, arising from the stretching vibrations of the carbonyl group of the β-lactam ring, appeared at 1758 cm^{-1}. This band appeared also in all studied complexes almost at the same wavenumber. This may suggest that the carbonyl oxygen atom from β-lactam ring is not involved in metal binding. The IR spectra of cefadroxil reveal a band at 1686 cm^{-1} due to the stretching vibrations of the amide group. This band vanished in metal complexes suggesting the coordination of metals

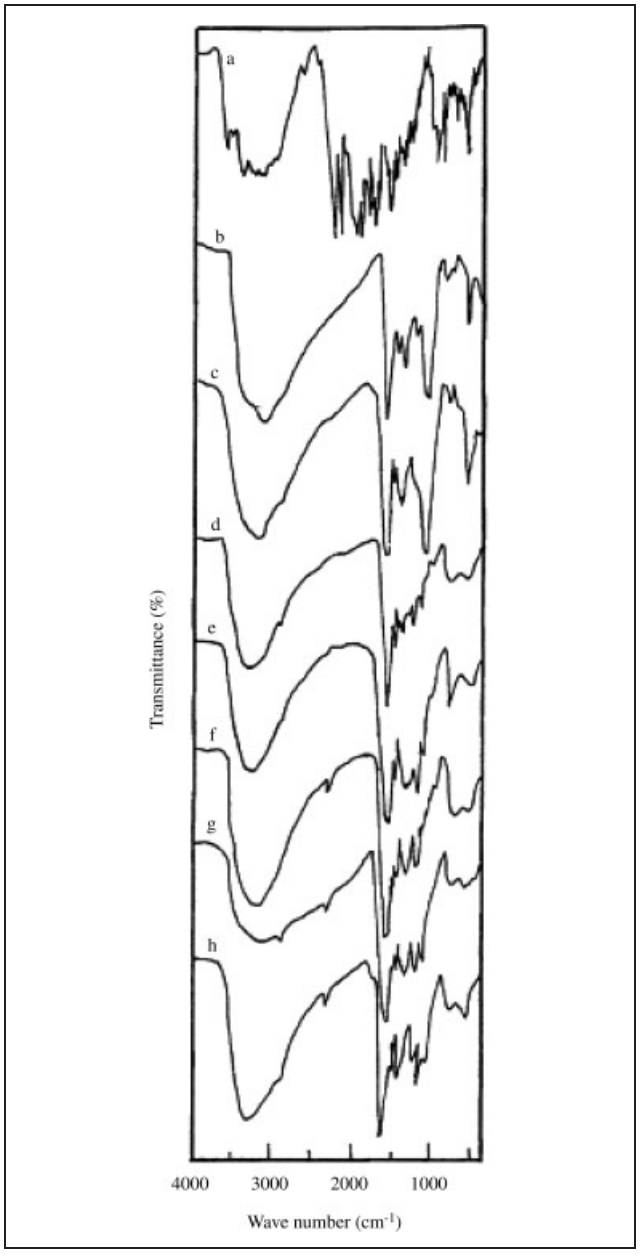

Fig. 1: IR spectra of: (a) cefadroxil; and its complexes with (b) Fe^{II}, (c) Fe^{III}, (d) Co^{II}, (e) Co^{III}, (f) Ni^{II}, (g) Cu^{II}, and (h) Zn^{II} (Zayed and Abdallah 2004)

through amide carbonyl group. The bands of antisymmetric (νas) and symmetric (νs) vibrations of carboxylate group appeared at 1563 and 1399 cm^{-1}, respectively. The bands assigned to νasCOO overlapped with II amide band (βNH, VCN). In spectra of metal complexes, νasCOO band is shifted towards higher wavenumbers, contrary to the νsymCOO bands which shift towards lower wavenumbers. This fact suggests interaction between metal ions and the carboxylate group of cefadroxil. The same author investigated the interaction of cefadroxil with cadmium. He found that the cadmium complex has a different mode of coordination. The principle difference in comparison to remaining spectra is the presence of a stretching vibration of carbonyl group at 1664 cm^{-1}. The antisymmetric and symmetric stretching vibrations of the carboxylate group are shifted towards frequencies 1568 and 1420 cm^{-1}, respectively. This may point to a coordination of the carboxylate group only while the carbonyl group from the amide group bond is not engaged in coordination.

