

**CHARACTERISATION OF
COPPER(II) DIMERS OF THE
NON-STEROIDAL ANTI-
INFLAMMATORY DRUG
INDOMETHACIN**

by

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A Thesis Submitted in Partial Fulfillment of the
Requirements for Admission to the degree of
Doctor of Philosophy

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2000



2000

DEDICATION

*Rebecca & David**In memory of L. Amalia*

The work presented in this thesis is my own, unless otherwise stated.



Jane Elizabeth Weder

DEDICATION

Rebecca & David

In memory of Leonidas

Charles Marwick

Medical World News, August 2, 1982

LIST OF PUBLICATIONS

Parts of the results described in this thesis have been or will be published elsewhere in a different format. A list of papers and conference abstracts are given below.

In the beginning there was aspirin for arthritis. And it begat the non-steroidal anti-inflammatory drugs and they begat more.

1. Anti-inflammatory Dinuclear Copper(II) Complexes with Indomethacin. Synthesis, Magnetic and EPR Spectroscopy. Crystal Structure of the N,N' -dimethylformamide Adduct, Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., MacLachlan, D., Bramley, R., Delfs, C. D., Murray, K. S., Mowbray, B., Warwick, B., Biffin, J. R., Ragnop, H. L. *Inorg. Chem.*, 1999, 38, 8, 1736-1744.

2. Anti-inflammatory Dinuclear Copper(II) Complex of Indomethacin. X-band EPR and X-ray Absorption Spectroscopy, Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., Fegan, G. J., Rich, A. M., Warwick, B., Biffin, J. R., Ragnop, H. L., *Journal of Pure Applied Research (Pharmaceuticals)*, Perth, University of Sydney, 1999.

3. Characterisation of Anti-inflammatory Dinuclear Complexes of Indomethacin, Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., MacLachlan, D., Bramley, R., Delfs, C. D., Murray, K. S., Mowbray, B., Warwick, B., Biffin, J. R., Ragnop, H. L., *New Zealand Institute of Chemistry/Royal Australian Chemical Institute*, Perth, Victoria University of Wellington, New Zealand, 1998.

4. XAFS Analysis of Anti-inflammatory Cu(II) Dinuclear of Indomethacin, Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., Fegan, G. J., Rich, A. M., *New Zealand Institute of Chemistry/Royal Australian Chemical Institute*, Perth, Victoria University of Wellington, New Zealand, 1998.

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2. Anti-inflammatory Dinuclear Copper(II) Complex of Indomethacin. X-band EPR and X-ray Absorption Spectroscopy, Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., Foran, G. J., Rich, A. M., Warwick, B., Biffin, J. R., Regtop, H. L., *Society for Free Radical Research (Australasia): Poster*, University of Sydney, **1999**.
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venipuncture 154

LIST OF SYMBOLS AND ABBREVIATIONS

$A_{\parallel}$	perpendicular A hyperfine parameter
AAS	atomic absorption spectroscopy
A_s/A'_s	back-scattering amplitude/effective back-scattering amplitude
AXAFS	atomic x-ray absorption fine structure
α -TocH	α -tocopherol
B	magnetic field
BM (β)	Bohr Magneton
BP	British Pharmacopoeia
BVR	Biochemical Veterinary Research
CAT	catalase
cgs	c/g/s
$\epsilon_{\max}$	molar extinction coefficient
ϵ	constitutive correction for electronic characteristics used in the calculation of the value of atomic diamagnetic susceptibility
COX I & II	cyclooxygenase isoenzymes I & II
d	day
D	number of dimension of the XAFS refinement unit
D	symmetric axial crystal field splitting parameter
DI	double integral
DIPS	diisopropylsalicylic acid
Δ_0	octahedral crystal field splitting
DMA	<i>N,N</i> -dimethylacetamide
DMF	<i>N,N</i> -dimethylformamide
DSA	dog serum albumin
E	asymmetric rhombohedral crystal field splitting parameter
ESI-MS	electrospray ionisation-mass spectroscopy
EPR	electron paramagnetic resonance
ESA	equine serum albumin
e.s.d.	estimated standard deviation

eV	electron Volts
FT	Fourier transform
g	anisotropic Lande splitting parameter
$g_{\perp}$	perpendicular g parameter
$g_{\parallel}$	parallel g parameter
G	gauss
GI	gastrointestinal
H_{ex}/H_s	exchange Hamiltonian/spin Hamiltonian
hr	hour
HSA	human serum albumin
I	I nuclear tensor
i.d.	inner diameter
IndoH	indomethacin
$-J$	singlet-triplet energy separation in dimers
I	ground state nuclear spin quantum number
IR	infrared
k	10^3
k	photoelectron wavevector
K	Kelvin
kHz	kiloHertz
L	ligand
<i>L</i> -Ala	<i>L</i> -alanine
<i>L</i> -Asa	<i>L</i> -asparagine
<i>L</i> -His	<i>L</i> -histidine
l.m.l.	low-molar-mass
<i>L</i> -Ser	<i>L</i> -serine
<i>L</i> -Thr	<i>L</i> -threonine
<i>L</i> -Tyr	<i>L</i> -Tyrosine
λ	photoelectron mean-free path
$\lambda_{\max}$	wavelength of maximum absorption
MCE	micellular capillary electrophoresis
MeCN	acetonitrile

min	minute
ms	millisecond
MS	multiple scattering
mT	MilliTesla
mW	milliWatt
MW	molecular weight
n	the number of legs in an XAFS scattering path
N	number of atoms in the XAFS model
N	MS XAFS model atom number
N_A	Avargardo constant
N_i	number of independent data points in the XAFS data set
N_α	temperature independent contribution to magnetic susceptibility
N_{fr}	number of free parameters in the Fourier-filtered XAFS data
NMP	1-methylpyrrolidone
NMR	nuclear magnetic resonance
NOS	nitric oxide synthetase
N_p	number of refinement parameters in the XAFS model
N_s	number of XAFS scattering atoms
NSAID	non-steroidal anti-inflammatory drug
Ω	ohm
o.d.	outer diameter
PBS	pharmaceutical benefits scheme
PG	prostaglandin
PGG ₂	prostaglandin G ₂
PGH ₂	prostaglandin H ₂
PMNLs	polymorphonuclear leukocytes
PPD	prescribed daily doses per 1000 of the population
R_{as}	distance of the metal absorber atom from the scattering atom
R_{eff}	total distance travelled by the photoelectron; being twice the value of R_{as}
ROS	reactive oxygen species
r.m.s	root mean square

R_{xaf}	XAFS model's goodness-of-fit parameter
σ^2	mean square deviation in the distance of the metal absorber atom from the scattering atom
s	second(s)
S_0^2/S	XAFS inelastic loss term/XAFS scale factor
S	spin multiplicity
$\hat{S}$	Spin tensor of a spin Hamiltonian
SDS	sodium lauryl sulfate
SOD	superoxide dismutase
SS	single scattering
ϕ_{as}	photoelectron phase shift
T	temperature
TGA	thermogravimetric analysis
μ	x-ray absorption coefficient
μ_{s}	smooth background x-ray absorption coefficient
μ_0	X-ray absorption coefficient for a free atom
μ_{eff}	effective magnetic moment
μ_{so}	spin-only magnetic moment
UV-Vis	ultraviolet-visible
W	XAFS weighting factor
x	fraction monomer
χ	XAFS oscillations
χ_{A}	paramagnetic susceptibility per Cu atom
χ_{D}	diamagnetic susceptibility
χ_{g}	gram magnetic susceptibility
χ_{M}	molar magnetic susceptibility
χ_{obsd}	observed magnetic susceptibility
χ_{calc}	calculated magnetic susceptibility
XAS	X-ray absorption spectroscopy
XAFS	X-ray absorption fine structure
XPD	X-ray powder diffraction

XRD

X-ray diffraction

ZF-EPR

zero-field electron paramagnetic resonance

Arthritis. Inflammation occurring in one or more joints, as a result of a number of different diseases; causing heat, redness, swelling and pain; may be long term in nature and progressively debilitating.

Ceruloplasmin. A Cu containing α -globulin (glycoprotein); of molecular weight 132,000 dalton, containing 6 Cu atoms per molecule; a Cu transport protein and "acute-phase" protein; responds to a number of physiological states e.g. inflammation, stress.

Cyclooxygenase I & II. Isoenzymes of the inflammatory response, involved in the synthesis of prostaglandins.

Cytokines. Water-soluble proteins released by participating and interacting cells in the immune response to communicate during an immune/inflammatory response.

Human Albumin. Water soluble protein of blood associated with drug binding and transport.

Iatrogenic. As a result of medical treatment.

Leukotrienes. A variety of inflammation mediators derived from arachidonic acid; mediates the immune response e.g. vasodilation, white blood cell migration.

Metallothionein. A ubiquitous low molecular weight sulphhydryl-rich protein, which binds a number of metals including Cu; rich in cysteine; "house-keeping" action in the biological detoxification, transport and storage of metal.

Non-steroidal anti-inflammatory drug (NSAID). Class of drugs, of the non-steroid type, exhibiting anti-inflammatory, analgesic and antipyretic activity by inhibition of prostaglandin synthesis at the cyclooxygenase step.

Osteoarthritis. A form of arthritis caused by degeneration of the cartilage of joints, especially in the elderly.

Plasma. A yellow supernatant formed on the standing of whole blood and an anticoagulant agent, resulting in the precipitation of red blood cells; free from visible product of red blood cell hydrolysis.

Prostaglandins. Naturally occurring 20-carbon cyclopentano-fatty acids derived from membrane phospholipids; ubiquitous and diverse in action; mediating the inflammatory action; synthesis inhibited at the cyclooxygenase step by NSAIDs.

Rheumatoid arthritis. A form of arthritis mediated by the immune system.

GLOSSARY

Serum. The supernatant of whole blood on the standing of whole blood in the absence of an anti-coagulant agent.

Tensor. Generalised form of vector involving an arbitrary number of indices.

Arthritis. Inflammation occurring in one or more joints, as a result of a number of different diseases; causing heat, redness, swelling and pain; may be long term in nature and progressively debilitating.

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Non-steroidal anti-inflammatory drugs (NSAIDs), of which indomethacin (Indol) is the most potent, are the mainstay of the treatment of inflammatory diseases in both animals and humans, with an estimated worldwide market of between \$3-7b per annum. However, NSAIDs have serious gastro-intestinal (GI) side-effects, which not only limit their applications in the treatment of inflammation, particularly in elderly patients in whom these effects are most injurious, but they are potential pro-mutative/dyspeptic agents in the treatment of other diseases such as colo-rectal cancer, multiple sclerosis and Alzheimer's disease.

A successful solution to the GI side effects was the development of $[Ca_2(Indo)_2(DMF)_2]$ which was patented in 1994 by Biochemical Veterinary Research (BVR). $[Ca_2(Indo)_2(DMF)_2]$ is available in Australia in a number of pharmaceutical formulations for veterinary use. The lack of GI effects is highlighted by the fact that dogs, in which the administration of Indol is usually fatal, can routinely be given the drug.

The purpose of this thesis was to develop analytical methods for the characterisation of a series of $[Ca_2(Indo)_2L_2]$ ($L = DMF, DMA, NMP, OH_2$) complexes in order that their applications could be extended into worldwide markets and into human clinical trials. Furthermore, DMF is not an ideal solvent for use in human pharmaceutical formulations, with alternate solvent adducts having the potential to alter both the nature and biological activity of the complex. Consequently, different solvent adducts of $Ca_2(Indo)_2$ were prepared and characterised, e.g., $[Ca_2(Indo)_2(OH_2)_2]$, for use in pharmaceutical formulations.

The synthesis and solid-state characterisation of $[Ca_2(Indo)_2L_2]$ ($L = DMF, DMA, NMP, OH_2$) by various techniques, including EPR and X-ray absorption spectroscopy, are reported. Moreover, for the first time, the stability of $[Ca_2(Indo)_2(DMF)_2]$ in a number of pharmaceutical and biological media, including artificial "gastric juice", was established.

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs), of which indomethacin (IndoH) is the most potent, are the mainstay of the treatment of inflammatory diseases in both animals and humans, with an estimated worldwide market of between \$5-9B per annum. However, NSAIDs have serious gastro-intestinal (GI) side-effects, which not only limit their applications in the treatment of inflammation, particularly in elderly patients in whom these effects are most injurious, but also as potential preventative/therapeutic agents in the treatment of other diseases such as colo-rectal cancer, multiple sclerosis and Alzheimer's disease.

A successful solution to the GI side effects was the development of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ which was patented in 1994 by Biochemical Veterinary Research (BVR). $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is available in Australia in a number of pharmaceutical formulations for veterinary use. The lack of GI effects is highlighted by the fact that dogs, in which the administration of IndoH is usually fatal, can routinely be given the drug.

The purpose of this thesis was to develop analytical methods for the characterisation of a series of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) complexes in order that their applications could be extended into worldwide markets and into human clinical trials. Furthermore, DMF is not an ideal solvent for use in human pharmaceutical formulations, with alternate solvent adducts having the potential to alter both the nature and biological activity of the complex. Consequently, different solvent adducts of Cu-IndoH were prepared and characterised, e.g., $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, for use in pharmaceutical formulations.

The synthesis and solid-state characterisation of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) by various techniques, including EPR and X-ray absorption spectroscopies, are reported. Moreover, for the first time, the stability of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in a number of pharmaceutical and biological media, including artificial "gastric juice", was established.

The chemical and physical properties of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) are virtually independent of the nature of the axial ligand; with the axial ligand undergoing rapid exchange. Zero-field, electronic, X-ray absorption and X-band EPR spectroscopies, magnetic measurements, and X-ray powder diffraction studies confirm that the green powdered microcrystalline complexes, obtained in high yield and purity, are isostructural and similar in structure to $\text{Cu}(\text{II})$ acetate monohydrate, with a strong anti-ferromagnetic exchange interaction ranging from -141 to -152 cm^{-1} . The only measurable difference found among all the complexes is in the value of the symmetric axial crystal field splitting parameter (D), as measured by zero-field EPR spectroscopy. The experiments show that the DMF and NMP ligands contribute to a greater extent to the exchange between the ground state of one Cu and the excited state of the other Cu, compared to the aqua ligand. There is, therefore, a relative insensitivity in the electronic and magnetic characteristics of the complexes as a result of axial ligand exchange. This is consistent with the lack of variation in the pharmaceutical activities of the Cu-IndoH drugs with different axial ligands, as reported by BVR.

Certain pharmaceutical formulations cause rapid degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to, as yet, unknown $\text{Cu}(\text{II})$ monomer species, while the dimer is quite stable in others. The dimeric structure of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is retained in *Cu-Algesic* tablets, micellular, i.e., Span 80 (20% w/w) + tetraglycol (40% v/w) (MCT paste) or cremophor (4% v/w) + NMP (4%) (*Avigesic Forte*), solutions and oily paraffin paste. However, the complex is substantially degraded by carbopol (1% w/w) (*Cu-Algesic* paste) and gelatin (*Cu-Algesic* capsules), and to a lesser extent α -tocopherol and UV-radiation.

The coordination of the $\text{Cu}(\text{II})$ dimer core and quantification of $\text{Cu}(\text{II})$ monomer contaminants were reliably determined by X-ray absorption and EPR spectroscopies, respectively. This work highlights the power of EPR and X-ray absorption spectroscopies for such studies. Paraffin and MCT pastes have been developed as a result of this work, with carbopol and *Avigesic Forte* formulations using the aqua complex instead of the DMF complex also emerging from this research. In addition, the gelatin capsules are also being reformulated by BVR as a result of research presented in this thesis.

Preliminary investigations of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in equine serum and artificial "gastric juice" shows rapid degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. The identification of a breakdown product of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in artificial "gastric juice", which is different from that of free IndoH, may lead on to the development of new anti-inflammatory drugs and help explain the widely different side effects of IndoH compared to $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$). Whether the GI sparing effects of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is due to IndoH breakdown, the hydrophobic nature of the exterior of the complex, or both, is yet to be resolved. The hydrophobic nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ may be important in facilitating its rapid dissolution in micelles in the GI tract and transport through biological membranes (irrespective of any hydrolysis), so minimising direct GI toxicity. While considerable progress has been made at characterising these drugs in the solid state, pharmaceutical preparations and biological fluids, a detailed explanation for the biological activity of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ remains to be elucidated.

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1 Introduction

From time immemorial, man and beast have been afflicted with inflammatory diseases such as arthritis and other related muscular skeletal conditions. One of the earliest pieces of evidence of osteoarthritis was found in human skeletal remains of the Pleistocene era, dating back 500,000 years.¹ As a consequence, humans have searched for safe remedies to relieve the painful symptoms thereof. Over thousands of years a wide variety of medicines, including Cu and extracts of willow bark (salacin; a glycoside of salicylic acid) have been investigated to treat such diseases.^{2,3}

Some of the earliest references to the use of the extracts of willow for the relief of pain and fever⁴ are by the physicians Hippocrates (~460-377 BC) and Dioscorides (~40-90 AD).⁴⁻⁶ Salacin is the prototype of many of today's non-steroidal anti-inflammatory drugs (NSAIDs), e.g., salicylates, indoles, propionic acids, fenamates and oxicam NSAIDs.^{7,8} Their action, whilst still uncertain,⁹ has been attributed primarily to the inhibition of prostaglandin (PG) synthesis⁹⁻¹¹ and more specifically inhibition of the cyclooxygenase (COX) enzyme system.¹²⁻¹⁵ Today, NSAIDs are the mainstay of the treatment of arthritis and other related muscular-skeletal disorders,¹⁶ despite sometimes serious gastrointestinal (GI) side effects.^{11,17-21} The first commercially available NSAID, aspirin (acetylsalicylic acid), was introduced into medicine in

1889.⁷ Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-

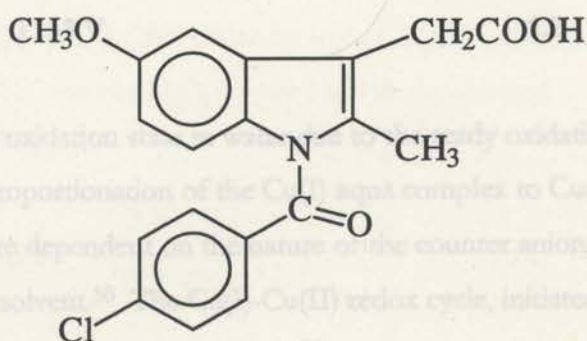
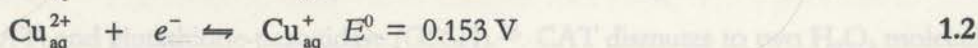
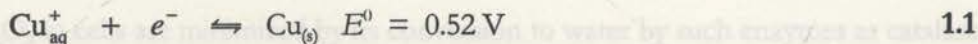


Figure 1.1. Indomethacin (IndoH).

methyl-1H-indole-3-acetic acid = IndoH, Figure 1.1), one of the most potent of the NSAIDs,⁹ was introduced in 1960 by Merck.⁷ Arylalkanoic acids, of the general formula ArCRHCOOH , (Ar = aryl or heteroaryl; R = H, CH_3 , alkyl) make up the largest group of NSAIDs - with IndoH just one of this group.^{11,22} A number of excellent references are available that detail the historical and structural aspects of NSAID development.^{7,8,22}

Likewise, the therapeutic properties of Cu and its compounds have been widely reported. As early as 3000 BC, the Egyptians used Cu as an antiseptic and to sterilize drinking water.²³ The Cu bracelet has long been used as a folk remedy for arthritis.²⁴ It has since been shown that metallic Cu may dissolve in sweat and be absorbed through skin.²⁴ Furthermore, it has been suggested that the mode of action of salicylates and other such anti-inflammatory drugs may involve the chelation of bioactive metal ions, e.g., Cu^{2+} ,²⁵⁻²⁷ so facilitating the transfer of the metal to and from a site of inflammation or pain.^{28,29}

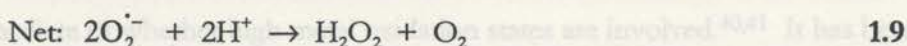
The importance of Cu in medicine and biology may be understood in terms of its basic redox chemistry. Although Cu ($[\text{Ar}]4s^1 3d^10$) is not an abundant metal in nature (55 ppm),³⁰ it is the third most abundant transition metal element in biological systems.³⁰ It has an occurrence, in the normal human body, of 80-120 mg, compared to 4-5 g for Fe and 1.4-2.3 g for Zn.³⁰ Copper is found principally in two different oxidation states; Cu(I) (d^10) and Cu(II) (d^9). The stabilities of the most common oxidation states, Cu(I) and Cu(II), are indicated by their standard reduction potentials:³⁰



with Cu(II) being the most common oxidation state in water due to the ready oxidation of Cu(I) to Cu(II) and the rapid disproportionation of the Cu(I) aqua complex to Cu(II) and Cu_0 in water.³¹ The potentials are dependent on the nature of the counter anion, the type of coordinating ligands and the solvent.³⁰ The Cu(I)-Cu(II) redox cycle, initiated by an oxidative-addition reaction, can be summarized as follows:³⁰



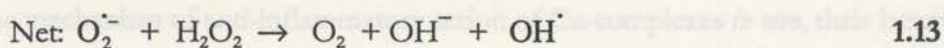
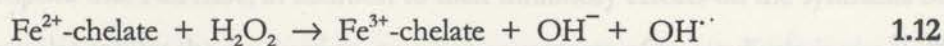
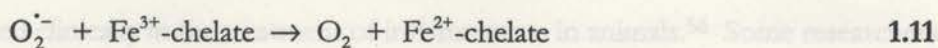
with the reduction of O_2 to water ($O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$) and an accompanying transfer of oxygen to a substrate.³⁰ Hence, many Cu enzymes in nature are either hydroxylases or oxidases.³² The beneficial role of Cu in minimizing inflammation may be attributed to its redox activity - in particular, the ability of Cu in such enzymes as superoxide dismutase (SOD) to remove the highly reactive pro-inflammatory superoxide anion $O_2^{\cdot-}$.^{33,34}



Superoxide, generated in biological systems by the one-electron reduction of O_2 , is capable of tissue damage by virtue of its ability to act as a weak base (undergoing protonation ($pK_a = 4.8$)) to the hydroperoxyl radical $HO_2^{\cdot}$ and as a one-electron reductant of oxidized metal enzymes, e.g., ferricytochrome c .³³ The damaging effects of H_2O_2 in cells are minimized by its conversion to water by such enzymes as catalase (CAT) and glutathione-peroxidase (GSSH).³⁵ CAT dismutates two H_2O_2 molecules to O_2 and two H_2O molecules, and GSSH reduces H_2O_2 to two H_2O molecules and disulphide bridged glutathione (GSSG), e.g.:



Whilst H_2O_2 is not a radical, it can cross cell membranes (unlike its precursor $O_2^{\cdot-}$) and is postulated to produce more potent oxidants, such as $OH^{\cdot}$ via the metal-catalyzed Haber-Weiss (or Fenton) reaction. The transition metals implicated in this catalyst are Cu(II)/Cu(I) and Fe(III)/Fe(II).^{33,34}



where the transition metal is reduced and subsequently re-oxidized by H_2O_2 yielding deleterious entities such as $\text{OH}^{\cdot}$ or $\text{FeH}_2\text{O}_2^{2+}$, Fe^{IV} and FeO^{2+} or the equivalent Cu compounds (CuH_2O_2^+ , Cu^{III} or CuO^{2+}).^{36,37} NO dramatically inhibits this $\text{O}_2^{\cdot-}$ driven Fenton reaction *in vitro*.^{38,39} While such equations are often used to imply the presence of $\text{OH}^{\cdot}$ radicals in such cycles, it is not clear whether $\text{OH}^{\cdot}$ exists as an oxidising intermediate or whether high metal oxidation states are involved.^{40,41} It has been suggested that NSAIDs and other ligands, i.e., $\text{OH}^{\cdot}$ inactivating ligands (IOL),⁴² inactivate the $\text{OH}^{\cdot}$ radical and so contribute to an anti-inflammatory action.^{43,44} It is paradoxical that Cu and Cu-complexes exert anti-inflammatory activity yet at the same time are postulated to produce the pro-inflammatory radical $\text{OH}^{\cdot}$ via the Fenton reaction.⁴² Furthermore, treatment of collagen with $\text{Cu(II)/H}_2\text{O}_2$ gives rise to degradation and fragmentation of collagen.^{45,46} However, under physiological conditions, there may be insufficient free Cu to catalyse the pro-oxidant pathways due to the avid binding of free Cu^{2+} to such biological proteins as ceruloplasmin, albumin and other low molecular weight plasma proteins.⁴⁷ The nature of the ligand bound to the active Cu ion may also determine the nature of the biological effect observed, with the ligand either enhancing or affording protection against Cu induced oxygen free radical damage.⁴²

Despite conflicting roles for Cu in inflammation, i.e., either in the production or dismutation of free radicals, the toxicity and inflammatory response to oxidants in the cell wall is due to damage of the cell's lipid membrane by the production of lipid superoxide radicals (lipid- $\text{O}_2^{\cdot}$) and peroxides (lipid- O_2H).^{33,47-49} Lipid peroxides may also augment the inflammatory process by promoting chemotaxis of immune cells⁵⁰ and activating the COX enzyme system.^{47,51,52} Cu-complexes including Cu-NSAIDs exhibit significant anti-inflammatory activities as well as SOD-mimetic activity⁵³⁻⁵⁵ and SOD is

used clinically in the treatment of inflammation in animals.⁵⁶ Some researchers propose that NSAIDs, in addition to their inhibitory effects on the synthesis of PGs, may also inhibit the production, or act as scavengers of free radicals *in vivo*.⁵⁷ Whatever the mechanism of anti-inflammatory action of Cu-complexes *in vivo*, their beneficial function has long been recognised and their clinical effect investigated extensively over the last 50 years.⁵⁸⁻⁶¹

Some of the earliest trials of the efficacy of Cu-complexes in the treatment of arthritis included the use of intravenous Cupralene (sodium meta-(allylcuprothiocarbamido)benzoate)⁶² (19% Cu content) and intravenous Dicuprene (diethylamine(cuprooxyquinoline)sulfonate)⁶³ (6.5% Cu content) in the 1940s. This research grew out of the hypothesis that arthritis was bacterial in nature and may respond to treatment with heavy metals, e.g., Cu and Au.^{23,64} Promising evidence of the benefit of Permalon, (an intravenous solution of 2.5 mM sodium salicylate and 39 μM CuCl_2) was reported in 1952⁶⁵ and 1977.⁶⁶ There was a decline in experimental work on Cu anti-inflammatory agents from the 1950s. This has been attributed to the appearance of the corticosteroids and later the NSAIDs.^{23,64}

Interest in the possible beneficial effects of Cu-complexes was refueled by Sorenson's report in 1976 that the active forms of anti-inflammatory drugs may well be the Cu complexes of such drugs *in vivo*.⁶⁷ Sorenson reported that Cu-complexes of these anti-inflammatory drugs were more active in animal models than either their parent inorganic Cu(II) salt or the parent NSAID.⁶⁷ The pharmacological activity was proposed to be due to the inherent physico-chemical properties of the complex itself rather than just that of its constituents, since the amount of Cu in such complexes does not correlate with anti-inflammatory activity.^{53,68} A salicylate complex of Cu(II) was at least 30 times more effective than aspirin (acetylsalicylic acid) as an anti-inflammatory agent.⁶⁹ In addition, Cu(II) complexes of many non-anti-inflammatory agents exhibited anti-inflammatory action.²³ Sorenson reported extensively on the anti-epileptic, anti-cancer, anti-diabetic, radiation injury protective, antibacterial and (most significantly for NSAIDs) the anti-ulcerogenic activity of Cu(II) complexes.^{53,60,68,70,71}

Sorenson's findings followed reports by others in the 1950s and 1960s^{25-27,72,73} on the potential of metal chelates in medicine. It was suggested as early as 1966 that salicylates may deliver Cu to target cells in the body.²⁵ Furthermore, the anti-inflammatory activity of the anti-rheumatic drug *d*-penicillamine⁷⁴ (an anti-inflammatory agent not of the NSAID class) was postulated to be due to *in vivo* chelation with endogenous Cu²⁺. The commitment of early researchers to the beneficial use of Cu-salicylate for the treatment of arthritis⁷¹ is evidenced by the comments of Hangarter on his comparison of Permalon therapy with NSAIDs, corticosteroids and gold:⁶⁶ "In general, Permalon therapy was superior to all of these".

The enhanced potency of Cu-NSAID complexes compared to their individual components was re-confirmed in 1989, when a Cu-salicylate complex was reported to be significantly more active than Cu(NO₃)₂ mixed with Na-salicylate.⁷⁵ There is now a wealth of literature documenting the potential benefits of Cu(II)-complexes and several patents have been issued, e.g., Cu(II)-complexes of amino-acids, metallothioneins (low molecular weight sulfhydryl-rich proteins) and *d*-penicillamine,²³ but no Cu(II) anti-inflammatory drugs are currently available for human use. This is despite the fact that many of today's anti-inflammatory drug therapies, including the NSAIDs, remain either largely inadequate and/or are associated with unacceptable side effects.⁷⁶⁻⁷⁸ GI side effects, with sometimes fatal perforations of gastric and/or duodenal ulcers, is one of the dose-limiting side-effects of all NSAIDs currently in use.^{21,79}

The search for safer NSAIDs is evidenced by the world-wide availability of over 35 NSAIDs.¹¹ Additional interest in NSAIDs lies in their possible therapeutic benefits in the prevention of colorectal⁸⁰⁻⁸⁴ and lung cancers⁸⁵ and even in the treatment of Alzheimer's disease.⁸⁶⁻⁸⁸ There is, therefore, a wide and expanding clinical use of NSAIDs - particularly in chronic diseases of the elderly, in whom the GI side-effects are most common and injurious.^{9,89-91}

Topical applications of the Cu(II) alcohol adduct of acetylsalicylic acid, Alcusal,²⁸ and the dimethylsulfoxide (DMSO) adduct, Dermusal,⁹² were developed in Australia in the late 1970s and early 1980s for human use but they are no longer commercially available. Apart from these initial reports of their anti-inflammatory activities, no further characterization of these products has been reported nor have oral preparations been

marketed. The quest for more effective, yet less toxic NSAIDs, has led to several studies in the last 10 years on the preparation, characterization, and veterinary and medical use of divalent metal salts of IndoH and a number of other NSAIDs.^{47,55,93-98} Table 1.1 lists some of the mono and divalent Cu-complexes of NSAIDs currently reported in the literature.

Table 1.1. Some Copper-NSAID Complexes.

Compound	Structure
$[\text{Cu}_2(\text{Asp})_4\text{L}_2]$ ^a ; L = pyridine, 3-picoline, 4-picoline, imidazole, 1-methylimidazole, diethylamine, nicotinamide, DMSO	dimer ⁹⁹
$[\text{Cu}(\text{Asp})_2\text{L}_2]$ ^a ; L = benzimidazole, 2-methylbenzimidazole, metronidazole ^b , 2-methylimidazole, 1,2-dimethylimidazole	monomer, ^{95,100}
$[\text{Cu}_2(\text{Sup})_4(\text{CH}_3\text{CN})_2]$ ^c	dimer ¹⁰¹
$[\text{Cu}_2(\text{Sup})_4(\text{OH}_2)_2]$	dimer ¹⁰²
$[\text{Cu}(\text{Tol})_2(\text{pyridine})_2]$ ^d	monomer ⁹⁸
$[\text{Cu}_2(\text{Tol})_4(\text{DMSO})_2]$	dimer ⁹⁸
$[\text{Cu}(\text{Nap})_2(\text{pyridine})_2]$ ^e	monomer ⁹⁸
$[\text{Cu}_2(\text{Nap})_4(\text{DMSO})_2]$	dimer ⁹⁸
$[\text{Cu}(\text{Ibu})_2(\text{pyridine})_2]$ ^f	monomer ⁹⁸
$[\text{Cu}_2(\text{Ibu})_4(\text{DMSO})_4]$	dimer ⁹⁸
$[\text{Cu}(\text{Ibu})_2(\text{imidazole})_2]$	monomer ⁹⁶
$[\text{Cu}(\text{Ibu})_2(2\text{-methylimidazole})_2]$	monomer ⁹⁶
$[\text{Cu}_2(\text{Ibu})_4(\text{caffeine})_2]$	dimer ⁹⁶
$[\text{Cu}_2(\text{Ibu})_4(\text{metronidazole})_2]$	dimer ⁹⁶
$[\text{Cu}_2(\text{Fen})_4\text{L}_2]$, ^g ; L = caffeine, papaverine.	dimer ¹⁰³
$[\text{Cu}(\text{Fen})_2\text{L}_2]$; L = nicotine, nicotinamide, N,N-diethylnicotinamide.	monomer ¹⁰³
$[\text{Cu}(\text{Nif})_2(3\text{-pyridylmethanol})_2]$	monomer ¹⁰⁴

^a Aspirin = 2-acetyloxybenzoic acid (AspH); ^b metronidazole = 2-methyl-5-nitrobenzimidazole; ^c Suprofen = α -methyl-4-(2-thienyl-carbonyl)phenylacetic acid (SupH); ^d Tolmentin = 1-methyl-5-(*p*-toluoyl)-1*H*-pyrrole-2-acetic acid (TolH); ^e Naprosyn = 6-methoxy- α -methyl-2-naphthaleneacetic acid (NapH);

^f Ibuprofen = α -methyl-4-(isopropylmethyl)benzeneacetic acid (IbuH);

^g Flufenamic Acid = N-(α , α , α -trifluoro-*m*-tolyl)anthranilic acid (FluH);

^h Niflumic Acid = 2-(3-trifluoromethyl)phenylamino)-3-pyridine-carboxylic acid (NifH).

Whilst a number of researchers reported the preparation and characterisation of Cu(II)-complexes of Indo,^{55,94,105} there had been no clinical applications of these complexes to animals or humans prior to patents issued to Biochemical Veterinary Research (BVR) in 1994⁹³ and 1995^{106,107} for bis(*N,N'*-dimethylformamide)tetrakis- μ -(*O,O'*-Indo)dicopper(II), $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (Appendix 1; Figure 1.2; L = DMF). $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is available in Australia in a number of pharmaceutical compositions for veterinary use.¹⁰⁸

The full advantage of the anti-ulcerogenic activity of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is illustrated by the fact that whilst IndoH cannot be used in dogs because of the fatal GI side-effects,^{17,19,20} $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ can be so used without deleterious effects.¹⁰⁸ The hypothesis is that NSAIDs may exert their anti-inflammatory effect *in vivo*

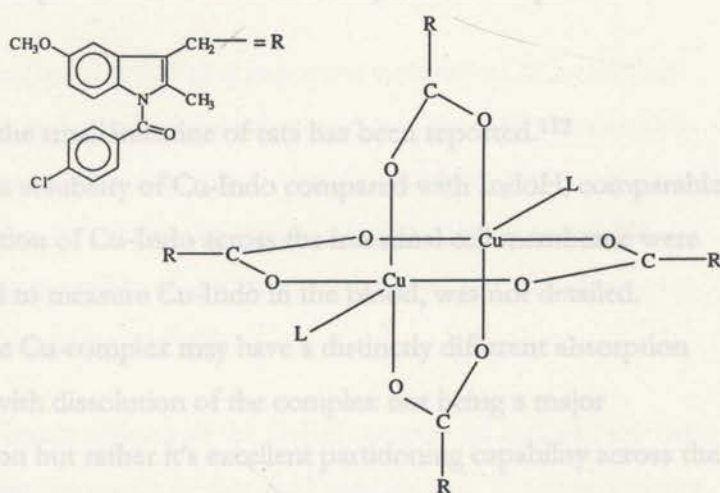


Figure 1.2. Structure of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$.

by chelation of endogenous Cu^{2+} , and that the Cu-NSAID complex remains intact as either a dimeric or monomeric entity^{109,110} rather than completely dissociating into its constituent elements in the GI tract and serum.

Doubts over the stability of Cu-NSAIDs in biological media follow from early computer simulations and *in vitro* studies that predicted for salicylic acid, that the Cu-NSAID could not exist in plasma.¹¹¹ Similar computer simulation work undertaken in 1997⁴³ on Cu(II)-anthranilic acid (anthranilic acid is not anti-inflammatory, whilst its Cu(II)-complex is) asserted the earlier prediction of an insignificant fraction of Cu(II) bound to its parent ligand in plasma.¹¹¹ However, it was proposed that there was a synergistic absorption of Cu(II) and anthranilic acid from the alkaline lower intestine, i.e., the major site of drug absorption, and formation of the Cu(II)-anthranilate complex in the acidic conditions of inflamed synovial fluid.⁴³ However, no therapeutic difference was found

between the administration of the pre-formed complex dissolved in solution and a mixture of its individual components at equivalent concentrations. Therefore, it was proposed that the pre-formed complex administered as suspensions exhibited a greatly enhanced bioavailability compared to the mixture of the individual reagents.⁴³ The observations of the potency and efficacy of Cu-complexes, along with the therapeutic use of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in dogs, where free IndoH can be fatal, indicates some intact Cu-Indo complex may survive in the stomach and perhaps be absorbed into plasma. This suggests the *in vitro* and computer simulation studies may not be adequate in predicting the *in vivo* situation.

Absorption of Cu-Indo from the small intestine of rats has been reported.¹¹² Notwithstanding poor aqueous solubility of Cu-Indo compared with IndoH, comparable blood levels and rapid penetration of Cu-Indo across the intestinal cell membrane were reported.¹¹² The method used to measure Cu-Indo in the blood, was not detailed. However, this suggests that the Cu-complex may have a distinctly different absorption profile compared to IndoH - with dissolution of the complex not being a major controlling factor for absorption but rather it's excellent partitioning capability across the intestinal cell membrane.¹¹²

Whilst the percent of a Cu-Indo dose that is absorbed across the gut lumen is uncertain, even less is known of its fate in plasma. A ternary human serum albumin-monomer or dimer complex of $[\text{Cu}(\text{DIPS})_2]$ or $[\text{Cu}_2(\text{DIPS})_4]$ (DIPS = diisopropylsalicylate) is proposed as the vector for distribution of this anti-inflammatory drug throughout the body.^{109,110} (A ternary complex is recognised as the mode of Cu binding to albumin).^{113,114} This hypothesis was based upon changes in the observed UV-Vis absorbance of serum albumin before and after the removal of $[\text{Cu}_2(\text{DIPS})_4]$ from a HSA sample by dialysis.¹¹⁰ Such direct interactions were not confirmed in subsequent studies by EPR, NMR and UV-Vis spectroscopies, where 95% of both Cu and DIPS are reportedly bound separately at different sites on the albumin.¹⁰⁹ The apparent dissociation of the Cu-complex on binding to HSA, while retaining its potent therapeutic action, led to the proposal that the action of the complex may be due to only small (<5%) amounts of either the mononuclear or dinuclear complex being bound intact to HSA.¹⁰⁹ It was concluded that either HSA delivers Cu(II) and DIPS to

the same cellular site, or that the administration of the Cu(II) complex of DIPS is only important in facilitating intestinal absorption. Moreover, it was proposed that chelates of Cu(II) formed with plasma/cellular proteins, e.g., histidine, following ligand exchange and that it was these plasma/cellular Cu(II) chelates which accounted for the pharmacological effects of drug rather than the administered Cu(II)-complex.¹⁰⁹ Further investigations of the ternary HSA complexes would require detection of very low concentrations of the Cu(II)-complex in the presence of substantially higher concentrations of their constituents, i.e., HSA, Cu and DIPS.

The full characterisation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is important because of its increasing use in veterinary pharmaceuticals and potential for use as a potent, yet safer, human anti-inflammatory drug. Little information is available on the bioavailability or pharmacokinetics of the complex, its stability, its structure in the currently available pharmaceutical formulations, or the effect of variations in the nature of the adduct on the chemical characteristics of the complex.^{93,106}

Consequently, a number of different adducts of the Cu(II) complex of IndoH have been prepared and investigated during the course of this PhD candidature. These complexes are reported as a prelude to the characterization of the DMF adduct in different pharmaceutical and biological media.

1.2 Inflammation and the Biological Role of Copper

As the pharmacology and pharmacokinetics of Cu-NSAID treatment of inflammation is uncertain, it is appropriate to briefly review the inflammation process, the mode of action of NSAIDs and Cu(II)-NSAIDs and role of Cu(II) in biology and inflammation.

1.2.1 Inflammation

Inflammation is a disease condition in which body tissues are affected by heat, redness, swelling and pain.¹¹⁵ John Hunter (1728 - 1793), one the early English physicians to scientifically study the reaction, described inflammation as such:¹¹⁶

"This operation of the body termed inflammation requires our greatest attention, for it is one of the most common and most extensive in its effect of any in the animal body".

The inflammatory response is a normal and essential response of the body to a harmful stimulus, and may vary from a localized reaction in affected tissue or joint, to a more generalized whole-body reaction.¹¹⁵ While inflammation may be a normal response, chronic inflammation results in destruction of normal connective tissue.¹¹⁷⁻¹²⁰ This destruction is due to the release of hydrolytic enzymes, such as collagenases and proteinases, that cause degradation of collagen and other extra-cellular components found in body joints and connective tissues.^{115,121} Inflammation is a feature of a number of diseases of which arthritis is just one – and the clinical features are described in standard medical textbooks.^{115,122}

The etiology of inflammation has been a subject of much research and debate over the years.^{57,61,119,120} Today, it is accepted that the process is initiated by the response of the body to an antigen.¹¹⁵ This antigen, having gained access to tissues, combines with antibodies from our defense system, so releasing a number of chemical mediators, such as PGs and leukotrienes. The release of these chemical mediators results in the heat, redness, swelling and pain. The inflammation process is summarised as:¹¹⁵

- i. initial injury causing the release of inflammatory mediators;
- ii. vasodilatation;
- iii. increased vascular permeability and exudation;
- iv. white blood cell migration, i.e., chemotaxis and destruction of antigenic material; and finally,
- v. proliferation of connective tissue cells as part of the healing process.

A general understanding of the pathology of inflammation; including the sequence of events, network of mediators and sites of action of the NSAIDs is well documented.^{4,10,14,47,123,124} This follows the discovery in the mid-1930s of PG¹²⁵ and the reporting of its structure in 1962.¹²⁶ The network of mediators, i.e., the inflammation cascade and sites of action of the NSAIDs, are shown in Figure 1.3.

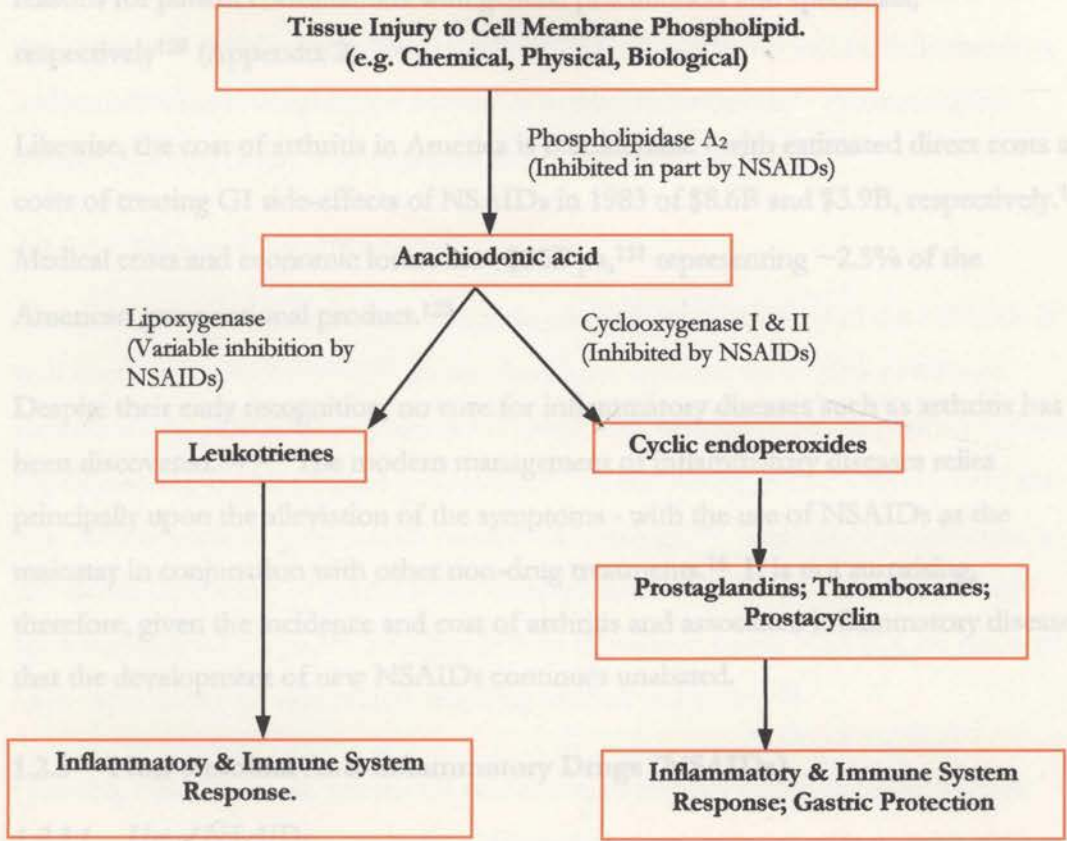


Figure 1.3. The Inflammation Cascade. Adapted from refs. 47 and 115.

1.2.2 Incidence and Costs of Inflammatory Diseases

Whilst arthritis and associated muscular skeletal diseases in themselves do not dominate the mortality statistics, they can account for considerable costs to both the patient and the community as a result of lost working hours, doctor visits and treatment. Their incidence¹²² and substantial socioeconomic costs have been well documented.^{127,128}

In Australia alone, the overall cost of arthritis to the community is \$1-5 billion pa.¹²⁷ Approximately 15% of the Australian population is affected by the disease, accounting for 22% of all visits to hospital outpatient departments and 7.5% of people having to leave the workforce.¹²⁷ Furthermore, more than 70% of these patients have used some form of medication for the relief of symptoms during the course of their illness.¹²⁷ Muscular skeletal diseases in Australia have been ranked fourth and fifth as the main

reasons for patient consultations with general practitioners and specialists, respectively¹²⁹ (Appendix 2).

Likewise, the cost of arthritis in America is considerable - with estimated direct costs and costs of treating GI side-effects of NSAIDs in 1983 of \$8.6B and \$3.9B, respectively.¹³⁰ Medical costs and economic losses are ~\$35B pa,¹³¹ representing ~2.5% of the American gross national product.¹²⁸

Despite their early recognition, no cure for inflammatory diseases such as arthritis has been discovered.^{16,122} The modern management of inflammatory diseases relies principally upon the alleviation of the symptoms - with the use of NSAIDs as the mainstay in conjunction with other non-drug treatments.¹⁶ It is not surprising, therefore, given the incidence and cost of arthritis and associated inflammatory diseases, that the development of new NSAIDs continues unabated.

