BioMetals **12:** 167–172, 1999.

Copper(II) complexes of diclofenac: Spectroscopic studies and DNA strand breakage

Angela Theodorou[1], Mavroudis A. Demertzis[1], Dimitra Kovala-Demertzi[1,*], Efthimia E. Lioliou[2], Anastasia A. Pantazaki[2] & Dimitrios A. Kyriakidis[2,*]
[1]*Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 451 10 Ioannina, Greece*
[2]*Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece; *Author for correspondence (Tel: (0651) 98425; Fax: (0651) 44825; E-mail dkovala@cc.uoi.gr)*

Received 7 October 1998; accepted 20 November 1998

Key words: diclofenac, copper (II) complexes, DNA breakage

Abstract

Copper(II) complexes of diclofenac with interesting anti-inflammatory profiles have been prepared and studied by infrared and electronic spectroscopy. In the solid state and in polar and coordinating solvents, all the complexes are solvated binuclear carboxylato-bridged complexes, $[Cu(L)_2(S)]_2$, where L is monodeprotonated diclofenac and S is the axially bonded solvent. The effect of the copper(II) complexes on the *in vitro* DNA strand breakeage was studied by agarose gel electrophoresis. Relaxation or double stranded scissions of pDNA were observed leading to the formation of linear pDNA. Treatment of pDNA with high concentrations of these compounds caused a disappearance of pDNA. For the parent drug, sodium diclofenac, no effect on the pDNA was observed. This study presents some indications that the binuclear copper(II) complexes, $[Cu(L)_2(S)]_2$, could have some relevance in the treatment of tumor cell lines.

Introduction

Diclofenac sodium {2-[(2,6-dichlorophenyl)amino]phenyl]acetate} is a potent nonsteroidal anti-inflammatory drug (NSAID), therapeutically used in inflammatory and painful diseases of rheumatic and non-rheumatic origin. The anti-inflammatory activity of diclofenac and most of its other pharmacological effects are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators of the inflammatory process (Sallmann 1986; Todd & Sorkin 1988; Moser *et al.* 1990). Diclofenac is a potent inhibitor of cyclo-oxygenase *in vitro* and *in vivo*, thereby decreasing the synthesis of prostaglandins, prostacyclin, and thromboxane products. The structure of diclofenac consists of a phenylacetic acid group, a secondary amino group, and a phenyl ring, both ortho positions of which are occupied by chlorine atoms causing an angle of torsion ($\alpha = 69$) between the two aromatic rings (Sallmann 1986) (Figure 1). Moser *et al.* (1990) studied 36 congeners of diclofenac as inhibitors of cyclooxygenase and the *in vivo* inhibition of rat adjuvant arthritis and found that both activities can be explained by lipophilicity and twisting of the two aromatic rings (angle of twist = 58 to 69). These findings allowed the rationalization of the high activity of diclofenac.

Copper, an essential element, has received considerable attention with regard to its presence in normal blood plasma and serum components. It has been established that copper-dependent enzymes are required for hemoglobin synthesis, growth, keratinization, pigmentation, bone formation, reproduction, fertility, development and function of the central and peripheral nervous systems, cardiac and nerve function, cellular respiration, extracellular connective tissue formation, vascularization, mental and behavioral development, and regulation of monoamine concentrations. Copper-dependent processes appear to be required for modulation of prostaglandin synthesis, lysosomal membrane stabilization, and modulation of histaminic activity. Reports abound in the literature concerning the active

role of copper complexes in the control of inflammatory diseases. Other pharmacological activities of copper complexes and their potential as antiarthritic, antiulcer, anticancer, antidiabetic and antiepileptic drugs have been reported (Sorenson 1982, 1989). In previous works (Sorenson 1989; Sorenson *et al.* 1993; Baquial & Sorenson 1995; Greenaway *et al.* 1998) is described that the copper(II) complex of 3,5-diisopropylsalicylic acid, $[Cu(II)(3,5\text{-}Dips)_2]_2$ exhibits much interest because of its radioprotectant, radiorecovery, anti-inflammatory, antiulcer, antineoplastic, anticarcinogenic, anticonvulsant, antidiabetic, and analgetic activities. The binuclear copper(II) complex of diclofenac, $[Cu(L)_2(H_2O)]_2.2H_2O$ was found to have an anti-inflammatory profile superior to diclofenac when inhibiting inflammations due mainly to the activation of lipooxygenase and/to the complement systems. Also, complexes of diclofenac with metalloelements offered significant protection against lipid peroxidation *in vitro*, acting as antioxidants, while diclofenac does not show this property (Konstandinidou *et al.* 1998).