Other studies on iron, cobalt, nickel, copper, and zinc complexes of cefadroxil were reported (Zayed and Abdallah 2004). Based on the comparison between the IR spectra of the free ligand and its complexes, the authors noticed that the coordination between the metal and the ligand occurred at three positions. The first is the β-lactam and thiazole ring nitrogen atom, since the IR spectra of cefadroxil exhibited a band at 1354 cm^{-1} due to ν(C−N) of this nitrogen atom. This band is absent in all studied complexes indicating participation of the β-lactam and the thiazole ring nitrogen atom in the bonding. The second position is oxygen from carbonyl group of the β-lactam ring. This suggestion was obtained from the disappearance of ν(CO) band of the β-lactam ring in all complexes while it appeared at 1759 cm^{-1} in the free ligand. The last one is the carboxylate group. This was observed from the significant shift in the bands of νsym(COO) at 1234 cm^{-1} which shifted to higher frequencies (about 30–50 cm^{-1}) in the investigated complexes. The IR spectra of this study are presented in Fig. 1.

Abdul Baqi (2004) investigated the IR spectra of Cu(II) and Zn(II) complexes of cefalexin. He observed a significant shift for νsymCOO from 1765 to 1760 cm^{-1}. A new absorption band appeared around 3300 cm^{-1} due to ν(MO) in the complexes (absent in cefalexin). This suggests that the carboxylic group is involved in the interactions with metal ions. A shift of the νas(COO) and δ(CO) was found. A new band, ν(MN), appeared in the spectra of the complexes indicating coordination of the ligand through N. Based on the IR spectra and other investigations, such as elemental and metal analysis, the author proposed the structure of Cu(II) and Zn(II)-cefalexin complex as shown in Fig. 2 B with a stiochiometry of 2 : 1 (L : M).

The IR spectra of cefradin and its metal complexes were assigned (Afzal 1998). It was found that metal ions coordinate to the carboxylate group and to the β-lactam carbonyl oxygen. This is indicated by frequency shifting of β-lactam C=O and O as well as symmetric and asymmetric

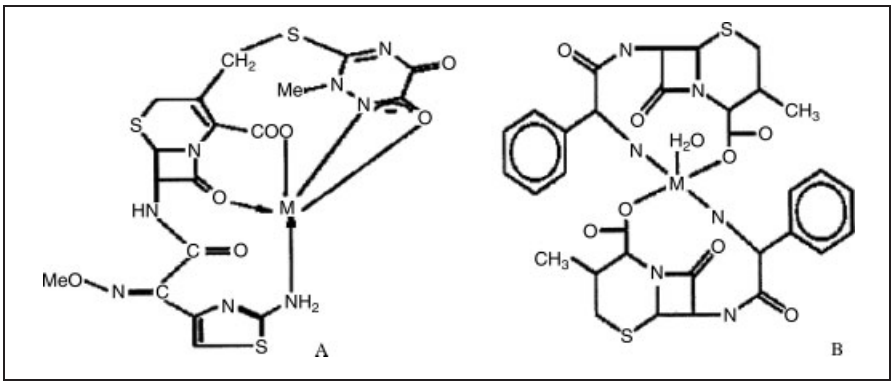

Fig. 2:
Proposed structure of A, ceftriaxone metal complexes (Anacona and Rodriguez 2005); B, cefalexin metal complexes (Abdul Baqi 2004)

Table 1: Main vibrational frequencies, υ (cm^{-1}) of cefalothin metal complexes

Compound	υ(C=O) lact.	υ(C=O) amide	υ(COO) asym	υ(COO) sym	Δυ (COO)
Cefalothin Na	1730	1650	1620	1400	220
[Mn(Cefalo)Cl]	1790	1660	1615	1380	235
[Co(Cefalo)Cl]	1790	1660	1610	1390	220
[Ni(Cefalo)Cl]	1780	1640	1610	1380	230

(Anacona and Jose 2003)

Table 2: Main vibrational frequencies, υ (cm^{-1}) of cefazolin metal complexes

Compound	υ(C=O) lact.	υ(C=O) amide	υ(COO) asym	υ(COO) sym	Δυ (COO)
Cefazolin	1745	1650	1585	1380	205
[Mn(Cefaz)Cl]	1760	1680	1595	1390	205
[Co(Cefaz)Cl]	1760	1665	1590	1370	220
[Ni(Cefaz)Cl]	1750	1680	1600	1380	220

(Anacona and Alvarez 2002)

COO-bands while most of the bands of the ligand remain unchanged on complexation.