1.2.3 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

1.2.3.1 *Use of NSAIDs*

It is asserted that NSAIDs are the most frequently prescribed drug in the world;^{77,132} with over 100M prescriptions pa.^{77,133} and resulting in sales worth between \$5B and \$9B pa.^{6,133,134} The ubiquitous use of acetylsalicylic acid, the prototype of the NSAIDs, is evidenced by its reported place as the most frequently used of all drugs world wide - with the exception of the use of non-medicinal caffeine and ethanol.¹²⁶ NSAIDs, other than aspirin, are used by ~30M people worldwide.¹³⁰

Approximately 66.5M prescriptions for NSAIDs were reported in the U.S. in 1983. This represented 4% of the total prescription market i.e., \$1B pa and approximately 13 prescribed daily doses per 1000 of the population (PDD).¹³⁵ By the 1990s, this use had increased to between 70 and 75 M prescriptions per annum, at an estimated cost of \$2.2 - \$ 2.3 B.²² In 1993, the U.S. NSAID prescription sales were estimated to be increasing at an annual rate of approximately 11%.²²

The use of NSAIDs is somewhat higher per head of population in Australia than the U.S., although the total number of prescription dispensed is considerably smaller.

Between 1990 and 1995, 30 to 40 PDDs of NSAIDs were reported accounting for approximately 4-6M prescriptions pa.¹³⁶ In 1995, the total cost of anti-inflammatory and anti-rheumatic drugs to the Australian Federal Government's Pharmaceutical Benefits Scheme (PBS), was estimated to be approximately \$60 M.¹³⁷

1.2.3.2 Iatrogenic Costs of NSAIDs

The incidence, pathogenesis and iatrogenic costs of the GI toxicity of the NSAIDs is well documented.^{21,78,133,138,139} Researchers have reported up to 30% to 40% of patients taking NSAIDs complain of GI symptoms, with up to 1% of patients suffering life-threatening GI bleeding or perforation.⁸⁹ At endoscopy, it has been reported that only 30% of patients present with normal GI histology.¹⁴⁰ The risk of hospitalization due to the GI toxicity of NSAIDs has been estimated at 1-2% and risk of death of approximately 0.13% pa.¹⁴¹ Roughly 25% of all patients taking NSAIDs are reported to have experienced GI side-effects requiring medical treatment.¹³⁰

Likewise, NSAIDs cause serious GI side effects in animals with some inter-species variations.^{108,142} Dogs are reportedly extremely susceptible to the GI toxicity of IndoH,¹⁴³⁻¹⁴⁵ with sometimes fatal consequences²⁰ resulting in recommendations that IndoH not be used in such animals.¹⁹ Consequently, much research has been undertaken into the development of newer and safer NSAIDs. This includes investigations of the specific COX-II inhibitors,^{12,15,146} nitric oxide releasing NSAIDs;^{21,147,148} leukotriene pathway inhibitors¹⁴⁹⁻¹⁵² and Cu(II) complexes of NSAIDs.^{95,96,153-155}

1.2.3.3 Mode of Action of NSAIDs: Inhibition of Prostaglandin Synthesis by the COX Enzyme System

It is now accepted that the NSAIDs work principally by inhibition of the COX enzyme, which has two catalytic sites (cyclooxygenase and peroxidase) sites.¹⁵⁶ In the first, arachidonic acid is converted into the cyclic endoperoxide PGG₂; while in the second PGG₂ is converted into PGH₂ by peroxidase activity.¹²³ The endoperoxides are further metabolized to form additional PGs, prostacyclin and thromboxane.²² The PGs and prostacyclin mediate and perpetuate inflammation by causing vasodilatation and enhanced vascular permeability - so promoting the inflammatory effects of histamine

and other cytokines.¹³⁴ The leukotrienes mediate inflammation by superoxide generation and increased vascular permeability.⁸ In addition, the leukotrienes act as potent chemotactic agents in the immune system and stimulate smooth muscle in the lungs.^{157,158}

There are two isoforms of COX, COX I and II, which have been well characterized since the early 1990s. The structure of COX II was first published in 1996¹⁵⁹ and shows a close resemblance to COX I,¹⁶⁰ except that the COX II active site is slightly larger and can accommodate larger substrates than the active site of COX I.¹⁶¹ The active COX I and COX II site is described as a hydrophobic channel of amino acids, culminating with serine 530, arginine 120 and tyrosine 385 at the apex.¹² Aspirin reportedly binds irreversibly to serine 580 by acetylation, so leaving the peroxidase activity unaffected.¹²³ This prevents access to arachidonic acid, whereas other NSAIDs, e.g., ibuprofen and mefenamic acid, are reported to sterically and reversibly bind to tyrosine 385 or arginine 120, thus blocking the COX action of the enzyme.^{12,156} Non-specific, instantaneous and reversible binding of other NSAIDs by a carboxylate bridge e.g., IndoH and flurbiprofen, to Arg 120 of both the COX-I and COX-II enzyme has been reported.¹⁵⁶ The *S*-enantiomer of flurbiprofen interacts through its carboxylate group with the arginine 120; allowing its second phenyl ring to come within van der Waals contact of the tyrosine 385.¹⁵⁶ Binding by the *S*-enantiomer of flurbiprofen is consistent with the much greater activity in blocking PG synthesis of the *S*-enantiomer than the *R*-enantiomer of NSAIDs.⁷

It is suggested that there are a number of sub-sites for binding in the narrow COX channel by the selective COX-II NSAIDs.^{123,161} These sites include the binding by the COX-II inhibitors, e.g., celecoxib or rofecoxib, through a phenylsulfonamide group to a side-pocket present in COX-II but not in COX-I¹⁵⁶ or to the apex of the more flexible COX-II channel e.g., by meloxicam.¹⁵⁶ It is the presence of a smaller valine (Val 523) in COX-II as opposed to isoleucine (Isoleu 523) in COX-I¹⁶² that allows access to the side pocket and binding by either a sulfonyl, sulfone or sulfonamide group, which is characteristic of the selective COX-II inhibitors.^{156,161}

Figure 1.4 shows the COX-I and COX-II binding channels, with the COX-II side pocket, non-selective Arg 120 binding site of COX-I and COX-II, prostaglandin synthesis site and the blocked entry of arachidonic acid.

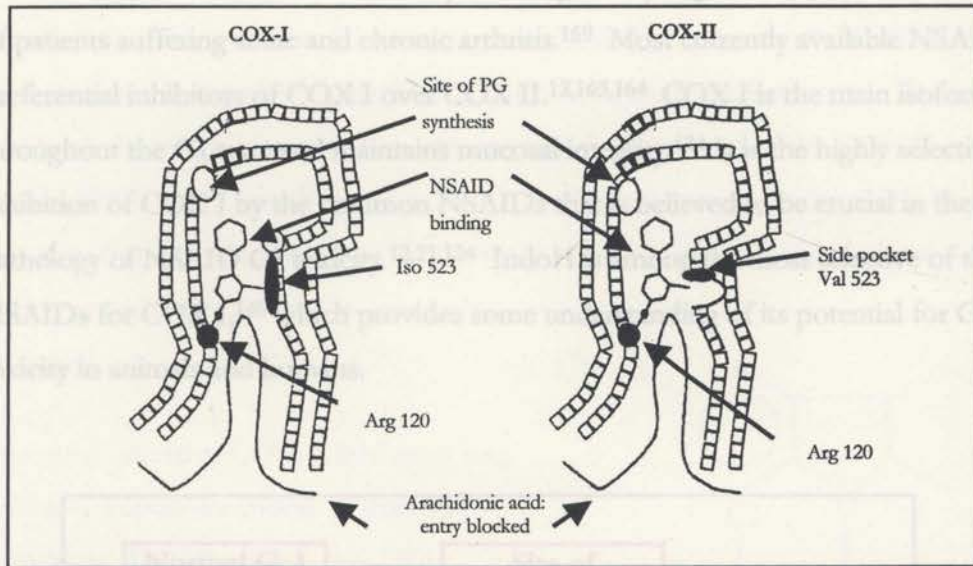


Figure 1.4. COX-I and COX-II binding channels. Adapted from ref. 161.

1.2.3.4 Cyclooxygenase II Inhibition and Gastric Toxicity of the NSAIDs

The two isoforms of COX explains the observation that the plasma and tissue concentrations of NSAIDs needed to obtain detectable anti-inflammatory effect *in vivo*, i.e. inhibition of constitutive COX I, are higher than those needed to inhibit cyclooxygenase *in vitro*, i.e., inhibition of inducible COX II.¹⁶³ COX I is believed to be involved in the maintenance of essential physiological functions, such as platelet aggregation, gastric protection and maintenance of renal function.¹⁶⁴ COX I activation leads to the production of prostacyclin, which when expressed by the endothelium, is anti-thrombogenic and when expressed by the gastric mucosa is gastric protective.¹⁴ The physiological and pathological roles of COX I and COX II, including the effect of various inflammatory mediators, corticosteroids and NSAIDs are illustrated in Figure 1.5.

Figure 1.5. COX I and COX II sites of action showing the enhancing (+) and inhibiting (-) effects of various inflammatory mediators, corticosteroids and NSAIDs. Adapted from ref. 12.

Unlike COX I, COX II is reportedly induced under inflammatory conditions,^{165,166} by cytokines and bacterial products such as liposaccarides.¹⁶⁷ Elevated expression of COX II is found in synovial tissue of patients suffering from rheumatoid arthritis,¹⁶⁸ however, both COX I and COX II have recently been reportedly expressed by synovial fluid cells of patients suffering acute and chronic arthritis.¹⁶⁹ Most currently available NSAIDs are preferential inhibitors of COX I over COX II.^{12,163,164} COX I is the main isoform throughout the GI tract and maintains mucosal integrity.¹³⁴ It is the highly selective inhibition of COX I by the common NSAIDs that is believed to be crucial in the pathology of NSAID GI toxicity.^{12,21,134} IndoH is among the most selective of the NSAIDs for COX I,¹⁴⁶ which provides some understanding of its potential for GI toxicity in animals and humans.

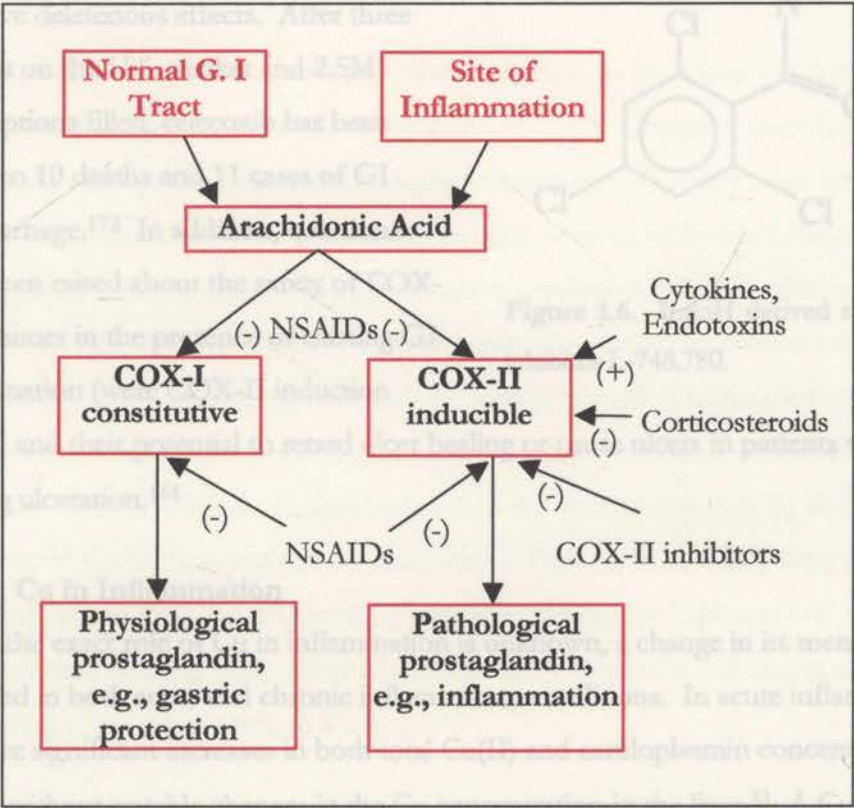


Figure 1.5. COX I and COX II sites of action showing the enhancing (+) and inhibiting (-) effects of various inflammatory mediators, corticosteroids and NSAIDs. Adapted from ref. 12.

Only two selective COX II inhibitors, celecoxib and rofecoxib, have been approved by the U.S. Food and Drug Administration for human use^{170,171} although a number of experimental compounds, e.g., nimesulide and flosulide are being developed.^{161,163} Of interest is the selective COX II inhibitory activity of the IndoH derivative L-748,780¹⁵ (Figure 1.6). Merck achieved the conversion of IndoH into a selective COX-II inhibitor by an increase in the steric bulk of IndoH with two chlorine substituents. The increase in steric bulk was proposed to take advantage of the larger size of the COX-II compared to the COX-I active site. This ability to convert IndoH into a selective COX-II inhibitor may have important implications for the study of possible mechanisms of anti-inflammatory action of the Cu-complexes of IndoH.

The use of selective COX-II inhibitors may still have deleterious effects. After three months on the U.S. market and 2.5M prescriptions filled, celecoxib has been linked to 10 deaths and 11 cases of GI haemorrhage.¹⁷² In addition, questions have been raised about the safety of COX-II inhibitors in the presence of existing GI inflammation (were COX-II induction occurs) and their potential to retard ulcer healing or cause ulcers in patients with pre-existing ulceration.¹⁶¹

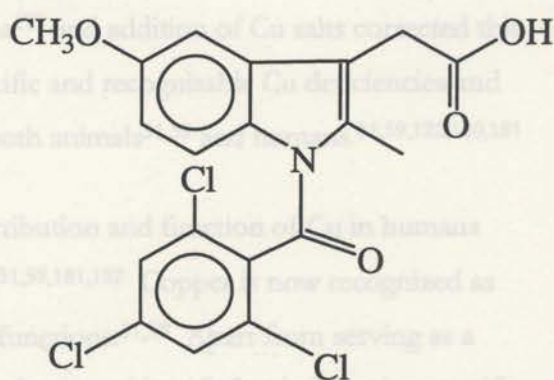


Figure 1.6. IndoH derived selective COX II inhibitor L-748,780.

1.2.4 Cu in Inflammation

Whilst the exact role of Cu in inflammation is unknown, a change in its metabolism is observed in both acute and chronic inflammatory conditions. In acute inflammation, there are significant increases in both total Cu(II) and ceruloplasmin concentrations in serum, without notable changes in the Cu concentration in the liver.³¹ A Cu-deficiency has a pro-inflammatory effect.¹⁷³ Likewise, in chronic inflammation, Cu serum concentrations are increased during the active phase, with appreciable ceruloplasmin levels in the synovial fluid of patients with rheumatoid arthritis,⁵⁹ with a net accumulation of Cu in an inflamed area.¹⁷³⁻¹⁷⁵ Furthermore, successful treatment with

exogenous Cu complexes leads to a reduction of serum Cu levels from their formerly elevated levels during the inflammation.⁵⁹ It is proposed that there is increased demand for Cu during inflammatory conditions, which is compensated for by enhanced intestinal absorption and/or decreased intestinal excretion of Cu.¹⁷⁴ The importance of Cu in preventing inflammation is further highlighted by its presence in such anti-oxidant metalloenzymes as Cu-Zn SOD.¹⁷⁶

1.2.5 Cu in Humans and Animals

1.2.5.1 *Biological Roles of Cu*

Copper was first shown to be essential for nutrition in the 1920s, when it was found that Cu-deficient diets in animals resulted in anemia¹⁷⁷ and addition of Cu salts corrected this deficiency syndrome.^{178,179} A number of specific and recognisable Cu deficiencies and disorders of Cu metabolism are described in both animals^{31,59} and humans.^{31,59,122,180,181}

Since the early 1920s, the metabolism, bio-distribution and function of Cu in humans and animals have been extensively researched.^{31,59,181,182} Copper is now recognized as an essential trace element for many biological functions.^{31,59} Apart from serving as a catalytic component in several enzymes, it has also been identified as influencing specific gene expression in mammalian cells,^{183,184} nerve myelination and endorphin action.¹⁸⁵

The primary role of Cu is as an important constituent of metalloproteins exhibiting oxidative reductase activity, e.g., oxidases or hydroxylases.³⁰ The function of the various Cu-enzymes includes radical scavenging activity (e.g., superoxidase dismutase, metallothionein, ferrioxidase I), ferrioxidase activity (ferrioxidase I & II) and metal transport.¹⁸³ Cu in these enzymes is classified as Type 1 ("blue" Cu), Type 2 ("non-blue" Cu); where Type 1 and Type 2 Cu are differentiated by EPR and UV-Vis characteristics, or Type 3 (dinuclear Cu-enzymes).¹⁸⁶ Some Cu containing enzymes and Cu-binding proteins are given in Table 1.2.¹⁸⁷

Table 1.2. Some Cu-containing Metalloenzymes and Cu-Binding Proteins. Adapted from ref. 187.

	<u>Cu Atoms Per Site</u>		
	Type 1	Type 2	Type 3
Mono (type 1) Cu Proteins			
Stellacyanin	1	—	—
Plastocyanin	1	—	—
Azurin	1	—	—
Amicyanin	1	—	—
Auracyanin	1	—	—
Planotacyanin	1	—	—
Mono (type 3) Cu Proteins			
Hemocyanin	—	—	2
Tyrosinase	—	—	2
Multi Cu Oxidases			
Ascorbic Oxidase	2	1	2
Laccase	—	—	2
Cu, Zn-Superoxidase Dismutase	—	1	—
Cu-binding proteins		Function	
Albumin	Cu transport		
Trancusprien	Cu transport		
Factor V, Factor VIII	Blood clotting factors (Cu may stabilise the structure of Factor VIII)		

1.2.5.2 Bioavailability of Cu

The intake of dietary Cu in humans amounts to approximately 2-4 mg per day; with the richest food sources being animal livers, shellfish, dried fruits, nuts and chocolate.¹⁸² The general bioavailability and metabolic fate of dietary Cu in humans is well known (Figure 1.7). Whilst whole-body Cu metabolism is difficult to study, isotropic tracers have enabled direct measurements. Of the seven isotopes of Cu, ⁶⁴Cu (t_{1/2} = 12.8 hr) and ⁶⁶Cu (t_{1/2} = 58.5 hr) are most commonly used for such metabolic and bio-distribution research.¹⁸⁸

Dietary Cu is absorbed into the body through the intestinal mucosa and transported to the liver. The major metabolic pathway involves the liver, where Cu is incorporated into ceruloplasmin then released into the blood and delivered to tissues. Most Cu is excreted via the bile into the GI tract. This Cu is excreted with small amounts of Cu from pancreatic and intestinal fluids and cells, and is then eliminated in the faeces.^{188,190,191} Little Cu is lost in the urine and sweat, or stored in the body. The mechanisms resulting in the excretion of Cu from the body have not yet been fully elucidated but most excreted Cu is in the form of ceruloplasmin.¹⁹² The body of an average healthy adult male (70 kg) contains ~110 mg of Cu, much of which is in the liver (10 mg), brain (8.8 mg), blood (6 mg), skeleton (46 mg) and skeletal muscle (26 mg).¹⁸⁹ Table 1.3 shows normal concentrations of Cu in various biological fluids and daily excretion in humans.

Table 1.3. Copper Concentrations in Various Biological Fluids and Daily Excretion in Humans.

Tissue	Humans	Daily excretion (µg)
Whole blood	800-1300 µg L ⁻¹ ³¹	—
Serum/plasma	800-1750 µg L ⁻¹ ³¹	—
Urine	12-80 µg L ⁻¹ ³¹	30-70 ¹⁹²
Gastric juice	0.39 µg g ⁻¹ ¹⁹²	1000 ¹⁹²
Bile	4.0 µg g ⁻¹ ¹⁹²	2500 ¹⁹²
Pancreatic fluid	0.13-0.9 µg g ⁻¹ ¹⁹²	400-1300 ¹⁹²
Duodenal fluid	0.17 µg g ⁻¹ ¹⁹²	400-2200 ¹⁹²
Synovial fluid	0.2-0.5 µg g ⁻¹ ¹⁹²	—

In mammals, Cu absorption varies inversely with uptake¹⁹⁰ and occurs from the stomach through to the small intestine,¹⁹³ predominantly in the stomach and jejunum.¹⁹⁴ While Zn, Fe, Mo reportedly decrease absorption, high protein diets increase absorption.^{193,195} The low pH value of the gastric juices is also claimed to contribute to the freeing of Cu bound to foodstuffs prior to GI absorption.¹⁹³ Once absorbed, approximately 70-80% of plasma Cu is in a non-exchangeable form bound to ceruloplasmin. The remainder is bound to albumin, transcuprien, metallothionein

and low-molar-mass (l.m.m) complexes, e.g., *L*-His-Cu(II)-*L*-Ser, *L*-His-Cu(II)-*L*Thr, as the exchangeable Cu fraction in blood.^{192,196}

Transfer of the Cu across the cell of the intestinal mucosa reportedly occurs by diffusion and carrier mediated systems.¹⁹² Once in the cytosol of the cell, ~80% of the Cu is bound to metallothionein.¹⁹² The intestinal mucosal cells then transfer Cu into the portal blood. Some Cu may be released into the portal blood with metallothionein.¹⁹⁷ Not a lot is known about the transport of Cu across the cell membrane, with mammalian genes encoding for Cu transporting P-type ATPases only recently identified.^{198,199} These enzymes are defective in Menkes syndrome,¹⁹² an inherited degenerative disease of the central nervous system involving Cu deficiency.³¹

1.2.5.3 *Cu Transport in the Blood*

Albumin appears to be the primary Cu carrier protein in the body - transferring a large amount of exchangeable Cu in the circulation and releasing it for specific cell uptake.¹⁸⁹ About 40 µg Cu can bind to the albumin in 1 mL of human plasma.¹⁸⁹

The Cu(II) transport site of serum albumin is one of the most extensively studied binding sites of any protein.²⁰⁰ The crystal structure and major NSAID ligand binding locations of human serum albumin (HSA) have been reported.^{201,202} The crystal structure of HSA complexed with the NSAIDs aspirin and ibuprofen identify hydrophobic cavities in sub-domains IIa and IIIA of HSA as the principal regions for NSAID binding.²⁰² The serum albumin binding site of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is as yet unidentified.