Most drugs bind to biopolymers, such as thioneins and human serum albumin (HSA), and are distributed to all tissues. HSA is generally involved in the transport and not in the mechanism of action of the drug. HSA binds strongly to small negatively-charged hydrophobic organic ligands, such as diclofenac, at its two subdomains, IIA and IIIA, not near its copper-binding N-terminus, as this was identified by crystallographic studies (Carter & He 1990, 1992).

There is a considerable interest in the DNA binding of metal complexes because of their potential applications as DNA probes and as possible antitumor agents (Billadeau & Morrison 1996). A large number of antitumor drugs interact with DNA and cause scission on the DNA (Ross *et al.* 1978; Waring 1981). Given the central role of DNA in the regulation of biochemical processes, it is not surprising that compounds capable of interacting with it exhibit a wide spectrum of antibacterial, antiprotozoal, antiviral, and antitumor activity. Among antitumor drugs many of them are inhibitors of nucleic acid synthesis and interact with DNA by an intercalative or a non-intercalative way. The effect of $Cu(O_2CMe)_2L_2$ where L was 1-methyl-4,5-diphenylimidazole on the *in vitro* DNA strand breakage was reported (Raptopoulou *et al.* 1998). High concentrations of this Cu(II) complex cause scissions on the supercoiled and the relaxed DNA. In this paper we would like to report the DNA strand breakage produced by Cu(II) complexes of diclofenac. Infrared and UV-visible spectroscopic studies were used to probe the copper-ligand environment for the copper(II) complexes in the solid state and in solution, respectively.

Materials and physical measurements

All chemicals and solvents used were of high purity and purchased from Fluka or Merck. Agarose was purchased from Sigma. Molecular weight markers λ DNA/Hind III came from BRL. Plasmid DNA Ptz18r was isolated from *E. coli* DH5a cells by the STET method (Ausubel *et al.* 1987). Diclofenac was a gift from 'HELP EPE' and was recrystallized twice from ethanol. Infrared spectra were recorded with a Perkin-Elmer 580 spectrometer with samples prepared as KBr pellets in the 4000 to 400 cm^{-1} region and with Nujol mulls supported by polyethylene windows in the 500 to 200 cm^{-1} region. UV-visible spectra were recorded on a JASCO V-570 spectrophotometer UV/VIS/NIR.

All plastics and glassware used in the experiments with nucleic acids were autoclaved for 30 min at 120 °C and 130 kPa. Heat-resistant solutions used were similarly treated, while heat-sensitive reagents were dissolved in autoclaved water.

Preparation of complexes. $[CuL_2(H_2O)]_2 \cdot 2H_2O$, **1**, and $[CuL_2(DMF)]_2$, **5**, were prepared using experimental methods as have been described in the literature (Kovala-Demertzi *et al.* 1997). $[CuL_2(H_2O)]_2.2H_2O$, **1** (0.1 g) was suspended to the appropriate solvent EtOH or MeCOMe (50 ml). The reaction mixture was stirred for 1 h. The resulting powder $[CuL_2(EtOH)]_2$, **2**, or $[CuL_2(MeCOMe)]_2$, **4**, respectively was washed with a small quantity of cold solvent and dried in vacuum over silica gel. Slow crystallization of $[CuL_2(H_2O)]_2.2H_2O$, **1**, from dimethylsulfoxide (DMSO) solution afforded green crystals of $[CuL_2(DMSO)]_2$, **3**. The elemental analyses of the complexes **2–4** confirm their stoichiometry. The complexes are microcrystalline or powder-like and stable in atmospheric conditions.

Formation of the $[Cu(L)_2(S)]_2$-pDNA complexes. Different concentrations of each compound were incubated with 10 μg of the plasmid DNA Ptz18r for 3 h at 37 °C in the presence of 50 mM Tris.HCl pH 8 and the samples were subjected to agarose gel electrophoresis (1% in agarose) containing 1% EtBr.