The IR spectra of Zn(II) complexes of cefalexin, cefapirin, cefamandole, cefuroxime, cefotaxime and ceftazidime were reported (Aly et al. 2004). Based on the comparison of the free ligand's spectra with that of metal complexes, the authors suggested that the metal coordinated to the free ligands through many sites. Shifting of νasCOO of the carboxylate group, ν(NH), υ(NC) and υ(C−S−C) gave evidence for the involvement of these groups in the coordination. On the other hand, Zn(II) ions form a complex of ratio 2 : 1 (M : L) with ceftazidime with molecular formula of $[Zn_2Cl_2(CFZ)H_2O] \cdot 3\,H_2O$. In this complex two Zn(II) ions are coordinated to the ligand, one metal ion is attached to the carboxylate group and the adjacent nitrogen atom; υas$(CO_2)-$, υs$(CO_2)-$ and υ(NC) are similar to the above frequency ranges. The other metal ion coordinates to the nitrogen atom of the group −C=N−O (which is manifested by the shift of υ(C=N) band from 1690 cm^{-1} in the ligand to 1655 cm^{-1} in the complex.

Anacona's group investigated the IR spectra of the free ligand and metal complexes of cefixime (Anacona and Gil 2006), ceftriaxone (Anacona and Rodriguez 2005), cephalothin (Anacona and Jose 2003), cefazolin (Anacona and Alvarez 2002) and cefalexin (Anaconaa and Rodriguez 2004). They found significant shifts in some main groups of cephalosporins on complexation indicating the coordination of the ligands with the used metal ions. The group reported a coordination of metal ions to the carboxylate groups in all studied complexes. In some cephalosporins, significant shifts in the stretching vibrations of lactam carbonyl group were observed indicating that the metal ions participate in the coordination through the oxygen atom from the lactam carbonyl group as in the metal complexes of cefixime (Anacona and Gil 2006), ceftriaxone (Anacona and Rodriguez 2005) and cephalothin (Anacona and Jose 2003) as shown in Table 1 and Fig. 2 A. In the others, metals were coordinated through the oxygen atom from the amide carbonyl group not from the lactam carbonyl group, where the shifting of the peaks of the lactam carbonyl group was not significant as in cefazolin (Anacona and Alvarez 2002) and cefalexin (Anaconaa and Rodriguez 2004) metal complexes as depicted in Table 2.

3.1.5. ^1H NMR studies

^1H NMR spectra of cefradine and its Co(II), Ni(II), Cu(II) and Zn(II)) complexes were investigated (Anacona and Faricar 2005). The spectrum of cefradine showed three groups of doublets due to CO−CH and N−CH on the β-lactam ring and NH appeared at 4.95, 5.48 and 9.03 ppm, respectively. A group of four resonance signals consistent with an AB system attributed to S−CH_2 on the dihydrothiazine ring was observed in the 3.18–3.45 ppm region with a coupling constant 17.2 Hz for J_{AB}. Otherwise, coupling between NH_2 and the adjacent CH could not be distinguished and a broad single signal due to NH_2 protons appeared at 3.84 ppm. A multiplet in the range 5.60–5.67 ppm attributed to 1,4-dihydrobenzene protons was also presented. On complexation, a downfield shift in the frequency of amino protons (3.96 ppm) was observed, confirming coordination of this group to the metal ion. It was found that all complexes have diamagnetic nature.

Furthermore, ^1H NMR spectra of Co(II), Ni(II), Pd(II) complexes with cefalothin were reported (Anacona and Jose 2003). The results presented that the complexes are paramagnetic and the peaks of protons of different groups in their ^1H NMR spectra were very broad and could not be easily distinguished.

Cefoxitine-Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II)] complexes were studied (Anacona and Gil 2005). With the exception of the Zn(II) and Cd(II) complexes the experimental results revealed that the complexes are paramagnetic and the peaks of the protons of different groups in their ^1H NMR spectra were very broad, so they are difficult to distinguish.