On entering the liver, Cu is incorporated into superoxide dismutase, cytochrome C oxidase, and ceruloplasmin. Most of the normal dietary Cu entering the liver re-emerges as ceruloplasmin.¹⁸⁹ Ceruloplasmin consists of 8 Cu sites, i.e. a trinuclear Cu cluster in domain 6, mononuclear Cu in domains 2, 4 and 6, and two labile Cu sites in domains 4 and 6.²⁰³⁻²⁰⁶ The carbon-chain representation of ceruloplasmin (Figure 1.8) shows the two labile Cu sites. Whilst ceruloplasmin is a major Cu-transport protein, it also exhibits a number of other physiological functions - including ferrioxidase and amine oxidase activity, and possible involvement in the regulation of blood clotting.²⁰⁷

Ceruloplasmin delivers Cu to specific receptors on the surfaces of cells of target tissues.²⁰⁸ On binding to the receptor, conformational changes in the protein lead to the release of the Cu in the cell where the fate of Cu in the target cell will be dependent upon cell type.^{207,208} The reduction of Cu(II) to Cu(I) seems to be a necessary step in the membrane transport of Cu into the cell.²⁰⁷

The metabolism, turnover and conservation of intracellular Cu are reportedly highly organ specific. Most intracellular Cu is recycled, with cells adapting to reduced bioavailability of Cu by reduced intracellular release.¹⁹⁸ The brain and heart, for example, are able to conserve Cu following minor decreases in total plasma Cu concentration but the sensors that activate this conservation have not been identified.¹⁹⁸

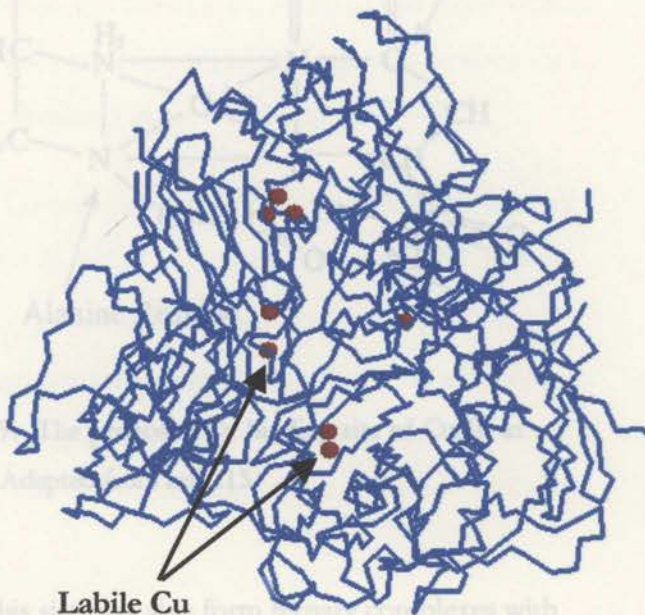


Figure 1.8. Carbon-chain representation of ceruloplasmin generated by the RasMol molecular visualisation program²⁰⁹ showing the two labile Cu-sites. Data taken from ref. 206.

1.2.5.4 Cu binding in Biofluids

Ternary complexes of Cu(II) reportedly form in plasma by the interaction between a l.m.m. species and albumin and these may provide a mechanism whereby Cu(II) is transferred between the various Cu binding substances in plasma.^{113,210} Cu binding to transcuprein is not well characterised but is reportedly greater than Cu binding to albumin, and rapidly exchanges with albumin.¹⁹⁶ Cu binding to metallothionein does not form a significant proportion of plasma Cu as it has a half-life of only a few minutes.¹⁹⁶

Copper binding to albumin is well characterised in a number of animal species.¹⁹⁶ The proposed structure of the Cu(II) binding site in human serum albumin (Figure 1.9) involves an *N*-terminus of albumin (*L*-Asp-*L*-Ala-*L*-His), which provides four N

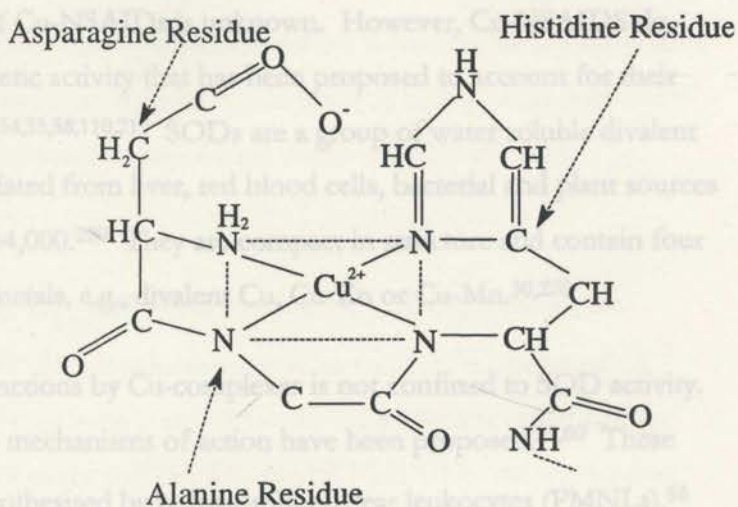


Figure 1.9. The proposed Cu binding site of Cu(II) in albumin. Adapted from ref. 213.

ligands: viz. the terminal amino group, two deprotonated peptide nitrogens and a *L*-His imidazole nitrogen.^{200,211} Copper in this site may also form ternary complexes with small ligands such as *L*-histidine and other endogenous ligands in plasma.^{113,212}

The study of Cu in biological fluids, e.g., kinetics and bio-distribution, can be complicated by the non-heterogeneous nature of biological fluids. A number of low molecular weight antioxidants, including ascorbic acid, α -tocopherol, β -carotene, bilirubin, uric acid and glutathione contribute to antioxidant defenses in serum.^{214,215}

1.2.5.5 *Cu(II) Binding Sites within Dogs*

The significance of Cu(II) binding sites within dogs is of interest given the reported absence of specific Cu(II) binding sites in dog albumin^{216,217} and the increased sensitivity of dogs to Cu intake compared with humans.¹⁹² The terminal amino acid sequence of X-Y-His, (the motif required for Cu binding in albumin, where X and Y are any amino acid) is altered in dog serum albumin (DSA),²¹⁸ with substitution of *L*-tyrosine by *L*-histidine.^{114,196}

1.2.6 Cu-NSAID Complexes and Proposed Modes of Action

The exact mode of action of Cu-NSAIDs is unknown. However, Cu-NSAIDs do exhibit a marked SOD mimetic activity that has been proposed to account for their anti-inflammatory activities.^{54,55,58,110,219} SODs are a group of water soluble divalent metal congener proteins isolated from liver, red blood cells, bacterial and plant sources of an approximate MW of 34,000.²²⁰ They are compact in structure and contain four atoms of chelated divalent metals, e.g., divalent Cu, Cu-Zn or Cu-Mn.^{30,220}

Modulation of biological functions by Cu-complexes is not confined to SOD activity. A number of other possible mechanisms of action have been proposed.^{23,60} These include inhibition of $O_2^{\bullet -}$ synthesised by polymorphonuclear leukocytes (PMNLs).⁵⁸ PMNLs are a part of the immune system and exert phagocyte activity. In addition, inhibition of lysyl oxidase activity²³ (a collagen cross-linking enzyme), modulation of nitric acid synthetase (NOS) activity¹⁵⁴ and the scavenging of free radicals¹³² has also been proposed.

The role of NO in explaining the mode of action of Cu-NSAIDs may be important. Nitric oxide has varied actions in the body - resulting in a number of normal and pathological actions.²²¹⁻²²⁴ However, studies of NO release are hampered by the short half-life of the molecule,²²⁵ with NO disappearing in blood within seconds because of avid binding with haemoglobin.²²⁵ Reported values of the *in vivo* half-life in humans of NO range from "very short"/"only a few seconds"²²⁶⁻²²⁹ to 5 s²³⁰ and an estimated half-life of 1.8 ms in whole blood.²³¹ The half-life of NO *in vivo* is also difficult to specify due to its dependence upon both location and tissue vascularity²³¹ and target, i.e. direct or indirect effects.³⁴ Direct effects, e.g., NO interactions with metal-containing proteins or organic free radicals, reputedly occur at lower concentrations or fluxes of NO, whereas higher NO fluxes result in indirect effect, e.g., effects mediated by reactive nitrogen oxide species derived from the interactions of NO with O_2 or $O_2^{\bullet -}$. The rapid and spontaneous interaction between $O_2^{\bullet -}$ and NO, with the $O_2^{\bullet -}$ anion rapidly inactivating NO to form the potent oxidants peroxynitrous acid (ONOOH) and peroxynitrite (ONOO):^{229,232}



is suggested to represent an important pathway by which tissue may be injured during inflammation.^{39,224,232} Peroxynitrite is an inorganic toxin of biological importance²³³ implicated in the pathology of several neuro-degenerative disorders²³⁴ and programmed cell death.²³⁵

SOD competes with NO for $\text{O}_2^{\cdot-}$, and by scavenging $\text{O}_2^{\cdot-}$, SOD promotes the vasodilatory action of NO.²²⁹ NO is now recognised as a critical mediator of GI mucosal defenses,^{236,237} and may even account for the GI protective effect of Cu-NSAIDs. NO-releasing derivatives of NSAIDs, i.e., NO-NSAIDs, are reported to limit the GI side effects of traditional NSAIDs.¹³⁴ In addition, small amounts of NO have been shown to stimulate the production of prostaglandins,²³⁸ with $\text{O}_2^{\cdot-}$ reputedly inhibiting COX, and possibly other enzymes involved in prostaglandin synthesis, by reducing the haem iron in COX to its inactive Fe^{2+} state.³⁴ It is postulated that the presence of small amounts of NO may prevent the reduction of haem by scavenging $\text{O}_2^{\cdot-}$, so maintaining the COX-associated haem iron in the Fe^{3+} state and resulting in the formation of GI protective prostaglandins.³⁴

Besides the disproportionation of $\text{O}_2^{\cdot-}$,²³ the anti-inflammatory Cu(II) dimer $[\text{Cu}_2(\text{DIPS})_4]$ oxidises nitric oxide synthase (NOS).¹⁵⁴ This observation adds weight to the proposal that the anti-inflammatory, anti-convulsant, anti-diabetic and other pharmacological activities of Cu-NSAIDs may be due to NO-mediated events.¹⁵⁴ However, whilst NO is recognised as a mediator of mucosal blood flow, mucus release, repair of mucosal injury and inhibitor of neutrophil activation,¹³² no significant difference is reported between the NOS activities stimulated by IndoH (~ 0.06 mM/kg) or $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (~ 0.01 mM/kg).¹³² This is despite the use of the Cu-complex at 20-100 times the therapeutic dose.¹⁰⁸

1.3 Aim and Outline of this Thesis

Despite the wealth of material on the potential applications of Cu(II) complexes of NSAIDs,^{53,239} only the DMF adduct of the Cu(II) complex of IndoH is currently patented as an anti-inflammatory agent and its application is limited to veterinary practice.^{93,106,107} Furthermore, little is understood about the possible mode of the action of the Cu-NSAIDs, including their nature, transport and binding characteristics in the body,¹⁰⁹ or properties in pharmaceutical products.

The characterisation of Cu(II) complexes of IndoH is important because of their increasing use as veterinary pharmaceuticals and potential to be used as potent human anti-inflammatory drugs. The axial DMF ligands may exchange during drug absorption and metabolism or following incorporation into pharmaceutical preparations so potentially affecting reactivities and efficacies of the drug. It is, therefore, important to establish the structure of the Cu-IndoH complex with a number of adducts and in different pharmaceutical preparations.

This thesis describes the synthesis and solid-state characterisation of Cu(II) complexes of IndoH as DMF, acetonitrile (MeCN), *N,N*-dimethylacetamide (DMA), 1-methylpyrrolidone (NMP), ethanol (EtOH) and aqua adducts. Furthermore, the stability and quantification of the DMF adduct in a number of proprietary veterinary formulations and biological samples will be presented and the results discussed in terms of advancing an understanding of the efficacy and pharmacology of these complexes.

Chapter 2 is a description of the materials and experimental conditions used in the course of the investigations; including the formulae of selected proprietary formulations of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and synthesis of the Cu(II) complexes of IndoH. Chapter 3 outlines the results and discussions of the solid-state characterisation of the Cu(II) complexes; including their magnetic and spectroscopic properties and X-ray absorption fine structure (XAFS). These studies are important to assist in identifying the changes, if any, in the nature of the Cu(II) complexes as a result of variations in the nature of the axial ligands. Results on attempts to separate $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ from IndoH by capillary electrophoresis and electrospray ionisation mass spectroscopy are

also discussed. In Chapter 4, the results and discussions of the characteristics and stability of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in various veterinary pharmaceutical preparations, i.e., tablets, an oral powder and pastes, and ophthalmic and parental formulations, are presented. In addition, the nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in a number of biological media and buffers is reported. Finally, Chapter 4 contains a discussion of the implications of the research on understanding the pharmacology and pharmaceutics of Cu(II) complexes of IndoH and points to future research directions.

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CHAPTER TWO

EXPERIMENTAL

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2.1 Materials

The therapeutic formulae of the proprietary products, *Cu-Algesic*TM and *Avigesic*TM, and a manufacturing “pre-solution” of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (BVR) (Figure 2.1) are listed in Table 2.1. The reagents and solvents (Table 2.2) are commercially available and were used as received unless otherwise stated.



Figure 2.1. *Cu-Algesic*TM and *Avigesic*TM.

The same batches of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{NMP}, \text{DMA}, \text{OH}_2$) were used in all analyses unless otherwise stated. All glassware used in the analyses was soaked for 24–48 hr in a 1:1 solution of 70% nitric acid and Milli-Q water and then rinsed in Milli-Q water before use. Deionized water (electrical resistivity: 0.04–0.1 $\text{M}\Omega \text{ cm}$; pH: 6; heavy metals $< 0.04 \text{ mg mL}^{-1}$ at 25°C) was obtained using a Milli-Q Plus water system.

Table 2.1. Therapeutic Formulae of the $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ Veterinary Products (BVR).

Product (Conc. $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$)	Emulsion ^a	Therapeutic Formula
<i>Avigesic Forte</i> TM (4 mg mL ⁻¹)	o/w	Cremophor (4%), ^b N-methyl-2-pyrrolidone (4%) in Milli-Q water.
<i>Cu-Algesic</i> TM tablets (2 mg)	–	Dextrose (24.8%), cellulose (35%), maize starch (25.6%), magnesium stearate (4.27%), Tixosil (4.27%), ^c purified starch (4.27%).
<i>Cu-Algesic</i> TM paste (200 mg/5 g)	o/w	Carbopol (1%), ^d Nippasol M (0.5%), ^e NaOH (8.5%) in water.
Medium chain triglyceride (MCT) paste (200 mg/5 g)	–	Tetraglycol (40%), Span 80 (20%), ^f MCT (29%), ^g Aerosil (7%). ^b
Paraffin paste (200 mg/5 g)	–	liquid paraffin B.P. (33%), white soft paraffin B.P. (67%).
<i>Cu-Algesic</i> TM Granules (200 mg/20 g)	–	Aerosil (2.5%), ^b castor sugar (96%).
<i>Cu-Algesic</i> TM Ophthalmic Capsules (0.5 mg 0.4 mL ⁻¹)	–	α -tocopherol acetate (0.025% w/w), Span 80 (1.272%), ^f tetraglycol (1.272%) in MCT. ^g
“Pre-solution” (48 mg mL ⁻¹)	–	Span 80 (1.272%), tetraglycol (1.127%) in MCT.

^a o/w = oil-in-water emulsion. ^b CremophorTM = castor oil derivative (emulsifying agent). ^c TixosilTM = Silica based flow enhancing agent (used in the bulk manufacture of powders). ^d CarbopolTM = hydroxypropyl methylcellulose (gel-point initiated by basic conditions). ^e Nippasol MTM = *n*-propyl-4-hydroxybenzoate (a preservative). ^f Span 80TM = sorbitan mono-oleate (oil-soluble water dispersible non-ionic emulsifying agent). ^g MCT = medium chain triglyceride.

^b AerosilTM = Silica based flow enhancing agent containing > 99.8% SiO₂.

Table 2.2. Reagents Used in this Work.

Compound	Purity	Supplier
Acetonitrile (MeCN)	99.5%	Sigma
Albumin (horse), desiccated (Fraction V)	99.5%	Sigma
Benzalkonium chloride (alkyldimethylbenzylammonium chloride)	> 98% (Cu < 0.005%)	Aldrich
Boric Acid	99.5%	Aldrich
Tetrahydroborate	99.5%	Sigma
Cremophor EL	—	Sigma
Copper(II) chloride	99.9%	Sigma
Copper(II) acetate	99%	Univar
Copper(II) nitrate	> 98.0%	Ajax
Dodecyl sodium sulfate (sodium lauryl sulfate) (SDS)	95%	Sigma
Glycerin (water-free)	98-100.5%	Merck
Hydrochloric acid	31-33%	Sigma
Hydroxypropyl methylcellulose (Carbopol)	10% Hydroxypropyl & 30% Methoxyl content	Aldrich
Iron(II) ammonium sulfate hexahydrate	< 0.002%Cu	Riedel-De Haën
N-(2-hydroxyethyl) piperazine- <i>N'</i> -(sodium 2-ethanesulfonate) (HEPES sodium)	99%	Aldrich

Table 2.2. Reagents Used in this Work. (cont.).

Compound	Purity	Supplier
<i>N,N</i> -dimethylformamide (DMF)	99.8%	Univar
<i>N,N</i> -dimethylacetamide (DMA)	99%	DuPont
<i>N</i> -methyl-2-pyrrolidone (NMP)	99.5%	ISP
3-[<i>N</i> -morpholino]propanesulfonic acid (MOPS)	99.5%	Sigma
<i>N</i> -tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)	99%	Sigma
Nitric acid conc. (68.5-69.5%)	—	Sigma
Nitric acid 65%	Suprapur (Cu < 0.001 µg/g)	Merck
<i>N</i> -propyl <i>p</i> -hydroxybenzoate (Nippasol M)	> 99%	Sigma
Sodium hydroxide	97%	Ajax
Sodium tetraborate (Borax)	> 99.5%	Univar
Sulfuric acid, fuming [1.84 g mL ⁻¹ (Cu < 0.001 g mL ⁻¹)]	96-100%	Sigma
Titrasol Cu(II) standard	1.000 g ± 0.002 g Cu	Merck
Triethanolamine	98%	Aldrich
Vitamin E (α-tocopherol acetate)	50% adsorbed onto a silicon dioxide substrate	Redox Chemicals

2.2 Synthesis

2.2.1 Cu(II) Complexes of Indomethacin

The C, H, N elemental analyses of the complexes were performed by the Department of Chemical Engineering, University of Sydney or the National Analytical Laboratories (NAL) within 1-3 weeks of their synthesis. Copper was determined using a Varian AA-800 air-acetylene flame atomic absorption spectrophotometer.

I. Bis(*N,N*-dimethylformamide)tetrakis- μ -(*O,O'*-Indo)dicopper(II) *N,N*-dimethylformamide dihydrate, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$. To a warm ($\sim 50^\circ\text{C}$) DMF (20 mL) solution of IndoH (14.3 g, 0.04 mol) was added a solution of Cu(II) acetate monohydrate (4 g, 0.02 mol) in DMF (25 mL) and the mixture was heated to 80°C . Ethanol (250 mL) was added to the mixture with vigorous shaking and, after ~ 1 d, a microcrystalline light green powder precipitated from the resulting deep green solution. The mixture was filtered under vacuum and the green product was washed exhaustively with ethanol (100 mL) and left to dry on the benchtop overnight at room temperature. Yield 13.9 g (76%). Anal. ($\text{Cu}_2\text{C}_{85}\text{H}_{85}\text{Cl}_4\text{N}_7\text{O}_{21}$) C, H, N, Cu: calcd., 56.4, 4.7, 5.4, 7.0; found, 56.4, 4.6, 5.4, 7.0%.

II. Bis(*N,N*-dimethylformamide)tetrakis- μ -(*O,O'*-Indo)dicopper(II) 1.6-*N,N*-dimethylformamide, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot 1.6(\text{DMF})$. This complex was crystallised by BVR from a 36% w/v solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ dissolved in a mixture of 20% DMF and 80% ethanol. Anal. ($\text{Cu}_2\text{C}_{86.8}\text{H}_{85.2}\text{Cl}_4\text{N}_{7.6}\text{O}_{19.6}$) C, H, N: calcd., 57.4, 4.7, 5.9%; found, 57.8, 4.8, 5.9%. A DMF adduct, containing two DMFs of crystallisation was obtained at the University of Sydney from a 1 mg mL^{-1} solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ dissolved in a mixture of 80% DMF and 20% water and left to stand for $\sim$ two years. The X-ray crystallographic data was measured on an Enraf-Nonius CAD4F four-circle diffractometer employing graphite-monochromated Mo $\text{K}\alpha$ radiation in a manner reported for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot 1.6(\text{DMF})$.¹ Insufficient quantities of crystals however, were obtained for microanalysis.

III. Bis(*N,N*-dimethylacetamide)tetrakis- μ -(*O,O'*-Indo)dicopper(II) trihydrate, $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2] \cdot 3\text{H}_2\text{O}$ was prepared as for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, except that DMA was used as the solvent. Ethanol was added and the solution was cooled overnight to yield a microcrystalline dark green precipitate. The mixture was filtered under vacuum and the product was washed exhaustively with ethanol (150 - 300 mL), left to dry on the benchtop overnight at room temperature, and finally oven dried at 40 °C for 8 hr. Yield 6.40 g (35%). Anal. ($\text{Cu}_2\text{C}_{84}\text{H}_{84}\text{Cl}_4\text{N}_6\text{O}_{21}$) C, H, N, Cu: calcd., 56.6, 4.8, 4.7, 7.1; found, 56.2, 4.9, 4.9, 7.5%.

IV. Bis(*N*-methyl-2-pyrrolidone)tetrakis- μ -(*O,O'*-Indo)dicopper(II) dihydrate, $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2] \cdot 2\text{H}_2\text{O}$ was prepared as for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ except that NMP was used as the solvent. Yield 12.08 g (66%). Anal. ($\text{Cu}_2\text{C}_{86}\text{H}_{82}\text{Cl}_4\text{N}_6\text{O}_{20}$) C, H, N, Cu: calcd., 57.8, 4.6, 4.7, 7.1; found: 57.8, 4.5, 4.7, 7.8%.

V. Diaquatetrakis- μ -(*O,O'*-Indo)dicopper(II) sesquihydrate, $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$. IndoH (4 g, 0.01 mol) in ethanol (50 mL) was added to Cu(II) acetate monohydrate (1 g, 0.005 mol) in ethanol (50 mL) and a pale green Cu complex began to precipitate within minutes of mixing. While vigorously shaking the resultant solution, ethanol (150 mL) was added and then the mixture was left to stand at room temperature overnight. The mixture was filtered under vacuum and left to dry on the benchtop overnight at room temperature. Yield 3.9 g (78%). Anal. ($\text{Cu}_2\text{C}_{76}\text{H}_{67}\text{Cl}_4\text{N}_4\text{O}_{19.5}$) C, H, N, Cu: calcd., 56.4, 4.2, 3.5, 7.9; found, 56.30, 4.26, 3.62, 8.10%.

VI. Diaquatetrakis- μ -(*O,O'*-Indo)dicopper(II), $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$. To an aqueous NaOH solution (0.1 M, 100 mL) was added IndoH (14.3, 0.04 mol) and the resulting solution was filtered to remove undissolved IndoH. Copper(II) chloride dihydrate (1 g, 0.006 mol) in water (25 mL) was added and the solution was heated to 50 °C during which time the Cu complex separated as a pale green powder. The mixture was filtered under vacuum and the green product was washed exhaustively with ethanol and water. Yield 11.9 g (78%). Anal. ($\text{Cu}_2\text{C}_{76}\text{H}_{64}\text{Cl}_4\text{N}_4\text{O}_{18}$) C, H, N, Cu: calcd., 57.4, 4.1, 3.5, 7.9; found, 57.6, 4.1, 3.9, 7.9%.

VII. (Acetonitrile)(Aqua)tetrakis- μ -(O,O'-Indo)dicopper(II) Monohydrate, $[\text{Cu}_2(\text{Indo})_4(\text{MeCN})(\text{OH}_2)] \cdot \text{H}_2\text{O}$ or Diaquatetrakis- μ -(O,O'-Indo)dicopper(II)

1.0 acetonitrile, $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot \text{MeCN}$ was prepared as outlined for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ except that MeCN was used as the solvent, no EtOH was added to the synthesis, and the mixture was set aside at $\sim 4^\circ\text{C}$ for 24-48 hr. Yield 11.52 g (63%). Anal ($\text{Cu}_2\text{C}_{78}\text{H}_{69}\text{Cl}_4\text{N}_5\text{O}_{19}$) C, H, N, Cu: calcd., 56.8, 4.2, 4.3, 7.7%; found: 57.5, 4.1, 4.3, 8.2%.