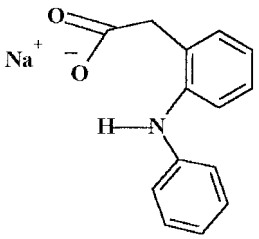

Figure 1. The structure of diclofenac.

Agarose gel electrophoresis. Agarose gel electrophoresis was performed as described elsewhere (Johnson & Grossman 1977).

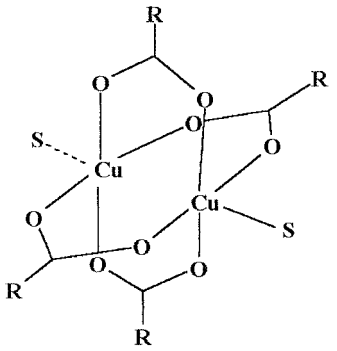

Figure 2. Structural representation of the complexes $[CuL_2(S)]_2$, where S is the axially bonded solvent molecule

Results and discussion

Synthesis. The complexes of Cu(II), were prepared according to the reactions (1)–(5)

$$2CuCl_2 + 4NaL + 4H_2O \xrightarrow{CH3OH,\ H2O,\ pH=6.5} [CuL_2(H_2O)]_2 \cdot 2H_2O + 4NaCl, \quad (1)$$

$$[CuL_2(H_2O)]_2 \cdot 2H_2O + EtOH \xrightarrow{EtOH} [CuL_2(EtOH)]_2, \quad (2)$$

$$[CuL_2(H_2O)]_2 \cdot 2H_2O + DMSO \xrightarrow{Dimethylsulfoxide} [CuL_2(DMSO)]_2, \quad (3)$$

$$[CuL_2(H_2O)]_2 \cdot 2H_2O + (CH_3)_2CO \xrightarrow{(Me)2CO} [CuL_2((CH_3)_2CO)]_2, \quad (4)$$

$$[CuL_2(H_2O)]_2 \cdot 2H_2O + DMF \xrightarrow{Dimethylformamide} [CuL_2(DMF)]_2. \quad (5)$$

The complex **5** is a binuclear molecule. The four carboxylato groups from four ligands are in a bidentate *syn,syn* $\eta^1{:}\eta^1$ bridging mode. The square pyramid geometry with an oxygen donor from a dimethylformamide molecule occupying both apical positions was established by single crystal X-ray study (Figure 2) (Kovala-Demertzi *et al.* 1997).

Infrared spectroscopy. The absence of large systematic shifts of the ν(NH) and δ(NH) bands in the spectra of all the complexes compared with those of the ligand indicates that there is no interaction between the NH group and the metal ions. The difference of bands of the prepared complexes ν_{as}(COO) and ν_s(COO) compared to that of sodium diclofenac characterizes the carboxylate ligation. The ν_{as}(COO) and ν_s(COO) bands of **1**–**5** are at 1620–1640 and 1450–1460 cm^{-1} respectively. The difference $\Delta[\Delta = \nu_{as}(COO) - \nu_s(COO)]$ 190–170 cm^{-1} is close to the ionic value (for sodium diclofenac the Δ value is 170 cm^{-1}), as expected for the bidentate bridging mode of carboxylato ligation (Kovala-Demertzi *et al.* 1993; Kovala-Demertzi *et al.* 1997; Nakamoto 1997: Kovala-Demertzi *et al.* 1998). The similar spectra of **1**–**5** support a structure analogous to **1** and **4** (Nakamoto 1997) with similar bidentate *syn, syn* $\eta^1{:}\eta^1{:}\mu_2$ bridging ligation. Complex **1** exhibits bands at 3580 and 3400 cm^{-1} attributed to the presence of coordinated and lattice water (Kovala-Demertzi *et al.* 1997). The dimethylsulfoxide adduct **3**, shows a S-O stretching vibration at 1040 cm^{-1}. This frequency is lower than that for free DMSO (1100–1055 cm^{-1}) and thus indicates that the binding of DMSO is through oxygen (Nakamoto 1997). The C=O stretching frequency of **5** and **4** from 1680 and 1710 cm^{-1} for free DMF and $(Me)_2CO$ are shifted to 1640 and 1690 cm^{-1} respectively indicating coordination through oxygen (Greenaway *et al.* 1988; Nakamoto 1997). The sharp band at 3320 cm^{-1} for **2** is assigned to bonded EtOH (Bellamy 1975). The multiple medium band at 400–360 cm^{-1} and the medium weak band at 320–320 $cm^{-1}$of the complexes **1**–**5** are assigned to the $\upsilon(Cu\text{-}O)_{COO}$ and $\upsilon(Cu\text{-}O)_S$ respectively (where S is the oxygen bonded solvent, H_2O, EtOH, $(CH_3)_2SO$, $(CH_3)_2CO$ and $(CH_3)_2NCOH$) (Kovala-Demertzi *et al.* 1993, Kovala-Demertzi *et al.* 1997, Nakamoto 1997, Kovala-Demertzi *et al.* 1998).