3.1.6. UV and Vis spectroscopy

The UV-spectrum of aqueous solution of cefaclor (Dimitrovska et al. 1996) exhibited two bands with a maximum absorption at 206 nm and 264 nm. The chemical interaction of the Cu^{2+} with cefaclor was determined by UV spectroscopy following the spectral changes in the range from 282 to 400 nm. The results indicated that in acidic medium, Cu^{2+} interacts with cefaclor to give the $Cu(CEF)^+$ complex which easily dissociates to give Cu(OH)(CEF). The hydroxo complex dominates at $pH > 7.5$. This complex species at $pH = 8$ shows absorption band in the range from 282 to 400 nm with a maximum at 300 nm as shown in Fig. 2.

The UV spectra of most investigated cephalosporins, ceftriaxone, cefoxitine, cefixime, cefradine, cefalothine, cefalaxin, ceforuxime and cefazolin, and their complexes in DMSO, tested by Anacona group, (Anacona and Alvarez

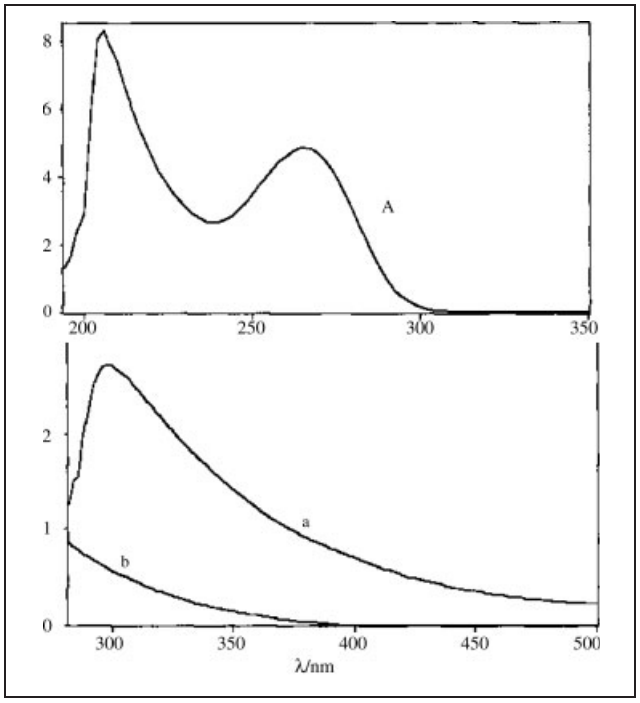

Fig. 3: UV-spectra of A: cefaclor (5×10^{-5} M), a: cefaclor (5×10^{-4} M) and Cu(II) perchlorate (1×10^{-4} M) reaction mixture and b: Cu(II) perchlorate (1×10^{-4} M), at pH 8 (Dimitrovska et al 1996)

2002; Anacona and Rodriguez 2004; Anacona and Gil 2006; Anacona and Rodriguez 2005) exhibited differences in the absorption when compared with the free ligands. Two or three absorption maxima ranging from 250 to 300 nm (approx.) were observed in the free ligands and their metal complexes indicating a $\pi \rightarrow \pi^*$ transition due to molecular orbital energy levels originating in the N−C−S moiety. Shoulders, corresponding to an intraligand $\pi \rightarrow \pi^*$ transitions attributed to a transition between energy levels originating in the S−C−S moiety, appeared. Bands at the 340 to more than 500 nm region (Vis range) are caused by the intraligand transition of the $n \rightarrow \pi^*$ type in accordance with the literature data for transitions between levels due to sulphur atoms (Franchini et al. 1985; Criado et al. 1990; Castillo et al. 1986). The complexes showed weak absorptions, probably due to spin–orbit forbidden transitions. Some complexes exhibited d–d electronic transition bands.

UV spectra of cefalexin, cefapirin, cefamandole, cefuroxime, cefotaxime and ceftazidime Zn(II) complexes were recorded in DMF (Aly et al. 2004). The complexes represented an intense band in the region 37.037–36.036 cm^{-1}, which is attributed to a π–π^* transition of the antibiotics moiety. A shoulder in the region 33.112–33.444 cm^{-1} was recorded, indicating the intraligand charge transfer transition. Due to the d^{10}-configuration of Zn(II), no d–d transition could be observed and the stereochemistry around Zn(II) in its complexes cannot be determined from ultraviolet and visible spectra.