However, the addition of EtOH during synthesis and leaving the sample aside for $\sim$ six months prior to elemental analyses resulted in $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$. Anal. ($\text{Cu}_2\text{C}_{76}\text{H}_{69}\text{Cl}_4\text{N}_4\text{O}_{20.5}$) C, H, N, Cu: calcd., 56.4, 4.2, 3.5, 7.9, found: 56.1, 4.2, 3.3, 8.0%.

2.2.2 Micelle-Buffer and "Artificial Gastric Juice" Solutions

Micelle solutions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ were prepared with SDS (40 mM) in 100 mM buffer solutions of TES, HEPES, MOPS or triethanolamine adjusted to pH $\sim$ 7.4 and SDS (40 mM) in borate buffer (50 mM) adjusted to pH $\sim$ 9.0. The SDS (40 mM) borate buffer (50 mM) was prepared using 200 mM boric acid (3.6 mL), 75 mM tetraborate (7.9 mL) and 200 mM SDS (12.5 mL). Sonication of the buffer samples for approximately 5 min was required to ensure homogeneous suspensions. Artificial "gastric juice"² (pH $\sim$ 1.6) was prepared by diluting 0.4% HCl (1 mL) in Milli Q water (6 mL). All solutions were freshly prepared prior to analyses unless otherwise stated.

2.3 Instrumentation

2.3.1 UV-Vis Spectroscopy

Diffuse reflectance and solution (DMF) UV-Vis electronic spectra were recorded over the range 200-1200 nm and 400-900 nm, respectively, using a Varian Cary 5E UV-Vis-NIR spectrophotometer. Solid-state spectra were acquired in a KBr matrix using a Cary Praying Mantis accessory (Part No. 00-100469-00). The spectra of the DMF solutions were acquired in 1-cm quartz cells in the double-beam acquisition mode. Spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the biological buffers and equine serum were recorded on a Hewlett-Packard HP 8452A Diode-array (180-800 nm) and Lambda Bio 40 spectrometers (180-800 nm), respectively. The reaction of a 4:1 molar ratio of

IndoH:[Cu₂(Indo)₄(DMF)₂] and IndoH in DMF and were recorded using the Bio-Sequential SX-17MV Stopped-Flow Reaction Analyser.

2.3.2 Infrared Spectroscopy

Solid-state IR (400-2000 cm⁻¹, KBr matrix) and far IR (75-500 cm⁻¹, polyethylene matrix) spectra were acquired from pressed disks on a Bruker IFS 66v FTIR spectrophotometer (resolution 1.0 cm⁻¹).

2.3.3 Fourier Transform Raman Spectroscopy

The solid and DMF solution state Raman spectra (100-4000 cm⁻¹) were recorded with a Renishaw Ramanscope and Renishaw Raman Imaging Microscope WiRE (v1.2), using the 514.5-nm line of an Ar⁺ laser for excitation (100% laser power, 20 s exposure time and 4 continuous scans).

2.3.4 Thermogravimetric Analysis

Dynamic thermogravimetric analyses (TGA) of IndoH and the aqua and MeCN adducts of Cu-Indo complexes were conducted with a TA Instruments HI-Res TGA 2950 Thermogravimetric Analyzer equipped with a Platinel II thermocouple. Experiments were performed using Pt crucibles and a constant heating rate of 20 °C min⁻¹ from room temperature to 800 °C. The weight loss of the sample was recorded every 2 s.

The sample pan was tared before each analysis, and cleaned between each experiment by a 20-min run through a thermal program to 900 °C. A weight, temperature and sample platform calibration was undertaken prior to the experimentation in accordance with the instrument manufacturer's recommendations. The TGA was purged for at least 30 min prior to the start of each experimental session. Plots of the TG decomposition steps were obtained following fast Fourier filter smoothing of the differentiated TG data and plotting the inverse values of the derivative data versus temperature.

2.3.5 Electrospray Ionisation Mass Spectroscopy

The electrospray ionisation mass-spectroscopy (ESI-MS) was undertaken using a Mat LCQ MS detector with LCQ Data Processing and Instrument Control Software. Samples were dissolved in MeCN and introduced through a loop injector with methanol (50%), water (50%) and acetic acid (0.1%) as the flow carrier. The ESI spray voltage was 5 kV, sheath gas (N₂) gas pressure, 60 psi; heated capillary temperature,

200 °C; voltage, 19 V; and tube lens offset, 27 V. Full mass and zoom mass spectrometry scans were run in the negative mass spectrometer operating mode. Samples investigated included $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, and a 4:1 molar mixture of $\text{IndoH}:[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. All samples were freshly prepared prior to analyses. Simulations of the electrospray mass spectra were undertaken using IsoPro MS/MS v3.01 software.³

2.3.6 Micellular Capillary Electrophoresis

The micellular electrophoresis was undertaken using a Beckman P/ACE System coated capillary system at 214 nm in the normal, i.e., inlet -ve and outlet +ve, and reverse polarity mode. Two pre-rinses of the capillary were undertaken prior to each sample analysis – consisting of a 1-min 0.1 M NaOH rinse followed by a 3-min rinse of the capillary with the running buffer solution. The capillary was equilibrated prior to each change of buffer by cleaning for 1 min with 0.1 M NaOH followed by a 30-min equilibrating rinse of the capillary with the new buffer solution.

2.3.7 X-ray Powder Diffraction

Powder X-ray diffraction patterns were collected at room temperature using $\text{CuK}\alpha$ radiation on a SIEMENS D5000 diffractometer with divergence and anti-scatter slits of 1 mm, and receiver and detector slits of 0.2 mm and 0.6 mm, respectively. The continuous scan data were collected over the range $2.0^\circ - 35.0^\circ$ in steps of 0.04° in 2θ , and a count time per step of 15.0 s. Profile fitting, indexing and refinement of the unit cell parameters of the powder diffraction patterns were undertaken using the PROSZKI System of Programs for Powder Diffraction Data Analysis (v2.4).⁴ Earlier measurements were collected for BVR at the University of Sydney by Alan McKenzie but were not used in this thesis.

2.3.8 Flame Atomic Absorption Spectroscopy (Flame AAS)

Copper analyses were determined using a Varian AA-800 air-acetylene flame atomic absorption spectrophotometer with a Cu hollow-cathode lamp. The main instrument parameters for the analysis were: wavelength, 324.8 nm; lamp slit width, 0.5 nm; normal slit height; lamp current, 4 mA; and acetylene and air gas flow of 2.00 and 13.5 L/min, respectively. The optimal working range of the instrument was $0.03\text{--}10\ \mu\text{g mL}^{-1}$. A matrix solution of 0.1% HCl was prepared from Milli-Q water. The standards, controls

and samples were diluted in the matrix using grade B glassware. A series of calibration standards (1, 2, 4, 6 and 8 mg Cu(II) mL⁻¹) were prepared by diluting a Titrasol™ Cu(II) standard solution (1000 g Cu) with 0.1 M HCl in Milli-Q water.

The Cu(II) dimer (~50 mg) was wet-ashed with Suprapur nitric acid (5 mL) and fuming sulfuric acid (10 mL) with subsequent additions of nitric acid over a 4-hr period until the samples were clear. Unknown and blank samples were volumetrically adjusted with the matrix solution to 100.00 mL prior to triplicate analysis of the Cu content. Wet ashing of the *Cu-Algesic*™ gelatin capsule contents (1 mL) and whole capsules (two capsules) were performed as above over a 24-48-hr period. Unknown and blank samples of the capsule and capsule contents were volumetrically adjusted to 25.00 mL with the matrix solution prior to triplicate analysis of the Cu content.

2.3.9 Graphite Furnace Atomic Absorption Spectroscopy (Graphite AAS)

Copper in equine whole blood samples was determined using a Varian Spectra AA 20 atomic absorption spectrometer, equipped with an auto-sampler, GTA 96 Graphite Tube Atomizer and a Cu hollow-cathode lamp. The instrumental parameters are listed in Appendix 3. Whole blood samples were collected in 10-mL sterile blood collection tubes (Venoject II) without anticoagulant. Once collected, the samples were packed in ice and refrigerated at 4 °C for a maximum of 5 days prior to analysis.

A working matrix (diluent) solution of 0.1 % HCl in Milli-Q water (plus 3 drops of Titron X100™ L⁻¹) was prepared. A working standard solution of CuCl₂ 50 µg L⁻¹ was prepared from this matrix solution. The standard curve (10, 20, 30, 40 and 50 µg L⁻¹) was obtained by pre-programming the automatic sampler to dilute aliquots of the working standard with the diluent solution. All samples were analyzed sequentially in triplicate according to the parameters listed in Appendix 3. Wet ashing of whole blood (~5 mL) was initiated with 65% Suprapur (13 mL) and fuming sulfuric acid (~5 mL); with subsequent additions of nitric acid over a 12-14-hr period until the samples were clear. Unknown and blank samples were adjusted volumetrically with the matrix solution to 25.00 mL, followed by a 1:10 dilution with the matrix solution. The dissolved Cu content was determined immediately.

2.3.10 Magnetic Susceptibility

The room-temperature gram magnetic susceptibilities (χ_g) and magnetic moments (μ_{eff}) were measured on a Sherwood Scientific Magnetic Susceptibility Balance using iron(II) ammonium sulfate hexahydrate as the calibrant.⁵ The calculated molar magnetic susceptibility (χ_M) was determined from the measured susceptibility per gram (χ_g) as per the Equation 2.1:⁶

$$\chi_M = \chi_g \times \text{MW} \quad 2.1$$

The per Cu paramagnetic susceptibility (χ_p) and magnetic moment (μ_{eff}) were subsequently determined from the following equations:⁶

$$\chi_M = \chi_D + \chi_p \quad 2.2$$

$$\mu_{\text{eff}} = 2.828\sqrt{\chi_p T} \text{ (BM)} \quad 2.3$$

respectively, where χ_D is the estimated diamagnetic susceptibility. In all cases, the value of χ_D was estimated as the sum of the measured or calculated value of the diamagnetic susceptibility of IndoH and adducts, respectively. The value of χ_D of the adducts was obtained by taking the sum of the atomic diamagnetisms of all the atoms present and a small constitutive corrections (ϵ) for specific electronic characteristics, e.g., π -bonds.⁵

2.3.11 Variable Temperature Magnetic Susceptibility

Variable temperature magnetic susceptibility data were collected over the range 5-300 K using a Quantum Design MPMS SQUID magnetometer by Professor Keith Murray and Dr Boujemaa Moubaraki at Monash University. The samples were cooled in zero field. In all cases the raw data were corrected for the diamagnetic sample holder. In the fitting procedure of the Bleany-Bowers equation,⁷ all of the experimentally observed susceptibilities were equally weighted and final fits were obtained by minimizing the function in Equation 2.4:³¹

$$E = \sum [(\chi_{\text{obsd}} - \chi_{\text{calcd}})T]^2 \quad 2.4$$

2.3.12 X-band Electron Paramagnetic Resonance Spectroscopy

Room and low temperature X-band EPR spectra of powdered and solution samples of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ were measured at X-band frequencies (~ 9.5 GHz) using a Bruker EMX EPR spectrometer equipped with a standard ER4120 X band cavity, EMX 035M NMR gaussmeter, EMX 032T field controller, EMX 081 magnet power supply, Bruker EMX 048T microwave bridge control and BVT2000 variable temperature unit and Oxford Instruments E900 continuous flow cryostat (for low temperature data collection). Low temperature X-band EPR Cu(II) spectra of solution samples of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ containing less than 0.47 mM Cu(II) were measured using a Bruker ESP 300 spectrometer equipped with a Hewlett Packard 5352B microwave frequency counter, Bruker ER 085C magnet power supply, Bruker ER 032 magnet field control, Bruker ER 023M signal channel and Bruker ESP 1600-1048 microwave bridge controller.

All Cu(II) monomer spectra were collected at 4 K. Powdered solids, 100- μL solution and approximately 20 mg paste samples were placed in quartz epr tubes (2-mm o.d., 1.5 mm i.d.) for both low and room temperature data collection. Paste samples were placed into polyethylene tubes cut from the tips of 1-mL transfer pipettes prior to insertion into the epr tube.

The Cu(II) monomer content relative to the total initial Cu(II) content of samples was calculated using the WINEPR data analysis program⁸ by determining the double integral (DI) of the Cu(II) monomer spectra of the solution/paste sample and comparing this to a CuCl_2 calibration curve. Samples for the CuCl_2 calibration were prepared in Milli-Q water using grade B volumetric glassware. Glycerin (20% w/w) or DMSO (20%) was added to the CuCl_2 calibration samples to produce vitrified samples suitable for EPR spectroscopy. A spectrum of the empty resonator cavity and polyethylene paste sample holder was recorded prior to any Cu(II) calibration experiment to confirm a negligible contribution of the cavity and polyethylene sample holder to the sample spectra. The absence of signal saturation for the Cu(II) monomer calibration spectra was checked by verifying a decrease in signal intensity by the square root of the microwave power with decreasing microwave power.⁹

Proprietary samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ for Cu(II) monomer determination were supplied by BVR while those containing $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ or $[\text{Cu}_2(\text{Indo})_4(\text{DMF}_2)_2]$ without Nippasol were prepared at the University of Sydney.

The values of $g_{\parallel}$ and $g_{\perp}$ were determined using the EPRPOW and SIMER simulation programs^{10,11} and the values of D and E were determined from the ZF-EPR measurements. Details of the X-band instrument parameters for the collection of the Cu(II) monomer and dimer spectra are given in Appendix 4.

2.3.13 Zero-field Electron Paramagnetic Resonance Spectroscopy (ZF-EPR)

The ZF-EPR data were collected using a resonant reflection ZF-EPR spectrometer^{12,13} at 60, 120 and 145 K by Drs Richard Bramley and Christopher Delfs at the Australian National University. The spectra were simulated by taking the difference between spectra, calculated in zero field and with an applied field (B) equal to the magnitude of a modulated field ($B = \pm 2$ mT). In the least-squares fitting procedure, all of the experimentally observed data were weighted equally and final fits were obtained by minimising the function:

$$R = \sum_k \left(\frac{S_k^0 - S_k^c}{\sigma_k} \right)^2 \quad 2.5$$

where S_k^0 and S_k^c are the observed and calculated ZF-EPR spectrum intensities and σ_k is the estimated error in the measured value.¹⁴ The value of D was obtained from the frequency at the centre of the spectrum, $2E$ from the distance between the two innermost peaks and, $A_{\parallel}$ (assuming $4A_{\parallel} \gg E$) from the distance between the two outermost peaks on one side of the spectrum.^{12,14}

2.3.14 X-ray Absorption Spectroscopy and X-ray Fine Structure Analysis

X-ray absorption spectroscopy (XAS) was performed in transmission or fluorescence modes for the powder and veterinary paste/solution samples, respectively. A brief description of the theory of XAS is given in Appendix 5.

2.3.14.1 Transmission and Fluorescence XAS Data Collection

Transmission, typically used for solid and solution samples > 2% w/w, and fluorescence, typically used for approximately 1-15 mM of absorbing atom, XAS measurements were performed at the Australian National Beamline Facility (ANBF) on bending magnet beamline 20B at the KEK, Photon Factory, Tsukuba, Japan using a Si (111) channel-cut monochromator. The storage ring delivered a current of 250-400 mA at 2.5 GeV. The samples for low temperature XAS were pre-chilled in liquid nitrogen prior to insertion into a Crydone REF-1577-D22 closed cycle cryostat and maintained at 10 K during XAFS data collection with a Neocera LTC-11 temperature controller unit.

The solid samples were ground to a fine powder and used without further dilution ($\approx 7.5\%$ wt. Cu). The powders were pressed into a pellet ~ 1.0 -mm thick and supported in an Al sample holder with Kapton™ windows. Triplicate scans were recorded in transmission mode at both room temperature and 10 K using standard N₂-filled ionization chambers, where the intensity of the X-ray beam is measured before and after the sample, and the resultant absorbance (μ) was determined using the expression:¹⁵

$$\mu x = -\ln(I_1/I_0)$$

2.6

where, x is the thickness of the sample, I_1 is the intensity after the sample, and I_0 is the intensity before the sample. The intensity of the X-ray beam measured before and after a Cu-foil standard (I_f) was similarly measured to standardise the energy of the Cu-edge.

The monochromator crystal was detuned 50% throughout the Cu K-edge XAS measurements. Energies were calibrated by means of the Cu foil; the first inflection point being assigned as 8980.3 eV.¹⁶

Fluorescence XAS experiments were undertaken on the veterinary paste samples (containing 0.7 mM of Cu) at 10 K using a Canberra™ 10-element Ge detector in an experimental set-up similar to that used in the transmission mode measurement, except that the sample and fluorescence detector were orientated at 45° and 90°, respectively to the incident beam. In the fluorescence XAS mode, the fluorescence excitation spectrum is proportional to μ versus X-ray energy - where the intensity of the X-ray fluorescence detected (I_f) is that produced when higher-shell electrons relax into the hole left by the

emitted photoelectron.^{17,18} A schematic of the transmission and fluorescence XAS experimental modes and Canberra 10-element Ge fluorescence detector set-up are shown in Figures 2.2 and 2.3, respectively. The fluorescence detector response is linear for the count rate employed (up to $\sim 150\text{k s}^{-1}$).^{17,18} All fluorescence spectra were collected with incoming count rates (ICR) per channel of $\sim 15\text{--}52\text{k counts s}^{-1}$.

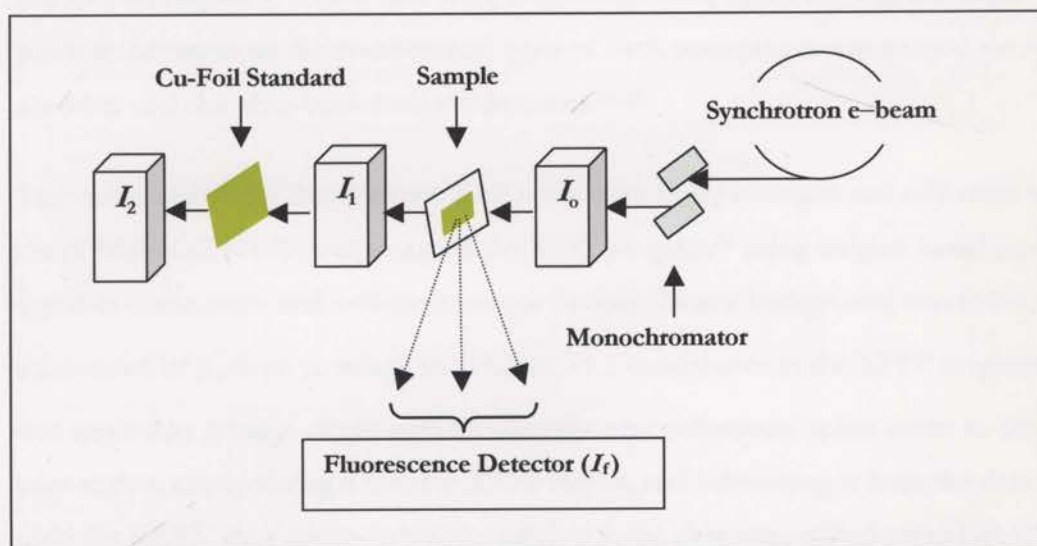


Figure 2.2. Transmission and fluorescence XAS experimental modes. In fluorescence mode, the sample and fluorescence detectors are orientated 45° and 90° , respectively, to the incident beam.

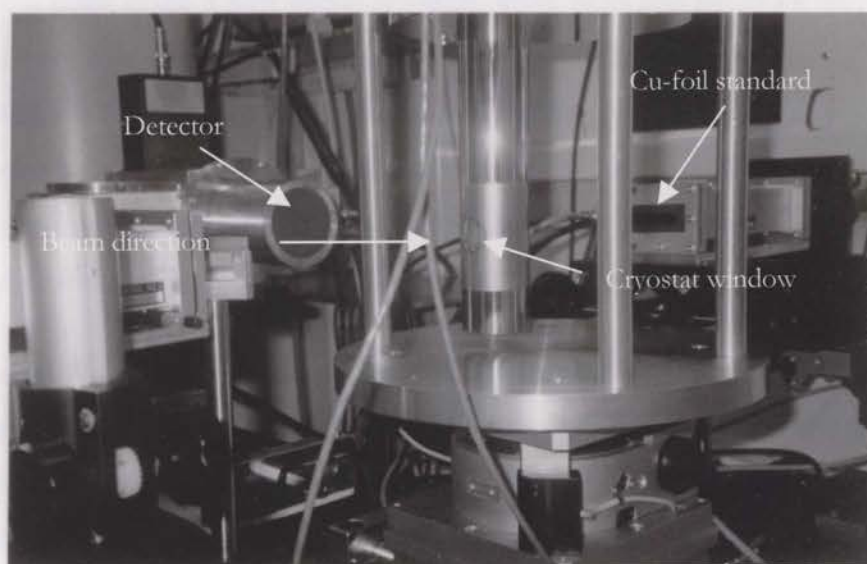


Figure 2.3. Canberra 10-element Ge fluorescence detector, Cu-foil standard and Kapton™ windows of the cryostat sample chamber.

2.3.14.2 XAS Data Extraction

The XAFS is derived from the XAS prior to any curve-fitting analysis of a parameterised XAFS model to the experimentally observed XAFS.^{15,19,20} A full description of XAFS data extraction and analysis technique is described elsewhere.¹⁹⁻²¹ XAFS data extraction and analysis was undertaken using the XFIT suite of programs AVERAGE, SPLINE, GRAPH and XFIT.²⁰ These allow for the optimisation of the parameters of an atomic model with respect to XAFS data using a non-linear least-squares fitting technique that yields information on the numbers and types of back-scattering atoms around the metal absorber and absorber-back-scatterer distances.^{21,22}

The transmission and fluorescence XAS scans were firstly averaged and calibrated using the AVERAGE (v1.1) component of the XFIT program²⁰ using weights based on the signal-to-noise ratios and calibrated using a Cu-foil. Next a background correction, i.e., subtraction of μ_s from μ , using the SPLINE v1.1 component of the XFIT program²⁰ was applied by fitting a single segment second-order polynomial spline curve to the pre-edge region, extrapolating it into the XAFS region, and subtracting it from the data to yield the XAFS. Any monochromator glitches in the data were edited using GRAPH (v 1.1)²⁰ following the calibration and subtraction of the background absorption.

Finally, for the removal of the underlying absorbance, a three-region, second-order spline was fitted to the XAFS region at ~ 9021 , 9225 , 9669 and 10127 eV and subtracted. The resultant data were normalised to an edge jump of 1.0 and compensated for decreasing absorbance past the edge with a k -weighting = 3 so that the final XAFS is relative to the edge (μ_0) and the amplitude enhanced at high k oscillations. The background-subtracted and normalized XAFS data were converted to k^3 -space, where k is the photoelectron wave vector, and plotted as a function k .