UV-visible spectroscopy The reflectance spectrum of 1 shows two bands having maxima at 14.100 cm^{-1} (Band I) and at 26.500 cm^{-1} (Band II), typical of square-pyramidal species with a CuO_5 chromophore.

It is recognized that band I is assigned to a ligand-field d-d transition of the copper ions and band II to a charge transfer from carboxylato-oxygen atoms to the metal ion recognized (Greenaway *et al.* 1988; Kato & Muto 1988; Kovala-Demertzi *et al.* 1997). Band I is expected to be sensitive to the nature of both ligands $RCOO^-$ and the axial, and shifts to a higher energy as the pk_a of the axial ligand decreases and the basicity of the axial ligand becomes weaker. The same pattern of spectrum is shown when compound 1 is dissolved in $(CH_3)_2SO$, MeOH, and $(CH_3)_2NCOH$, suggesting that the axially bonded water molecules are exchanged with solvent molecules according to reactions (3)–(5). $[CuL_2(H_2O)]_2.2H_2O$ is not appreciably soluble in EtOH and $(CH_3)_2CO$ to obtain visible spectra. The ν_{max} values of band I tend to increase according to the terminal group: water (14.100) > $(CH_3)_2NCOH$ (13.870) > MeOH (13.700) > $(CH_3)_2SO$ (13.530). In general the greater the tetragonality, e.g. the longer the axial bond, the greater will be the energy of the $d_{z2} \rightarrow d_{x2-y2}$. The broad visible band envelope will probably contain $d_{xy} \rightarrow d_{x2-y2}$ and $d_{xz}, d_{yz} \rightarrow d_{x2-y2}$. As the axial bond lengthens, the $d_{xy} \rightarrow d_{x2-y2}$ and $d_{xz}, d_{yz} \rightarrow d_{x2-y2}$ transitions shift to the blue because of a synergistic effect (Lever 1984). According to this, the bond length of the axially bonded ligand should be decreased with this order; $Cu\text{-}O_{(H2O)} > Cu\text{-}O_{(DMF)} > Cu\text{-}O_{(MeOH)} > Cu\text{-}O_{(DMSO)}$. The bond distances $Cu\text{-}O_{(H2O)}$ and $Cu\text{-}O_{(EtOH)}$ are 2.147(2) and 2.141(2) Å respectively, for the binuclear copper(II) salicylate $[Cu(C_7H_5O_2)_2(H_2O)(C_2H_5OH)]_2$ complex (Yoneda *et al.* 1993), while the bond distance $Cu\text{-}O_{(DMF)}$ for the binuclear $[CuL_2(DMF)]_2$, **5**, is 2.122(2) Å (Kovala-Demertzi *et al.* 1997). In polar and coordinating solvents the binuclear structure of **1** is retained, while for $[Cu(II)(3,5\text{-}Dips)_2]_2$ the dimer dissociates into monomers $[Cu(II)(3,5\text{-}Dips)_2(solvent)_2]$ (Greenaway *et al.* 1998).

Direct effect of the Cu(II) complexes of Diclofenac on the plasmid DNA. The effect of the newly synthesized complexes of Diclofenac $[Cu(L)_2(H_2O)]_2 \cdot 2H_2O$ (1), $[Cu(L)_2(EtOH)]_2$(2), $[Cu(L)_2(DMSO)]_2$(3), $[Cu(L)_2(MeCOMe)]_2$ (4), $[Cu(L)_2(DMF)]_2$ (5) as well as of NaL (6) on the integrity and supercoiling of the plasmid DNA was examined. Different concentrations of the above compounds were incubated with the plasmid Ptz18r DNA and their effect on pDNA was tested by agarose gel electrophoresis.