3.2. Biological and biopharmaceutical methods

3.2.1. In vitro release study

The release of cefdinir from a cellulose membrane was measured by rotary basket method in the presence and absence of iron(III) citrate and calcium chloride. The results showed that the release profile of cefdinir from the cellulose membrane in the presence of iron ions was slower than that in the absence of metal ions. However, no difference was observed in drug release between the presence and absence of calcium ions. The results suggest that although the formation of chelate complexes occurred between iron ions and cefdinir, but not between calcium ions and cefdinir (Kato et al. 2002).

Table 3: Minimum inhibitory concentrations (μg/mL) of the Cu(II) and Zn(II) complexes of cefalexin against bacteria

Std. Culture	Cefalexin sodium (MIC) μg/mL	Cefalexin Cu complex (MIC) μg/mL	Cefalexin Zn complex (MIC) μg/mL
S. aureus	10.0	2.5	3.5
E. coli	100.0	7.0	10.0
P. aeruginosa	Resistant	Resistant	Resistant
K. pneumoniae	Resistant	150.0	10000

(Afzal 1998)

3.2.2. Antibacterial activity tests

In vitro antibacterial activities of the investigated cephalosporins and their complexes were tested using the paper disc diffusion method. Most of cephalosporin metal ion complexes gave bactericide diameters larger than 10 mm indicating that they are effective (Shungu et al. 1983). Anti-bacterial activity of cephalosporin metal ion complexes depends mainly on the type of cephalosporin used, the type of metal and the type of microorganism.

The anti-bacterial activity tests of Cu(II)-cefalexin and Zn(II)-cefalexin complexes demonstrate that the complexation of cefalexin with these metals enhances its activity significantly (Afzal 1998). The results of minimum inhibitory concentration study (Table 3) revealed that copper(II)-cefalexin was four times more active against *Staphylococcus aureus* than free cefalexin sodium and about fourteen times more active against *Escherichia coli* than cefalexin sodium. At concentrations up to 150 μg/mL, the copper complex exhibited a remarkable antibacterial activity against *Klebsiella pneumoniae*, in contrast cefalexin sodium was not active. Cefalexin sodium and copper(II)-cefalexin up to 10 mg/mL were not active against *Pseudomonas aeruginosa*. Zinc(II)-cefalexin seemed to be three times more active against *S. aureus* than cefalexin sodium and ten times more active against *E. coli* than cefalexin sodium. The zinc complex was found to be active at 10 mg/mL against *K. pneumoniae* while cefalexin sodium had no effect. At 10 mg/mL, the zinc complex and cefalexin sodium were not active against *P. aeruginosa*.

Table 4: Antibacterial activity data of cefaclor metal complexes

Cefaclor and its complexes	a	b	c	d
Cefaclor	+	+ +	+ +	+
Cefaclor-Co(II) complex	+ +	+ + +	+ + +	+ +
Cefaclor-Ni(II) complex	+ + +	+ + +	+ + +	+ + +

a = Streptococcus pyogenes, b = S. pneumoniae, c = S. aureus, d = Escherichia coli. Inhibition zone measured in diameter +, 6–10 mm; ++, 10–16 mm; +++, 16–10 mm (Chohan 1991)

Table 5: Comparison between some cephalosporins and their copper (II) complexes studied by Anacona's group (inhibition zone measured in mm of diameter)

Compound	*S. aureus*	*E. Coli*	Reference
Cefalothin	35	26	(Anacona and Jose 2003)
Cefalothin-Cu(II) complex	36	39	
Cefazolin	27	23	(Anacona and Alvarez 2002)
Cefazolin-Cu(II) complex	27	25	
Ceftriaxone	42	40	(Anacona and Rodriguez 2005)
Ceftriaxone-Cu(II) complex	36	35	
Cefalexin	22	10	(Anaconaa and Rodriguez 2004)
Cefalexin-Cu(II) complex	24	13	
Cefoxitin	40	14	(Anacona and Gil 2005)
Cefoxitin-Cu(II) complex	19	10	
Cefradine	38	0	(Anacona and Acosta 2005)
Cefradine-Cu(II) complex	21	10	

Similar results were obtained by Chohan (1991) who studied the antibacterial activity of cefaclor and its Co(II) and Ni(II) complexes against the bacterial species *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *S. aureus* and *E. coli* in comparison with pure, uncomplexed cefaclor. The results indicated that the cefaclor metal complexes were more active against all the tested bacterial species. The results of this study are depicted in Table 4.