In addition, the XFIT program calculates a Fourier transform (FT) of the resultant k -weighted XAFS spectrum as a function of the distance of the back-scattering atoms from the metal absorber distance ($\text{\AA}$). The FT (of which the inverse of the FT is the original XAFS)²⁰ displays peaks near R_{ex} and at magnitudes comparable with the number of back-scattering atoms at that distance. A phase correction is required to directly relate the peak positions of the FT plot to the true inter-atomic distances.²³

2.3.14.3 XAS Data Analysis

Optimisations of the XAFS model parameters were undertaken with respect of the experimentally observed and extracted XAFS data using the XFIT v1.1 component of the XFIT package,²⁰ i.e., a curve-fitting analysis with *ab initio* phase and amplitude parameters calculated using the Single Scattering (SS) FEFF 4.06 program and the curved-wave Multiple Scattering (MS) program FEFF 6.01.²⁴⁻²⁶

In the XAFS curve-fitting analysis, a parameterised model is “fitted” to the observed XAFS by adjusting the structure-dependent parameters of the theoretical XAFS expression to optimise agreement with the experimentally observed XAFS. The parameters treated as variables in order to optimize this agreement, were the positional parameters (x, y, z) of all the atoms in the model, the Debye-Waller factors σ^2 , scale factor S_0^2 and the threshold energy E_0 . The final values of these parameters yield information on the coordination and geometry around the absorber. The XAFS model’s goodness of fit parameter (R_{xaf}) is calculated as:²⁰

$$R_{\text{xaf}} = [\Sigma(k^3(\text{data} - \text{fit}))^2 / [\Sigma(k^3(\text{data}))^2]^{1/2} \quad 2.7$$

Both the observed and calculated XAFS and FT data were filtered. The XAFS (k -space) and FT (r -space) window parameters used in this work were typically set at 0-12.94 $\text{\AA}^{-1}$ and 0.7-4.7 $\text{\AA}$, and 0-12.94 $\text{\AA}^{-1}$ and 0.8-2.4 $\text{\AA}$ for MS and SS analyses, respectively. The k - and r -windows used in the XAFS analyses are shown in the XAFS and FT figures. The degree of determinacy of the system²⁷ (N_i / N_p) was determined from the number of independent data points (N_i) and parameters (N_p) of the XAFS data set and model, respectively, where:²⁷

$$N_i = \frac{2(\Delta r)(\Delta k)}{\pi} + \sum_i D(N - 2) + 1 \quad 2.8$$

Δr and Δk are the ranges of the FT and XAFS filtered data, respectively, and D and N are the number of dimensions (1 for single scattering, 2 for planar groups) and atoms per refinement unit, respectively. Refinements with a value of $N_i / N_p < 1$ were rejected as they correspond to less than one data point per refinement parameter.²⁷ Thus, the MS model used in the current thesis, with values of Δr and Δk of 3.9 $\text{\AA}$ and 12.40 $\text{\AA}^{-1}$,

respectively, $N_p = 20$ (E_o , S_o^2 , 11 and 7 positional parameters and Debye-Waller thermal parameters, respectively), $\sum_i = 7$, and degree of determinancy ≈ 1.89 , is supportable.

Statistical errors in bond distances and bond lengths due to noise in the XAS were determined by Monte-Carlo calculations.^{20,28} Typical systematic errors (σ_s) in the metal-nitrogen/oxygen distances in XAFS analysis of 0.01 – 0.02 Å²⁹ were combined with the Monte-Carlo calculations of statistical errors (σ_r)²⁸ to obtain the estimated maximum root mean square (r.m.s) error $([\sigma_r^2 + \sigma_s^2]^{1/2})$.³⁰ The value of σ_s for the bridging O–C–O bond angle was calculated using the e.s.d.'s of the O–O and O–C bond lengths. Typically, systematic errors in bond angles of 1° were calculated for bond lengths with an e.s.d. of 0.02 Å. The estimated maximum r.m.s errors are shown in the thesis as standard deviations in the reported bond distances and bond angles.

2.3.14.4 SS and MS XAFS Models

SS and MS XAFS analyses of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) were undertaken. Based on these results, only MS analyses of the veterinary formulations and solutions were performed. The numbering system for the MS XAFS model is shown in Figure 2.4. A representation of the 3-shell SS model and examples of two-, three- and four-leg MS pathways around the Cu absorber (Cu_0), which give rise to the XAFS signals, are shown in Figures 2.5(a) and (b), respectively.

The path $\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$ represents the strongest contribution to the MS XAFS analyses (Table A9.1). The importance of other MS paths to the model is represented as a contribution expressed as a percentage relative to the strongest path. For example, paths $\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$ and $\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$, have 45% and 15% of the contributions relative to the strongest pathway, respectively (Table A9.1).

A description of the two- and three-SS XAFS model including shell atom type, and the number of scattering atoms (N_s) are given in Table 2.3. A table of the crystal structure atom numbers of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot 1.6\text{DMF}$ and the corresponding MS XAFS model atom numbers (N), distances (Å) from the Cu absorber (Cu_0) and positional parameters

Figure 2.4. MS XAFS model for the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ series.

of the MS XAFS model atoms < 5.5 Å from Cu₀ and figures of the crystal structure numbering of the Indo groups are given in Appendix 8.

Table 2.3 The Two- and Three-Shell SS Model.

Shell No.	2-shell SS Model		3-Shell SS Model	
	Atom Type	N _s	Atom Type	N _s
1	O	5	O _{Ac}	4
2	Cu	1	O _L	1
3	—	—	Cu	1

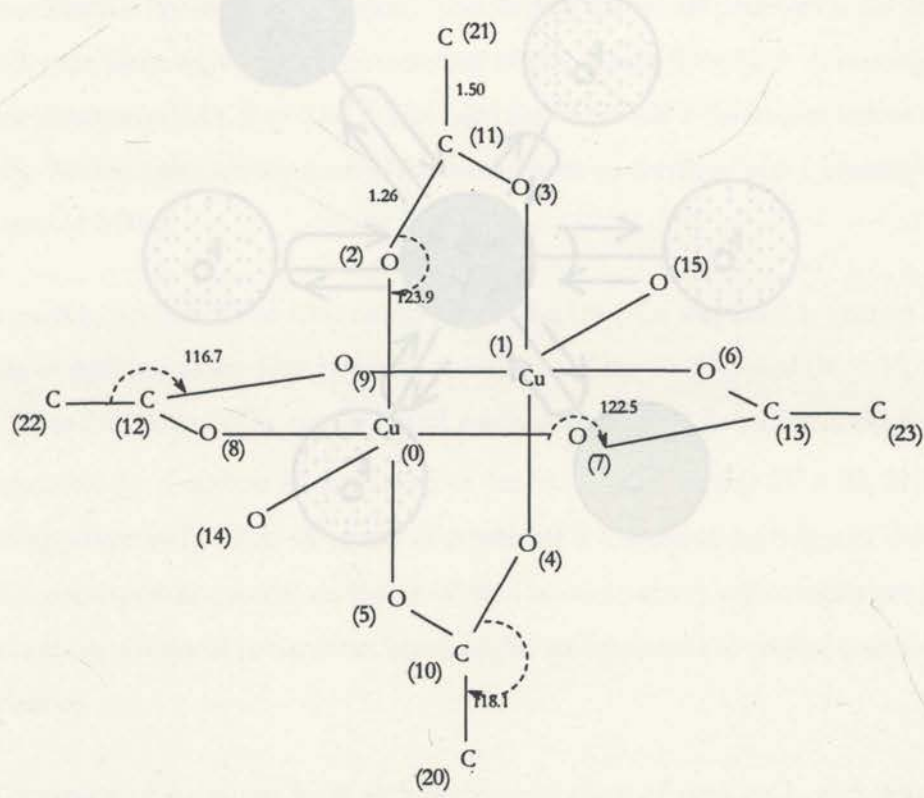


Figure 2.4. MS XAFS model for the [Cu₂(Indo)₄L₂] core.

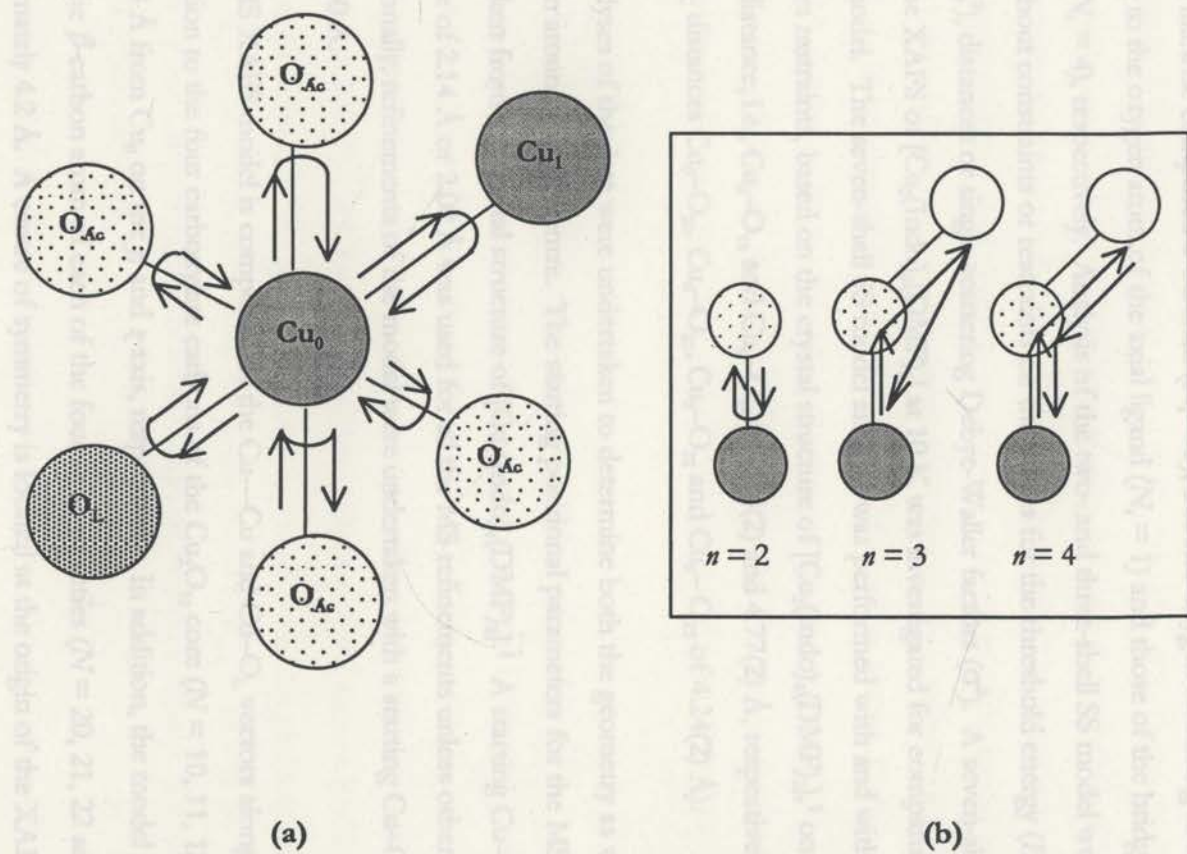


Figure 2.5. (a) 3-shell SS model, i.e., Cu-Cu₀, Cu-O_{Ac} and Cu-O_L shells, and (b) MS pathways around the central Cu absorber atom (Cu₀) of [Cu₂(Indo)₄L₂] for $n = 2, 3$ and 4 legs per scattering pathway.

SS analysis was initially undertaken to determine any similarities in the co-ordination of the Cu in the various powder samples, and to determine if it was possible to distinguish the axial Cu–O_L bond from the mean Cu–O_{Ac} bond length. The two-shell SS model comprised a Cu scattering shell ($N_s = 1$) and an oxygen shell ($N_s = 5$). The three-shell SS model likewise comprised a Cu shell ($N_s = 1$), but two oxygen scattering shells corresponding to the oxygen atom of the axial ligand ($N_s = 1$) and those of the bridging Indo groups ($N_s = 4$), respectively. Analysis of the two- and three-shell SS model were performed without constraints or restraints on the values for the threshold energy (E_0), scale factor (S_0^2), distances or single-scattering Debye-Waller factors (σ^2). A seven-shell SS model of the XAFS of [Cu₂(Indo)₄(DMF)₂] at 10 K was investigated for comparison with the MS model. The seven-shell SS model analysis was performed with and without vector distances restraints, based on the crystal structure of [Cu₂(Indo)₄(DMF)₂],¹ on the axial Cu₀–O_L distance, i.e., Cu₀–O₁₄ and Cu₀–O₁₅ of 2.14(2) and 4.77(2) Å, respectively, and the Cu–C_C distances Cu₀–O₂₀, Cu₀–O₂₁, Cu₀–O₂₂ and Cu₀–O₂₃ of 4.24(2) Å.

MS XAFS analyses of the data were undertaken to determine both the geometry as well as coordination around the Cu centre. The starting positional parameters for the MS model were taken from the crystal structure of [Cu₂(Indo)₄(DMF)₂].¹ A starting Cu–O_L vector distance of 2.14 Å or 2.00 Å was used for all the MS refinements unless otherwise stated. Additionally, refinements of the model were undertaken with a starting Cu–O_L distance of 2.00 Å.

The starting MS XAFS model is composed of the Cu---Cu and Cu–O_L vectors along the *x*-axis, in addition to the four carboxylate carbons of the Cu₂O₁₀ core ($N = 10, 11, 12$ and 13) at 2.84 Å from Cu₀ on the *y*, and *z* axis, respectively. In addition, the model incorporates the β-carbon atom of each of the four Indo moieties ($N = 20, 21, 22$ and 23) at approximately 4.2 Å. A centre of symmetry is located at the origin of the XAFS model (corresponding to the midpoint of the Cu---Cu vector) with constraints and restraints on positional parameters, bond angles and distances to ensure such a symmetry restriction.

The deviation of the atoms from each orthogonal plane of the Cu₂O₁₀ core was restrained to be less than 0.1 Å, with deviations in the angles restrained to be less than 2°. The bridging O–C–O angles of the Cu₂O₁₀ core were not restrained. All

refinements were undertaken without bond distance symmetry restraints on Cu–O_L unless otherwise stated, but constraints were incorporated to ensure an equi-distance of the two Cu–O_L bonds in the dimer.

All Debye-Waller factors were restrained to be greater than zero

($\sigma > 0.001$ (0.0005) ($\text{\AA}^2$)) and less than 0.08 ($\sigma < 0.08$ (0.001) ($\text{\AA}^2$)). The Debye-Waller factors were set to increase with increasing distance away from the Cu absorber, with identical shells constrained to have the same Debye-Waller factor. The incorporation of constraints and restraints was used to reduce the degrees of freedom in the model and hence result in better defined minima and a faster convergence of the refinements.²⁷

The MS XAFS model bond angle ($^\circ$) and bond length ($\text{\AA}$) restraints, Debye-Waller restraints (σ^2 ($\text{\AA}^2$)) and Debye-Waller (σ^2 ($\text{\AA}^2$)) and positional parameter (x, y, z) constraints are given in Tables 2.4, 2.5 and 2.6, respectively. MS refinement models investigated also included scattering atoms of the DMF ligand and Indo groups within 5.0 $\text{\AA}$ of Cu₀.

The current work excludes contributions from the axial ligand other than the coordinated oxygen atom. Attempts to include these atoms into the MS model proved unsuccessful. A description of these investigations is given in Chapter 3 and Appendix 7. As there were no significant changes in the refined bond distances and angles of [Cu₂(Indo)₄(DMF)₂] at RT as a result of the exclusion of the atomic XAFS (AXAFS) region (r -values < 0.7 $\text{\AA}$), but a significant improvement in the goodness-of-fit parameter; (i.e. $R_{\text{xafs}} = 20.7$ % (r -window 0.2-4.6 $\text{\AA}$) compared to $R_{\text{xafs}} = 12.6$ % (r -window 0.7-4.6 $\text{\AA}$) (see Chapter 3), all refinements used an r -window Fourier filter of 0.7-4.6 $\text{\AA}$ unless otherwise stated.

The MS calculations included ~ 65 unique paths when the total distance traveled by the photoelectron (R_{eff}) was ≤ 10.0 $\text{\AA}$ and not more than 5 legs ($n = 5$) in each MS pathway. To reduce the refinement time for each MS calculation, curve-wave (CW) and plane-wave (PW) filters were set to 3% and 2%, respectively. These filters ensured CW and PW paths with importance factors of less than 3% and 2%, respectively, of the most important path (Cu₀ $\rightarrow$ O₅ $\rightarrow$ Cu₀), were rejected from the final refinement during the analysis. A table of the MS paths and their importance factors is given in Appendix 9 (Table A9.1). For [Cu₂(Indo)₄L₂] (L = DMF, OH₂) at 10 K, MS refinements also

included paths with $R_{\text{eff}} \leq 10.2 \text{ \AA}$, a maximum of 6 legs per pathway and CW and PW filters set at 0 %, i.e., representing ~ 130 unique MS pathways. A table of the resulting MS paths and importance factors of the paths is given in Appendix 9 (Table A9.2).

Table 2.4. The MS XAFS Model Angle ($^\circ$) and Bond Length ($\text{\AA}$) Restraints.^a

$\text{Cu}_0\text{-O}_7\text{-C}_{13} \approx 123(2)$	$\text{C}_{10}\text{-C}_{20} \approx 1.50(0.02)$	$\text{C}_{20}\text{-C}_{10}\text{-O}_4 \approx 118(2)$
$\text{O}_2\text{-C}_{11} \approx 1.26(0.02)$	$\text{Cu}_0\text{-O}_2\text{-C}_{11} \approx 124(2)$	$\text{C}_{22}\text{-C}_{12}\text{-O}_9 \approx 117(2)$

^a All dihedral angles and their inverted symmetric mirror image are set at zero ($\pm 0.1 \text{ \AA}$) and zero ($\pm 2 \text{ \AA}$), respectively.

Table 2.5 The MS XAFS Model Debye-Waller Restraints ($\sigma^2 (\text{\AA}^2)$).

$\sigma_{13}^2 > (\sigma_7^2 + 0.001) (0.001)$	$\sigma_{23}^2 > (\sigma_{13}^2 + 0.001) (0.001)$	$\sigma_{10}^2 > (\sigma_5^2 + 0.001) (0.001)$
$\sigma_6^2 > (\sigma_7^2 + 0.001) (0.001)$	$\sigma_{20}^2 > (\sigma_{10}^2 + 0.001) (0.001)$	$\sigma_4^2 > (\sigma_5^2 + 0.001) (0.001)$
$0.001(0.0005) < \sigma_n^2 < 0.08(0.001); n = 1\text{-}23$		

Table 2.6 The MS XAFS Model Debye-Waller ($\sigma^2 (\text{\AA}^2)$), and Positional Parameter (x, y, z) Constraints.

$y_{O1} = 0$	$z_{O2} = 0$	$z_{C12} = -z_{C13}$	$x_{O6} = -x_{O7}$
$z_{O1} = 0$	$z_{O3} = 0$	$z_{C22} = -z_{C23}$	$z_{O6} = -z_{O8}$
$y_{O6} = 0$	$z_{O4} = 0$	$x_{O2} = -x_{O4}$	$z_{O7} = -z_{O9}$
$y_{O7} = 0$	$z_{O5} = 0$	$x_{O3} = -x_{O5}$	$z_{O6} = z_{O7}$
$y_{O8} = 0$	$z_{C10} = 0$	$x_{O2} = -x_{O3}$	$x_{O2} = x_{O8}$
$y_{O9} = 0$	$z_{C11} = 0$	$y_{O2} = -y_{O4}$	$y_{O2} = z_{O8}$
$y_{C12} = 0$	$z_{C20} = 0$	$y_{O3} = -y_{O5}$	$y_{C10} = z_{C13}$
$y_{C22} = 0$	$z_{C21} = 0$	$y_{O2} = y_{O3}$	$y_{C20} = z_{C23}$
$y_{C13} = 0$	$x_{C10} = 0$	$y_{C10} = -y_{C11}$	$x_{O15} = -x_{O14}$
$y_{C23} = 0$	$x_{C20} = 0$	$y_{C20} = -y_{C21}$	$y_{O15} = 0$
$x_{C12} = 0$	$x_{C11} = 0$	$x_{O6} = -x_{O8}$	$z_{O15} = 0$
$x_{C22} = 0$	$x_{C21} = 0$	$x_{O7} = -x_{O9}$	$x_{Cu0} = -x_{Cu1}$
$x_{C13} = 0$	$y_{O14} = 0$	$\sigma_{10}^2 = \sigma_{11}^2 = \sigma_{12}^2 = \sigma_{13}^2$ $\sigma_{20}^2 = \sigma_{21}^2 = \sigma_{22}^2 = \sigma_{23}^2$ $\sigma_3^2 = \sigma_4^2 = \sigma_6^2 = \sigma_9^2$ $\sigma_{20}^2 = \sigma_{21}^2 = \sigma_{22}^2 = \sigma_{23}^2$	
$x_{C23} = 0$	$z_{O14} = 0$		

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CHAPTER THREE

SOLID-STATE CHARACTERISATION

3.1 The Chemistry of Copper

The magnetic and electronic properties of Cu(II) allow the application of a range of analytical techniques to assist in the characterisation of the coordination of Cu(II) in IndoH complexes. The results of some of these techniques are presented in this chapter, along with the results of attempts to distinguish IndoH from $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ using micellar capillary electrophoresis (MEC) and electrospray ionisation mass spectroscopy (ESI-MS).

Excellent reviews on the general chemistry of Cu and the properties of mononuclear and dinuclear Cu(II) species are reported elsewhere.¹⁻⁵ A brief description of the electron-spin electron-spin dipolar interaction for two coupled Cu(II) centres and equations used in this thesis for the determination of the electron paramagnetic resonance (EPR) and zero-field EPR (ZFEPR) parameters⁶⁻⁸ are given in Appendix 10. The magneto-structural characteristics of Cu(II) dimers are reviewed in the following section.

3.1.1 Cu(II) Dimer Compounds and Their Magnetic Properties

The dimeric and polymeric Cu(II) complexes formed from multidentate ligands, may have Cu...Cu distances short enough to show metal-to-metal super-exchange interactions.⁹⁻¹² A weak coupling of the unpaired electrons of the two Cu(II) atoms, gives rise to a singlet ground state ($S = 0$; spin multiplicity $(2S + 1) = 1$) with a triplet state ($S = 1$; spin multiplicity $(2S + 1) = 3$) lying only a few kJ mol^{-1} above it.¹³⁻¹⁵

The triplet state is appreciably populated at normal temperatures and the compounds are paramagnetic. At 25 °C, the magnetic moment (μ_{eff}) of these dimeric Cu(II) complexes is typically reduced from the spin-only value of 1.7 BM to 1.4 BM/Cu atom, with pronounced temperature dependence.^{1,16}

Significant advances in the study of Cu(II) carboxylate dimers began with the measurement of the EPR spectra,⁸ X-ray crystal diffraction¹⁷ and variable temperature magnetic susceptibility¹⁶ of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. In the solid state, it exhibits a cage-type or “paddle-wheel” dimeric structure with a Cu...Cu distance of 2.64 Å, an octahedral stereochemistry tetragonally elongated along the Cu-O_L axis due to the Jahn-Teller effect¹⁷⁻¹⁹ (Figure 3.1).