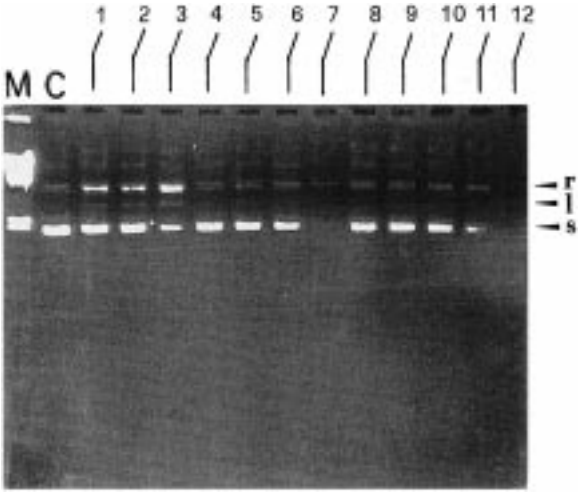

Figure 3. Agarose (1%) gel electrophoresis pattern of EtBr stained plasmid DNA treated with increasing concentrations of $[Cu(L)_2(H_2O)]_2 \cdot 2H_2O$ (0.5, 1.0 and 2.5 mM, lanes 1–3), $[Cu(L)_2(EtOH)]_2$ (0.1, 0.5, 1.0 and 2.5 mM, lanes 4–7) and $[Cu(L)_2(DMSO)]_2$ (0.05, 0.1, 0.5, 1.0 and 2.5 mM, lanes 8–12). Lane M: λDNA/Hind III markers: 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, 0,564 and 0,120 kb, lane C (control): plasmid DNA not treated with the compound. Form *s* represents the supercoiled pDNA. Form *r* represents the relaxed pDNA. Form *l* represents the linear pDNA.

Figure 3 shows that treatment of plasmid DNA with increasing concentrations of compound **1** causes gradual relaxation of supercoiled DNA, which is observed by the increase of the intensity of the band that represents the relaxed form of the plasmid DNA. Concentrations of the complex above 0.5 mM (Figure 3, lanes 2 and 3) cause double stranded scissions which lead to the formation of linear DNA, similarly to our previous report (Raptopoulou *et al.* 1998). Compound **2** causes double stranded scissions and formation of linear pDNA at concentrations 0.5–1.0 mM (Figure 3, lanes 5 and 6). A decrease of the initial amount of the pDNA was observed with 2.5 mM of compound **2** (Figure 3, lane 7), due to the strong degradative effect on the DNA substrate. A disappearance of pDNA is observed at 2.5 mM of compound **3** (Figure 3, lane 12) attributed to the same effect. The appearance of a higher molecular weight band with treatment of pDNA with the above three compounds even at the lowest concentrations suggests that these compounds cause the formation of catenanes.

Treatment of pDNA with increasing concentrations of compound **4**, which is shown in Figure 4, causes gradual relaxation of supercoiled DNA (lanes 1–5). Double stranded scissions and progressive

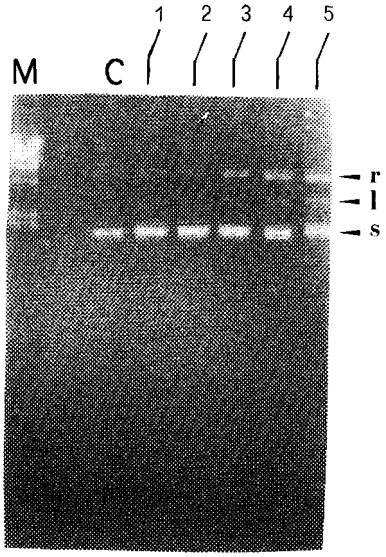

Figure 4. Agarose (1%) gel electrophoresis pattern of EtBr stained plasmid DNA treated with increasing concentrations of $[Cu(L)_2(MeCOMe)]_2$ (0.05, 0.1, 0.5, 1.0 and 2.5 mM, lanes 1–5). Lane M: λDNA/Hind III markers: 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, 0,564 and 0,120 kb, lane C (control): plasmid DNA not treated with the compound. Forms *s*, *r* and *l* as in Figure 3.

formation of linear pDNA is observed as well. Compound **5** has the same effect on the relaxation of pDNA and the formation of linear DNA as compound **4** (data obtained but not shown). Additionally, compound **5** at concentrations above 1 mM causes a decrease on the amount of pDNA which gradually leads to the complete disappearance of pDNA.