Antibacterial screening of many cephalosporins and their metal complexes was carried out by Anacona's group. The used bacterial strains in their studies were Gram positive (+) *S. aureus* and some types of Gram negative strains as *Proteus mirabilis*, *Shigella sonnei*, *Salmonella enteritidis*, *P. aeruginosa* and *E. coli*.

As an example, we will take here the anti-bacterial effect of copper (II) complex of cefalothin (Anacona and Jose 2003), cefazolin (Anacona and Alvarez 2002), ceftriaxone (Anacona and Rodriguez 2005), cefalexin (Anaconaa and Rodriguez 2004), cefoxitin (Anacona and Gil 2005) and cefradine (Anacona and Faricar 2005) on Gram positive (+) *S. aureus* and Gram negative (−) *E. coli* in comparison with their free ligands. The doses were 400 μg/disc in the cases of cefalexin, cefalothin, ceftriaxon and cefoxitin complexes, 100 μg/disc in the case of cefazolin and 800 μg/disc in cefradin complexes. Selected results are presented in Table 5. These results indicate that while some of Cu-complexes had more anti-bacterial activity against the two organisms or one of them than the uncomplexed cephalosporins, other complexes exhibited less activity than the free ligands.

3.2.3. Anti-inflammatory activity

The anti-inflammatory activity of cephalexin-copper and zinc complexes was studied using Kaolin paw oedema that was induced in male Wistar rats of weight 100–110 g (Iqbal et al. 1999). Inhibition of oedema was evaluated by comparing the swelling obtained in the treated animals with that in controls and was expressed as percentage of inhibition. The results of the paw oedema test are presented in Table 6. The copper complex was found to be active while the zinc complex and cefalexin sodium had no significant activity. This confirmed the previously reported role of copper in inflammation (Brown et al. 1979; Iqbal 1982).

Table 6: Anti-inflammatory effect of cefalexin complexes in kaolin paw oedema and LD_{50} values

Complex	Dose (mg kg^{-1})	Inhibition of oedema (%)	LD_{50}[a] (g kg^{-1})
Cu(II)-cefalexin complex	50	35*	8.12
Zn(II)-cefalexin complex	50	5*	9.07
Cefalexin Na alone	50	2*	5.25

[a] Quantity resulting in the death of half the rats; *$p < 0.05$ compared with control (Iqbal 1999)

3.2.4. Toxicity study

The toxicity of cefalexin and its Cu(II) and Zn(II) complexes was tested (Iqbal et al. 1999). The investigations were performed on albino Wistar male rats, 180–200 g. The complexes under investigation were administered orally in 0.15% agar suspension in a dose of 50 mL kg^{-1} to four groups of ten rats. After treatment, the animals were monitored every hour for several hours and then every day for two weeks. The LD_{50} values (quantities resulting in the death of half the rats) are given in Table 4. Toxicity was reduced (lower LD_{50} values) by complexation.

4. Conclusions and outlook

As demonstrated above, the metal ion complexes of the cephalosporins were characterized by different analytical and biological methods. The characterization methods revealed that the investigated cephalosporins exhibited modification in both solubility and bioactivity when they interacted with the metal ions. Although a lot of information is available on the influence of metal ion complexation on the pharmacokinetic parameters of tetracyclines, there is only little information about the influence of the complexation with metal ions on the pharmacokinetics of cephalosporins. Hence, much work remains to be done. Research work is clearly required in the future (a) to better understand and quantify the influence of metal ions on the pharmacokinetics of cephalosporins; (b) to better understand under which circumstances dynamic models are better suited to predict mass transport limitation, absorption limitation, etc.; (c) to determine the relationship between the concentration and the bioactivity of cephalosporin metal complexes; (d) to study the cephalosporins – metal complex formation in the gastrointestinal tract with external metal sources, for example, with the used multivitamins, powdered milk or with other food products containing metal ions as supplements.

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