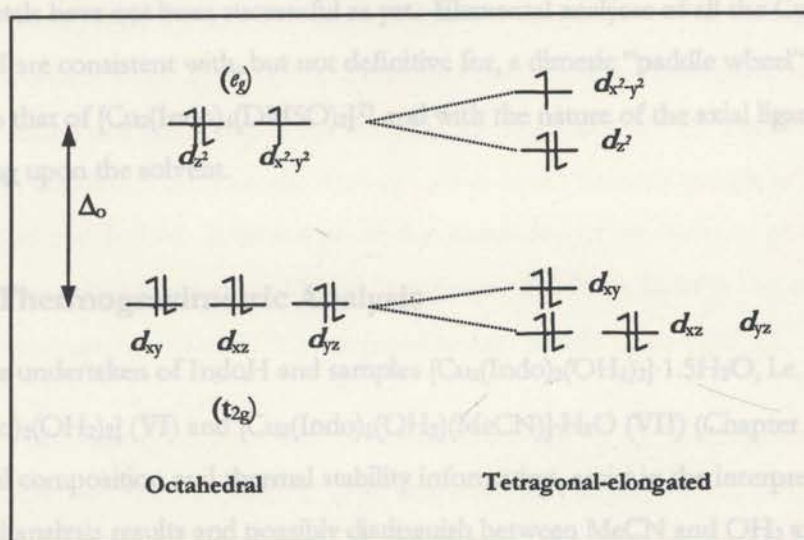


Figure 3.1. The electronic configuration of the energy levels of d^9 Cu(II) ion in octahedral and tetragonal crystal fields.²⁰

3.2 Synthesis

The complexes $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$ and $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$, ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$) were prepared as green crystalline or powdered solids by heating a 2:1 molar ratio of IndoH:Cu(II) acetate monohydrate (or 6.7:1 molar ratio of IndoH:Cu(II) chloride dihydrate for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$) in the appropriate solvent. The dimers were precipitated by the addition of excess ethanol. A dimeric species was also prepared using MeCN as the solvent, but on precipitation with or without EtOH, microanalysis demonstrated that the resulting complexes were the aqua or aqua-MeCN adducts $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$ and $[\text{Cu}_2(\text{Indo})_4(\text{MeCN})(\text{OH}_2)] \cdot 2.5\text{H}_2\text{O}$, respectively. Solvent molecules may become trapped in the cavities of the “paddle-wheel” structure, with additional atmospheric water becoming absorbed in the matrix upon storage.

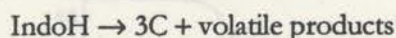
All complexes are soluble in DMF, tetrahydrofuran (THF) and MeCN ($\sim 0.1\%$ w/v at 25°C) but insoluble in water. $[\text{Cu}_2(\text{Indo})_2(\text{DMF})_2]$ is also readily soluble in NMP and DMA at concentrations of $\sim 0.1\%$ w/v at 25°C , but only sparingly soluble to insoluble in EtOH and MeOH. Saturation of DMF solutions of $[\text{Cu}_2(\text{Indo})_2(\text{DMF})_2]$ occurs at greater than 3×10^{-3} mM following sonication of the samples for ~ 20 min at $35\text{--}40^\circ\text{C}$. Small irregular crystals were obtained from neat NMP or DMA solutions upon cooling to 4°C but these were unsuitable for X-ray crystallography. Well-formed crystals obtained from neat MeCN

disintegrated during data collection at ambient temperature and further attempts to obtain such crystals have not been successful as yet. Elemental analyses of all the Cu(II) complexes of IndoH are consistent with, but not definitive for, a dimeric “paddle wheel” structure similar to that of $[\text{Cu}_2(\text{Indo})_4(\text{DMSO})_2]^{21}$ and with the nature of the axial ligand varying depending upon the solvent.

3.3 Thermogravimetric Analysis

TGA was undertaken of IndoH and samples $[\text{Cu}_2(\text{Indo})_2(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$, i.e. sample **V**, $[\text{Cu}_2(\text{Indo})_2(\text{OH}_2)_2]$ (**VI**) and $[\text{Cu}_2(\text{Indo})_2(\text{OH}_2)(\text{MeCN})] \cdot \text{H}_2\text{O}$ (**VII**) (Chapter 2) to provide additional composition and thermal stability information, assist in the interpretation of their elemental analysis results and possibly distinguish between MeCN and OH_2 axial coordination (Figure 3.2 and Table 3.1). TGA of samples **V** and **VI** were undertaken ~six months, and sample **VII** ~one month, after synthesis.

IndoH undergoes little decomposition prior to a single thermogravimetric step located between 250-320 °C (Figure 3.2). This corresponds to an almost complete vaporisation of the sample. No other decomposition peaks were observed. The thermogravimetric reaction pattern for IndoH is consistent with:



3.1

(calculated mass loss, 89.92%; experimental value, 90.24%).

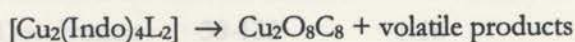
The thermograms of samples **V**, **VI** and **VII** all display a five-step decomposition profile from ambient temperature to 800 °C, although they are different from the five-step profile with steps at 100-130, 130-250, 250-320, 320-475 and 475-850 °C reported for $[\text{Cu}_2(\text{Sup})_4(\text{OH}_2)_2]$ ($\text{SupH} = (\alpha\text{-methyl-4-(2-thienyl-carbonyl)phenylacetic acid; SupH})$).²²

An initial weight loss step was observed from ambient temperature to 130 °C for samples **V** and **VI**. This corresponded to the loss of loosely bound waters of solvation. No similar step was observed for sample **VII**. A complex series of decomposition steps was observed after desolvation; proposed stoichiometries for these are given in Table 3.1. Differences between samples **V**, **VI** and **VII** were most notable in the region of steps 3, 4, and 5 and are attributed to variations in the decomposition of the benzene groups of the Cu(II) dimer.

Figure 3.2. TGA plots (—) and derivative plots (---) of the thermal decomposition of (a) IndoH, (b) **V**, (c) **VI** and (d) **VII**.

The decomposition appears to be consistently initiated by demethylation in step 2 over the temperature range 183-217 °C. Demethylation is then followed by the loss of Cl atoms in the temperature range 217-260 °C. Decomposition of the benzene group in IndoH, however, occurs as a single step in the temperature range 200-350 °C. The binding of Indo to Cu(II) in the dimers modifies the decomposition of the benzene groups of Indo compared to neat IndoH. Initiation of the decomposition of the benzene group in the Cu(II) dimer complex occurs at ~183 °C (compared ~250 °C in IndoH) and over a greater temperature range, i.e., 183-500 °C, compared to neat IndoH (250--350 °C).

No further decomposition steps were observed above 500 °C for all samples apart from a gradual weight loss from 500-800 °C to an observed mass residue of ~21.5%. The proposed stoichiometry for the mass residue at 800 °C corresponds to a $\text{Cu}_2\text{O}_8\text{C}_8$ core, which is in good agreement with the calculated values of ~21.2%. The overall reaction pattern for the decomposition of samples **V**, **VI** and **VII** to ~800 °C is consistent with:



3.2

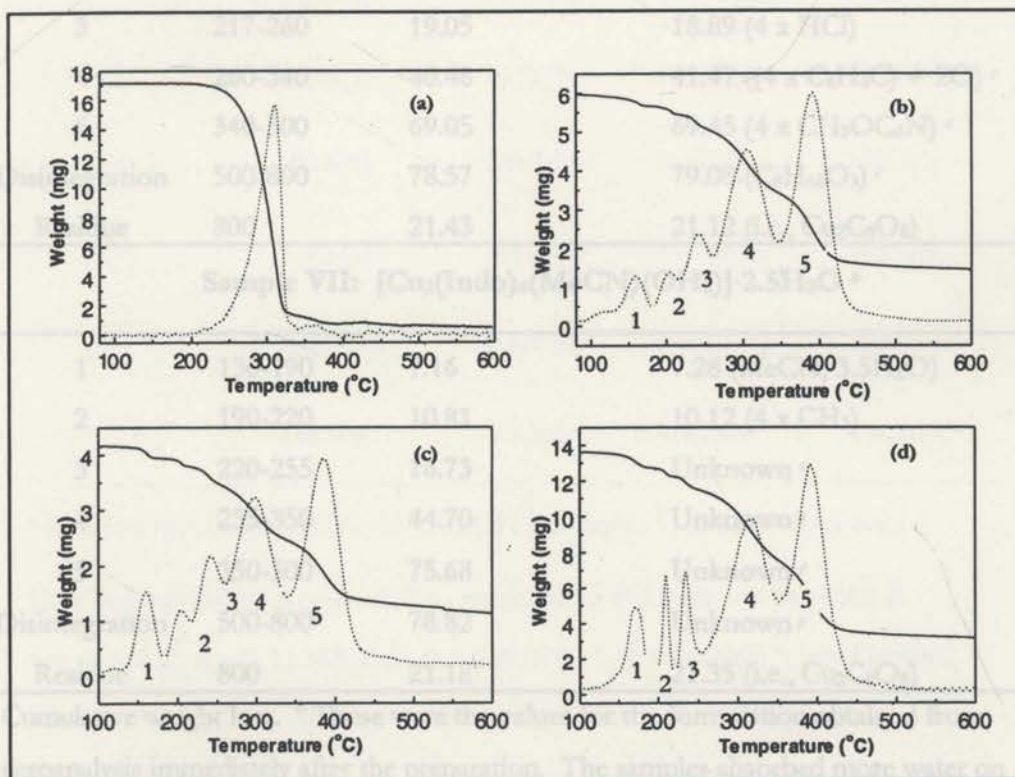


Figure 3.2. TGA plots (—) and derivative plots (···) of the thermal decomposition of (a) IndoH, (b) **V**, (c) **VI** and (d) **VII**.

Table 3.1. Thermogravimetric Analysis Results

Step	T (°C)	% Weight loss obs. ^a	% Weight loss calc.
Sample V: $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$ ^b			
Desolvation	39-130	2.23	2.19 (2 x H ₂ O)
1	130-183	6.53	6.50 (4x H ₂ O)
2	183-217	10.01	10.16 (4 x CH ₃)
3	217-260	16.72	16.56 (3 x HCl)
4	260-345	43.26	42.54 ((4 x C ₆ H ₄ C) + 2O + Cl) ^c
5	345-500	74.86	Unknown ^c
Disintegration	500-800	78.16	Unknown ^c
Residue	800	21.85	21.12 (i.e., Cu ₂ C ₈ O ₈)
Sample VI: $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ ^b			
Desolvation	30-133	1.19	1.08 (H ₂ O)
1	133-183	6.43	6.50 (5 x H ₂ O)
2	183-217	9.52	10.12 (4 x CH ₃)
3	217-260	19.05	18.89 (4 x HCl)
4	260-340	40.48	41.47 ((4 x C ₆ H ₄ C) + 2O) ^c
5	340-500	69.05	69.45 (4 x CH ₃ OC ₆ N) ^c
Disintegration	500-800	78.57	79.08 (C ₈ H ₁₆ O ₃) ^c
Residue	800	21.43	21.12 (i.e., Cu ₂ C ₈ O ₈)
Sample VII: $[\text{Cu}_2(\text{Indo})_4(\text{MeCN})(\text{OH}_2)] \cdot 2.5\text{H}_2\text{O}$ ^b			
1	130-190	7.16	7.28 (MeCN, 3.5H ₂ O)
2	190-220	10.81	10.12 (4 x CH ₃)
3	220-255	16.73	Unknown ^c
4	255-350	44.70	Unknown ^c
5	350-500	75.68	Unknown ^c
Disintegration	500-800	78.82	Unknown ^c
Residue	800	21.18	21.35 (i.e., Cu ₂ C ₈ O ₈)

^a Cumulative weight loss. ^b These were the values for the formulation obtained from microanalysis immediately after the preparation. The samples absorbed more water on standing. ^c Decomposition of the benzene rings of Indo.

3.4 X-ray Powder Diffraction Patterns

X-ray powder diffraction patterns for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$ and $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ are shown in Figure 3.3 and the derived lattice parameters are listed in Table 3.2. With the exception of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, the samples were reasonably crystalline and gave well-resolved X-ray diffraction patterns over the range $2^\circ < 2\theta < 35^\circ$. The small number of well-resolved lines observed in the diffraction pattern of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ prevented indexing of this pattern, the others were readily indexed on the basis of a triclinic cell.

Table 3.2. Lattice Parameters and Selected Details of Refinements of the Powder Diffraction Patterns of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$.

Parameters	L			
	DMF	OH ₂ ^a	NMP ^b	DMA
<i>a</i> (Å)	10.850(7)	11.17(3)	22.104(6)	11.10(2)
<i>b</i> (Å)	13.360(7)	14.84(4)	25.941(9)	12.98(2)
<i>c</i> (Å)	16.56(1)	16.26(7)	15.375(7)	15.97(12)
α (°)	105.8(1)	103.1(3)	91.80(3)	100.7(3)
β (°)	101.1(1)	104.8 (3)	107.17(3)	101.2(3)
γ (°)	106.9 (1)	100.0 (3)	108.48 (4)	109.7(2)
	Triclinic	Triclinic	Triclinic	Triclinic

^a $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$ from EtOH synthesis. ^b An NMP complex of $[\text{Zn}_2(\text{Indo})_4(\text{NMP})_2]$ is reported²³ to crystallise in $P\bar{1}$ with $a = 14.143(3)$ Å, $b = 14.521(2)$ Å, $c = 11.558(2)$ Å, $\alpha = 109.07(1)^\circ$, $\beta = 90.80(2)^\circ$, $\gamma = 116.40(1)^\circ$, $\text{Zn} \cdots \text{Zn} = 2.935(1)$ Å, $\text{Zn}-\text{O}_{\text{Ac}} = 2.051(3)$ Å, $2.0048(3)$ Å, $2.027(3)$ Å, $2.01(3)$ Å, $\text{Zn}-\text{O}_{\text{L}} = 1.987(3)$ Å.

The cell dimensions of the DMF complex are similar to those obtained from the single crystal measurements,²⁸ with the small differences probably arising from different DMF/H₂O solvents of crystallisation in the two samples. The cell dimensions of the DMF complex are similar to those of the DMSO complex.²¹ It is clear that $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2] \cdot 1.5\text{H}_2\text{O}$ are isostructural, and the DMF and DMA complexes are isostructural, but the two pairs of compounds are not isostructural, e.g., compare the strong 002 reflection near 7.5°.

The diffraction pattern of the NMP complex is unique when compared to those observed for either the DMF/DMA and the H₂O/ $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2] \cdot 1.5\text{H}_2\text{O}$ complexes. For the NMP complex, there appears to be a doubling of both the *a* and *b* axis, whilst *c* remains unchanged, when compared to the DMF complex. No clear relationship, however, is observed between the *c* axis for the NMP complex when compared to the unit cells of the other complexes. The cell dimensions of $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2] \cdot 2\text{H}_2\text{O}$ are also dissimilar to those reported for $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$.²⁵

3.5 UV-Vis

The solid-state spectra in a KBr matrix, solid state Raman spectra using an excitation frequency of 514 nm and diffuse reflectance UV-Vis spectra in a KBr matrix of IndoH, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{OAc})_4(\text{OH})_2]$ are shown in Figures 3.4 and 3.5, respectively. A summary of the electronic absorption spectral data for the $[\text{Cu}_2(\text{Indo})_4]$ complexes and $[\text{Cu}_2(\text{OAc})_4(\text{OH})_2]$ is shown in Table 3.1. The diffuse reflectance UV-Vis spectra of DMF solutions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, $[\text{Cu}_2(\text{OAc})_4(\text{OH})_2]$ and IndoH and a summary of the data are shown in Figure 3.6 and Table 3.4, respectively. The spectra obtained from stopped-flow mixing of a 1:1 molar mixture of IndoH/ $[\text{Cu}_2(\text{OAc})_4(\text{OH})_2]$, i.e., equivalent to the stoichiometry of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, and a similar concentration of $[\text{Cu}_2(\text{OAc})_4(\text{OH})_2]$ in DMF at 69 °C and 2.6 s are shown in Figure 3.7.

Relative Intensity

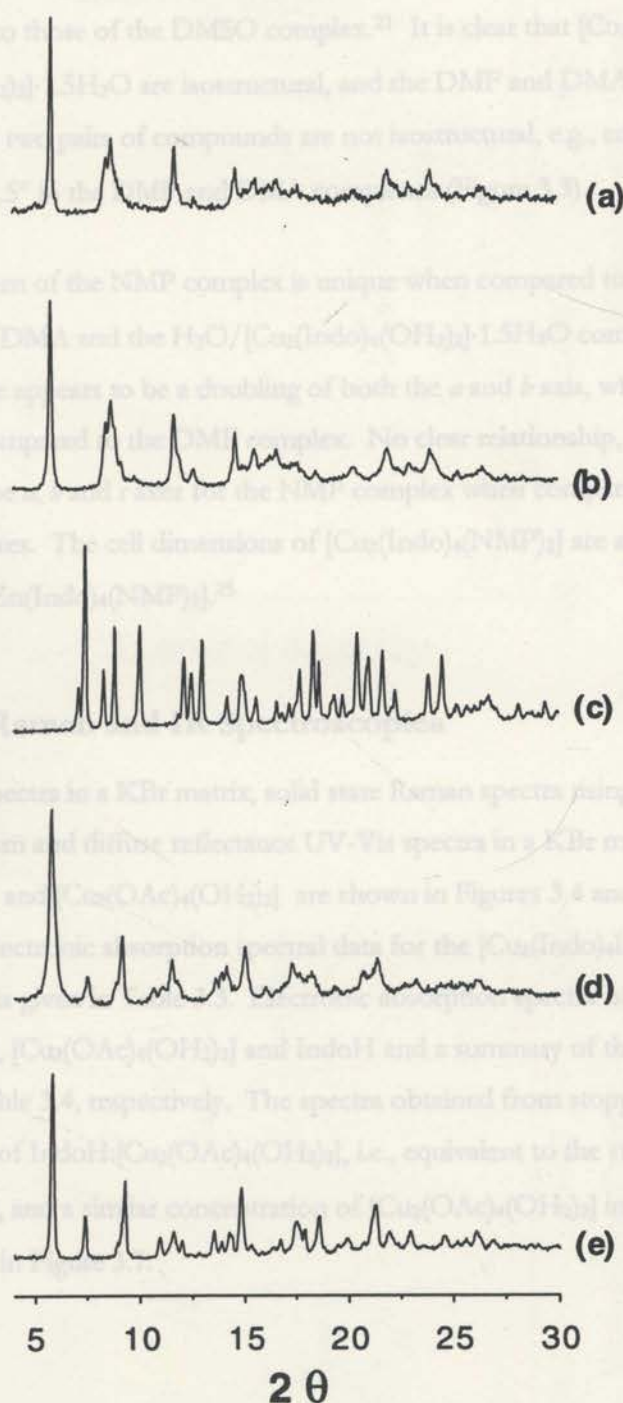


Figure 3.3. X-ray powder diffraction patterns of (a) $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]_2$, (b) $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2] \cdot 1.5\text{H}_2\text{O}$, (c) $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2] \cdot 2\text{H}_2\text{O}$, (d) $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2] \cdot 3\text{H}_2\text{O}$ and (e) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$.

The cell dimensions of the DMF complex are similar to those obtained from the single crystal measurements,²⁴ with the small differences probably arising from different DMF/H₂O solvents of crystallisation in the two samples. The cell dimensions of the DMF complex are similar to those of the DMSO complex.²¹ It is clear that [Cu₂(Indo)₄(OH₂)₂] and [Cu₂(Indo)₄(OH₂)₂].1.5H₂O are isostructural, and the DMF and DMA complexes are isostructural, but the two pairs of compounds are not isostructural, e.g., compare the strong 002 reflection near 7.5° in the DMF and DMA complexes (Figure 3.3).

The diffraction pattern of the NMP complex is unique when compared to those observed for either the DMF/DMA and the H₂O/[Cu₂(Indo)₄(OH₂)₂].1.5H₂O complexes. For the NMP complex, there appears to be a doubling of both the *a* and *b* axis, whilst *c* remains unchanged, when compared to the DMF complex. No clear relationship, however, is observed between the *a*, *b* and *c* axes for the NMP complex when compared to the unit cells of the other complexes. The cell dimensions of [Cu₂(Indo)₄(NMP)₂] are also dissimilar to those reported for [Zn(Indo)₄(NMP)₂].²⁵

3.5 UV-Vis, Raman and IR Spectroscopies

The solid-state IR spectra in a KBr matrix, solid state Raman spectra using an excitation frequency of 514.5 nm and diffuse reflectance UV-Vis spectra in a KBr matrix of IndoH, [Cu₂(Indo)₄(DMF)₂] and [Cu₂(OAc)₄(OH₂)₂] are shown in Figures 3.4 and 3.5, respectively. A summary of the electronic absorption spectral data for the [Cu₂(Indo)₄L₂] complexes and [Cu₂(OAc)₄(OH₂)₂] is given in Table 3.3. Electronic absorption spectra of DMF solutions of [Cu₂(Indo)₄(DMF)₂], [Cu₂(OAc)₄(OH₂)₂] and IndoH and a summary of the data are shown in Figure 3.6 and Table 3.4, respectively. The spectra obtained from stopped-flow mixing of a 1:1 molar mixture of IndoH:[Cu₂(OAc)₄(OH₂)₂], i.e., equivalent to the stoichiometry of [Cu₂(Indo)₄(DMF)₂], and a similar concentration of [Cu₂(OAc)₄(OH₂)₂] in DMF at 6.9 °C and 9.6 s are shown in Figure 3.7.

Table 3.3. Selected Diffuse Reflectance UV-Vis, Solid-state IR and Solid-state Raman Data for $[\text{Cu}_2(\text{Indo})_4]\cdot\text{d} \cdot \text{IndoH}$ and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.

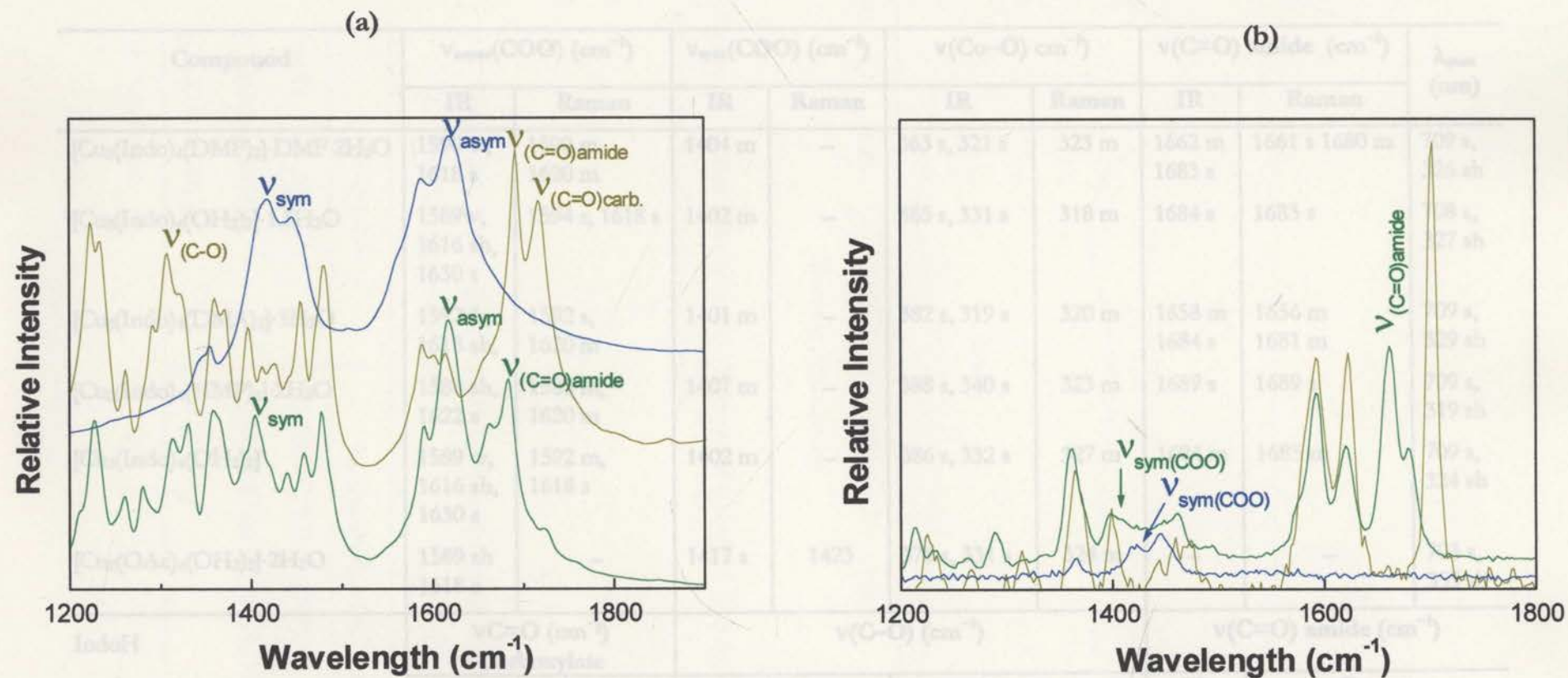


Figure 3.4. Solid-state (a) IR and (b) Raman spectra (excitation frequency of 514.5 nm) of IndoH (—), $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (—) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ (---).