Treatment of pDNA with increasing concentrations of compound **6**, which represents the effect of diclofenac sodium on the plasmid DNA, does not show any effect (data not shown).

It is known that human serum albumin and metallothioneins bind to copper. Thus, a competition between an administered drug, such as $Cu_2(diclofenac)_4$, and albumin will take place and the relative stability constants of the two chelating agents for copper, as well as the physiological pH, will determine the final equilibrium among the complexes (Frausto da Silva & Williams 1991; Greenaway *et al.* 1998). Stability constants of Cu(II)-diclofenac and Cu(II)-albumin were found to be $10^{6.8}$ and 10^{28}, respectively (Agatonovic-Kustrin *et al.* 1991; Sawada *et al.* 1996). Based on these values, human serum albumin will practically bind the total amount of copper of the Cu(II)-diclofenac complex. For a 10^{-5} M Cu(II)-diclofenac concentration in physiological pH, the free diclofenac anion will be just less than 10^{-5} M, while the ionic copper (II) concentration ranges from 10^{-17} to 10^{-14} M (Cowan 1977; Sorenson 1982). Therefore, the Cu(II)-diclofenac concentration which remains intact in serum will be around 10^{-20} to 10^{-17} M. Therefore, the reported therapeutic action of $Cu_2(diclofenac)_4$ (Konstandinidou *et al.* 1998) could be either due to this small proportion of the binuclear complex that can bind intact to HSA and can be transported to the site of action, or to a ternary Cu-diclofenac-HSA complex which is also the transporting agent (Yuan *et al.* 1996).

Acknowledgement

D.K.D. thanks HELP EPE for the donation of diclofenac sodium.

References

Agatonovic-Kustrin S, Zivanovic Lj, Radulovic D, Vasiljevic M, 1991 Experimental design applied to a spectrophotometric study of a diclofenac sodium-copper(II) complex. *Analyst* **116**, 753–756.

Ausubel FM, Brent R, Kingston RE *et al.* 1987 *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, p. 1.6.1.

Baquial JGL, Sorenson JRJ, 1995 Down-regulation of NADPH-diaphorase (nitric oxide synthase) may account for the pharmacological activities of $Cu(II)_2(3,5\text{-diisopropylasalicylate})_4$. *J Inorg Biochem* **60**, 133–148.

Bellamy LJ, 1975 *The Infrared Spectra of Complex Molecules*. London: Chapman and Hall; 107–121.

Billadeau MA, Morrison H, 1996 In Sigel A & Sigel H, eds. *Metal Ions in Biological Systems*. New York: Dekker; Vol 33, 269.

Carter DC, He XM, 1990 Structure of human serum albumin. *Science* **249**, 302–303.

Carter DC, He XM, 1992 Atomic structure and chemistry of human serum albumin. *Nature* **358**, 209–215.

Cowan JA, 1977 *Inorganic Biochemistry*, 2nd edn, New York: Wiley, 161–162.

Frausto da Silva JJR, Williams RJP, 1991 *The Biological Chemistry of the Elements. The Inorganic Chemistry of Life*, Oxford: Oxford Press; 396.

Greenaway FT, Norris LJ, Sorenson JRJ, 1988 Mononuclear and binuclear copper(II) complexes of 3,5- 3,5-diisopropylsalicylic acid. *Inorg Chim Acta* **145**, 279–284.

Greenaway FT, Hahn JJ, Xi N, Sorenson JRJ, 1998 Interaction of Cu(II) 3,5-diidopropylsalicylate with human serum albumin-an evaluation of spectroscopic data. *Biometals* **11**, 21–26.

Johnson PH, Grossman LI, 1977 Electrophoresis of DNA in agarose gels. Optimizing separations of conformational isomers of double- and single-stranded DNAs. *Biochemistry* **16**, 4217–4225.

Kato M, Muto Y, 1988 Factors affecting the magnetic properties of dimeric copper(II) complexes. *Coord Chem Rev* **92**, 45–83.

Konstandinidou M, Kourounaki A, Hadjipetrou L *et al.* 1998 Antiinflammatory Properties of Complexes of Diclofenac with Transition Metals. *J Inorg Biochem* **70**, 63–69.

Kovala-Demertzi D, Mentzafos D, Terzis A, 1993 Metal Complexes of the Anti-inflammatory Drug, Sodium[2(2,6-Dichlorophenyl)Amino]Phenyl]Acetate (Diclofenac Sodium). Molecular and Crystal Structure of Cadmium Diclofenac. *Polyhedron* **11**, 1361–1371.

Kovala-Demertzi D, Theodorou A, Demertzis MA *et al*, 1997 Synthesis and characterization of tetrakis-μ-2-[(2,6-dichlorophenyl)amino] benzenacetodiaquodicopper(II) dihydrate and tetrakis-μ-2-[(2,6-dichlorophenyl) amino]benzeneacetodimethylformamidodicopper(II). *J Inorg Biochem* **65**, 151–157

Kovala-Demertzi D, Hadjikakou SK, Demertzis MA *et al.*, 1998 Metal Ion-drug interactions. Preparation and properties of manganese (II), cobalt (II) and nickel (II) complexes of diclofenac with potentially interesting anti-inflammatory activity. Behavior in the oxidation of 3,5-di-*tert*-butyl-*o*-catechol. *J Inorg Biochem* **69**, 223–229.

Lever ABP, 1984 *Inorganic Electronic Spectroscopy*, 2nd edn, Amsterdam: Elsevier, pp. 553–572.

Moser P, Sallmann A, Wisenberg I, 1990 Synthesis and quantitative structure-activity relationships of diclofenac analogues. *J Med Chem* **33**, 2358–2368.

Nakamoto K, 1997 *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 5th edition, New York: Wiley; 57–62, 102–104.

Raptopoulou CP, Paschalidou S, Pantazaki AA *et al.* 1998 Bis(Acetato)Bis(1-Methyl-4,5-Diphenylimidazole) Copper(II): Preparation, Characterization, Crystal Structure, DNA Strand Breakage and Cytogenetic Effect. *J. Inorg Biochem* **71**, 15–27.

Ross WE, Glaubiger DL, Kohn KW, 1978 Protein associated DNA breaks in cells treated with adriamycin or ellipticine. *Biochim Biophys Acta* **519 (1)**, 23–30.

Sallmann A, 1986 The history of diclofenac. *Amer J Med* **80**, 29–33.

Sawada T, Fukumaru K, Sakurai H, 1996 Coordination-dependent ESR spectra of copper(II) complexes with a CuN_4 type coordination mode: relationship between EPR parameters and stability constants or redox potentials of the complexes. *Chem Pharm Bull* **44**, 1009–1016.

Sorenson JRJ, 1982 *Metal Ions in Biological Systems*; Sigel H. Edit.; New York: Dekker; Vol. 14, 77–113.

Sorenson JRJ, 1989 Copper complexes offer a physiological approach to treatment of chronic diseases. *Prog Med Chem* **26**, 437–568.

Sorenson JRJ, Soderberg LSF, Chang LW *et al.* 1993 Copper, iron manganese, and zink 3,5-diisopropylasalicylate complexes increase survival of gamma irradiated mice. *Eur J Med Chem* **28**, 221–229.

Todd PA, Sorkin EM, 1988 Diclofenac sodium: A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. *Drugs* **35**, 244–285.

Waring MJ, 1981 DNA modification and cancer. *Ann Rev Biochem* **50**, 159–192.

Yoneda K, Uchiyama K, Boettcher B, *et al*. 1993 Preparation and crystal structure of aqua(ethanol)tetrakis(μ-salicylato-*O*,*O*′)dicopper(II)-ethanol-water(1/1/1). *Bull Chem Soc Jpn* **66**, 3815–3817.

Yuan H, Antholine WE, Subczynski WK, Green MA, 1996 Release of CuPTSM from human serum albumin after addition of fatty acids. *J. Inorg Biochem* **61**, 1251–1259.