Table 3.3. Selected Diffuse Reflectance UV-Vis, Solid-state IR and Solid-state Raman Data for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$, IndoH and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.

Compound	$\nu_{\text{asym}}(\text{COO}) (\text{cm}^{-1})$		$\nu_{\text{sym}}(\text{COO}) (\text{cm}^{-1})$		$\nu(\text{Cu-O}) \text{cm}^{-1}$		$\nu(\text{C=O}) \text{ amide } (\text{cm}^{-1})$		λ_{max} (nm)
	IR	Raman	IR	Raman	IR	Raman	IR	Raman	
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$	1590 w, 1618 s	1592 m, 1620 m	1404 m	—	363 s, 321 s	323 m	1662 m 1683 s	1661 s 1680 m	709 s, 326 sh
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$	1589w, 1616 sh, 1630 s	1594 s, 1618 s	1402 m	—	385 s, 331 s	318 m	1684 s	1683 s	708 s, 327 sh
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2] \cdot 3\text{H}_2\text{O}$	1592sh, 1618 sh,	1592 s, 1620 m	1401 m	—	382 s, 319 s	320 m	1658 m 1684 s	1656 m 1681 m	709 s, 329 sh
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2] \cdot 2\text{H}_2\text{O}$	1588 sh, 1622 s	1588 m, 1620 m	1407 m	—	388 s, 340 s	323 m	1689 s	1689 s	709 s, 319 sh
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$	1589 w, 1616 sh, 1630 s	1592 m, 1618 s	1402 m	—	386 s, 332 s	327 m	1684 m	1685 m	709 s, 324 sh
$[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2] \cdot 2\text{H}_2\text{O}$	1589 sh 1618 s	—	1417 s	1423	376 s, 331 s	324 m	—	—	703 s, 355 sh
IndoH	$\nu\text{C=O } (\text{cm}^{-1})$ Carboxylate		$\nu(\text{C-O}) (\text{cm}^{-1})$				$\nu(\text{C=O}) \text{ amide } (\text{cm}^{-1})$		
	IR	Raman	IR		Raman		IR	Raman	
	1717 s	1594 s 1622 s	1307 m		—		1682 sh 1691 s	1702 s	

s = strong; sh = shoulder; m = medium; w = weak.

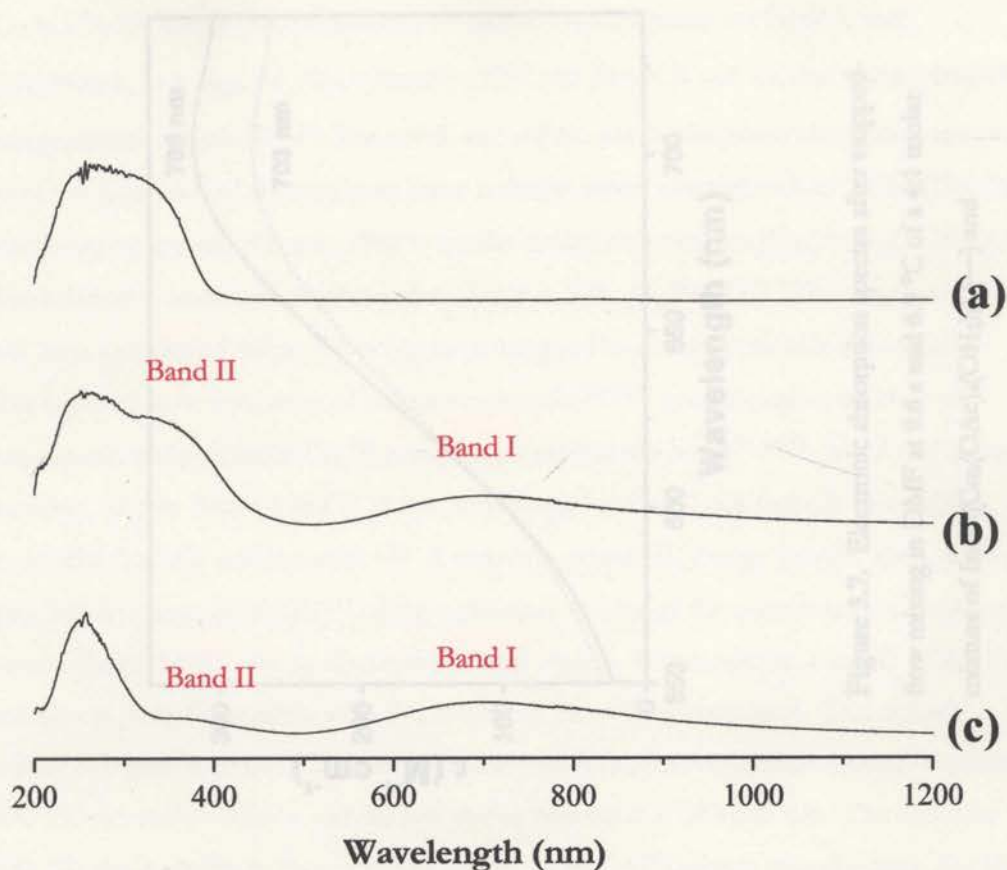


Figure 3.5. Diffuse reflectance UV-Vis spectra of (a) IndoH, (b) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and (c) $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ in a KBr matrix.

Table 3.4. UV-Vis Data of DMF Solution Spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and IndoH.

Compound	$\lambda_{\text{max}}(\text{nm})$ ($\epsilon_{\text{max}} (\text{M}^{-1} \text{cm}^{-1})$)
IndoH	270 (15464); 320 sh (6355)
$[\text{Cu}_2(\text{Indo})_2(\text{DMF})_2]$	270 (56640), 320 sh (22508), 708 s (418)
$[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$	703 (390)

sh = shoulder

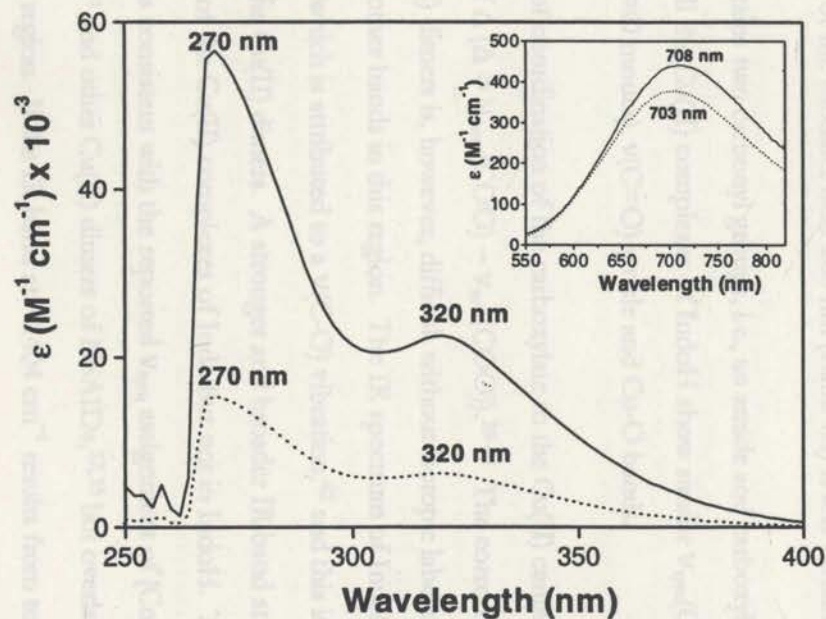


Figure 3.6. Electronic absorption spectra of DMF solutions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (—) and IndoH (---) with insert plot of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (—) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ (···).

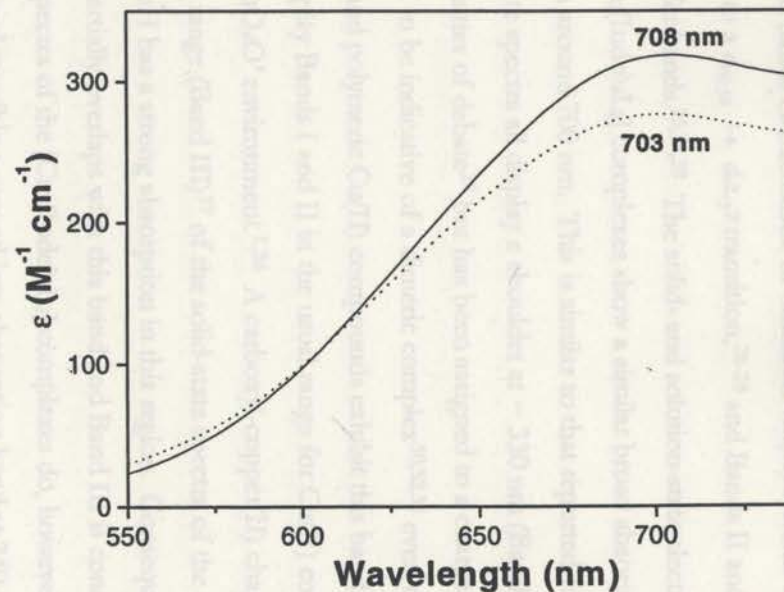


Figure 3.7. Electronic absorption spectra after stopped-flow mixing in DMF at 9.6 s and 6.9 °C of a 4:1 molar mixture of Indo: $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ (—) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ alone (···).

Three UV-Vis bands characteristic of dinuclear Cu(II) systems are Band I, that corresponds to a $d_{xy, yz} \rightarrow d_{x^2-y^2}$ transition,²⁶⁻²⁸ and Bands II and III that correspond to charge-transfer bands.^{26,29,30} The solid- and solution-state electronic absorption spectra of all of the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes show a similar broad absorption band (Band I) in the visible region around 700 nm. This is similar to that reported for $[\text{Cu}_2(\text{Indo})_4(\text{DMSO})_2]$.²¹ The solid-state spectra all display a shoulder at ~ 330 nm (Band II). The origin of Band II has been a matter of debate²⁹ but has been assigned to a charge-transfer absorption.³¹ Band II is believed to be indicative of a dimeric complex^{30,32,33} even though a number of monomeric and polymeric Cu(II) compounds exhibit this band.^{31,34,35} All of the complexes, however, display Bands I and II in the usual range for Cu(II) compounds in a square-pyramidal $\text{CuO}_4\text{O}'$ environment.^{1,36} A carboxyl-copper(II) charge transfer absorption in the 240–280 nm range (Band III)³⁷ of the solid-state spectra of the complexes is not observed because IndoH has a strong absorption in this region. Consequently, Band II of the Cu(II) complexes partially overlaps with this band and Band III is concealed. The diffuse reflectance spectra of the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes do, however, show a small shoulder at 300–330 nm and possibly a second low absorption band at 240–280 nm. The shoulder at 300–330 nm is similar to those present in spectra of Cu(II) acetate monohydrate, Cu(II) cyanoacetate and Cu(II) cyanoacetate monohydrate³⁸ and may be due to Band II. The assignment of the shoulder near 260 nm (Band III) is less certain.

IndoH contains two carbonyl groups, i.e., an amide and carboxylic acid. The Raman and IR spectra of all the Cu(II) complexes of IndoH show similar $\nu_{\text{sym}}(\text{COO})$, $\nu_{\text{asym}}(\text{COO})$ (albeit not pure C=O modes), $\nu(\text{C=O})$ amide and Cu-O bands.

The mode of coordination of the carboxylate to the Cu(II) centre can be determined from the value of Δ ($\Delta = \nu_{\text{asym}}(\text{COO}) - \nu_{\text{sym}}(\text{COO})$).³⁹⁻⁴¹ The correct assignment of $\nu_{\text{sym}}(\text{COO})$ in the Cu(II) dimers is, however, difficult without isotopic labeling due to the presence of a number of other bands in this region. The IR spectrum of IndoH shows a band at 1307 cm^{-1} , which is attributed to a $\nu(\text{C-O})$ vibration,⁴² and this is of lower intensity in the spectra of the Cu(II) dimers. A stronger and broader IR band at $\sim 1401\text{ cm}^{-1}$ is observed in the spectra of the Cu(II) complexes of Indo but not in IndoH. This broad IR band at 1401 cm^{-1} is consistent with the reported ν_{sym} assignment of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 1417 cm^{-1} ⁴¹ and other Cu(II) dimers of NSAIDs,^{22,33} but overlaps with other IR bands of Indo in the region. If the IR band at $\sim 1404\text{ cm}^{-1}$ results from a mode that is purely ν_{asym} ,

then all values of $\Delta \nu_{\text{asym}} - \nu_{\text{sym}}$ for the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ would be $> 200 \text{ cm}^{-1}$. This value is associated with unidentate coordination³⁹ and is inconsistent with the magneto-structural results of the Cu(II) dimers of IndoH described in the following sections. The criterion of using the value of $\Delta \nu_{\text{asym}} - \nu_{\text{sym}}$ to determine the mode of coordination of the carboxylate to the central Cu(II) cannot, therefore, be used with confidence for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}$, DMA , NMP , OH_2). The values of $\Delta \nu_{\text{asym}} - \nu_{\text{sym}}$ are, however, not too dissimilar from $175\text{--}200 \text{ cm}^{-1}$ ³⁹ reported for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.

Two IR bands due to Cu–O in the spectra of the Cu(II) complexes of Indo are similar to those reported in the $300\text{--}400 \text{ cm}^{-1}$ region for Cu(II) acetate type dimers,^{43,44} which are due to Cu–O vibrations.⁴⁵ The solid-state IR spectral data of the Cu(II) complexes of IndoH are, therefore, suggestive rather than definitive of bridging bidentate carboxylate coordination similar to $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.^{40,41,46} The Raman $\nu_{\text{sym}}(\text{COO})$ band observed in the Cu(II) dimers of IndoH at 1423 cm^{-1} is similar to that reported for $[\text{Cu}_2(\text{OOCDD}_3)_4(\text{OH}_2)_2]$,⁴⁷ at 1421.5 cm^{-1} .

Solid-state Raman spectral data were likewise, inconclusive in defining the coordination around the Cu(II) centres of the complexes. Consistent with previous results,⁴⁷ no Raman $\nu_{\text{asym}}(\text{COO})$ band was observed in the spectrum of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. This is because strong IR bands correspond to weak Raman bands due to the different selection rules for which Raman and IR modes are active.⁴⁰ Symmetric modes produce no change in dipole moment and, therefore, have the strongest Raman intensity whilst asymmetric modes produce a change in dipole moment and, therefore, lead to the strongest IR intensity.^{40,48}

IndoH and its Cu(II) dimers exhibit two bands at $\sim 1592 \text{ cm}^{-1}$ and 1620 cm^{-1} in their IR and Raman spectra. The intensities of these bands are very low in IndoH but are more pronounced in the Cu(II)-Indo dimer complexes. From IR spectra, these bands are assigned as $\nu_{\text{asym}}(\text{COO})$ and have similar wavenumbers to those of $[\text{Cu}_2(\text{Sup})_4(\text{OH}_2)_2]$ (1606 cm^{-1}).²² The unexpected presence of these two peaks in the Raman spectra of IndoH and Cu(II) complexes of Indo suggests either an overlap of the $\nu_{\text{asym}}(\text{COO})$ band with other yet-to-be assigned vibrational modes of IndoH and/or a possible polarisation of the Indo moiety resulting in a Raman active $\nu_{\text{asym}}(\text{COO})$ stretch.

IndoH exhibits a strong acid carbonyl/asymmetric acid $\nu(\text{C}=\text{O})$ IR stretch^{48,49} at 1717 cm^{-1} that is absent in the Raman spectrum of carboxylic acids known to form cyclic dimers.⁵⁰ The reported Raman spectra of IndoH includes a very strong amide $\nu(\text{C}=\text{O})$ band at 1698 cm^{-1} .⁴⁸ This band is observed at 1701 cm^{-1} in the current work.

Only one Cu-O band is observed at $\sim 320\text{ cm}^{-1}$ in the Raman spectrum of the Cu(II) complexes of Indo and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$, which is assigned to a symmetric stretch, whilst two are observed in the IR spectra at $\sim 320\text{ cm}^{-1}$ and $\sim 386\text{ cm}^{-1}$. Similarly, only one Raman Cu-O vibration has been reported for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 323 cm^{-1} .⁴⁷ No Raman absorption data is reported in this region for $[\text{Cu}_2(\text{Sup})_4(\text{OH}_2)_2]$.²²

Very weak IR bands are observed at 1718 , 1720 , and 1721 cm^{-1} for $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$, respectively, but not for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2 \cdot 1.5\text{H}_2\text{O}]$ or $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. A splitting of the $\nu(\text{C}=\text{O})$ amide band is observed in the IR and Raman spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ but not in $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{NMP}, \text{OH}_2$). These are assigned to $\nu(\text{C}=\text{O})$ of the axial solvent ligands. Only solvent peaks were observed in the solution Raman spectra (excitation frequency 514.5 nm) of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 3 \times 10^{-3}\text{ mM}$) in DMF.⁵¹

3.6 Magnetic Susceptibility

The values of anti-ferromagnetic exchange constant (J), fraction of Cu(II) monomer impurity, g values, and room temperature magnetic moment (μ_{eff}) for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{NMP}, \text{DMA}, \text{OH}_2$) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ are summarised in Table 3.5. Brief descriptions of the experimental determinations of the values of g , J and the fraction monomer are given in Appendix 10. The room temperature observed per Cu gram (χ_g), molar (χ_M), and paramagnetic susceptibility (χ_P) of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{NMP}, \text{DMA}, \text{OH}_2$) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and calculated diamagnetic susceptibilities (χ_D) of the ligands are given in Appendix 11. Plots of the variable temperature magnetic data for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2 \cdot 1.5\text{H}_2\text{O}]$ are shown in Figures 3.7 (a), (b), and (c), respectively, along with the lines of best fit.

Table 3.5. Room Temperature Magnetic Moment and SQUID Variable Temperature Magnetic Data for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.

Compound	μ_{eff}^a (B.M.)	g^a	J^b (cm^{-1})	Fraction monomer b
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$	1.55	2.10	-152.5(1)	0.006
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$	1.45	2.06	-149.8(1)	0.002
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2] \cdot 3\text{H}_2\text{O}$	1.52	2.16	-142.3(1)	0.09
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2] \cdot 2\text{H}_2\text{O}$	1.42	2.25	-142.3(1)	0.01
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$	1.58	2.17	-144.0(1)	0.065
$[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$	1.46	2.10	-142.0(1)	-

^a Calculated per Cu. ^b $T = 300 \text{ K}$ and $N_{\alpha} (\text{cgs}) = 0.00006$.

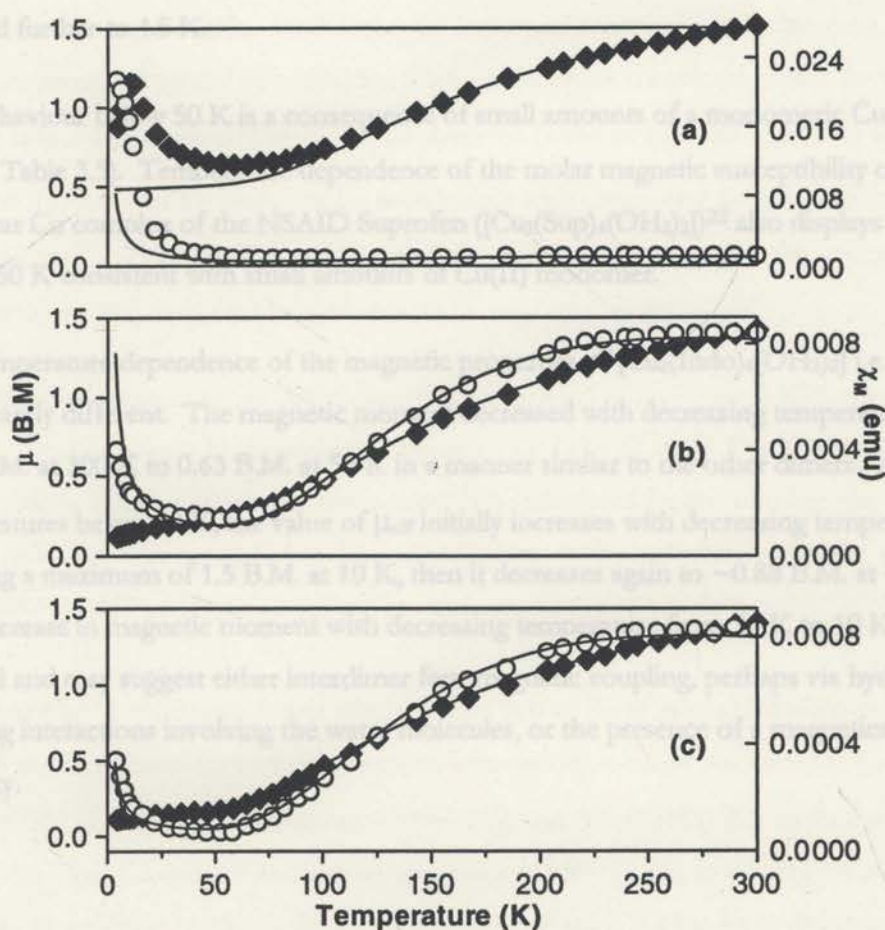


Figure 3.8. Plots of χ_M (o, observed; —, calculated) and μ (◆, observed; —, calculated) versus T for (a) $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, (b) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$, and (c) $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2] \cdot 1.5\text{H}_2\text{O}$.

Consistent with anti-ferromagnetic exchange,^{8,16} the room temperature magnetic moments per Cu ($\mu_{\text{eff}} = 1.42 - 1.55$ B.M.) are typical of those observed for other $[\text{Cu}_2(\text{RCOO})_4\text{L}_2]$ dimers,^{27,31,52-54} (including dinuclear Cu(II) complexes of anti-inflammatory drugs with bridging carboxylate ligands),^{27,55} and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ($\mu_{\text{eff}} = 1.53$)² and are lower than the d^9 spin-only magnetic moment of 1.73 B.M.^{1,8}

The variable temperature data (Figure 3.8) show a decrease in the value of the magnetic moments of the Cu-Indo complexes from around 1.50 B.M. at 300 K to approximately 0.2 B.M. at 4.5 K, as a consequence of depopulation of the excited triplet ($S = 1$) state.^{16,52,56} This observation is consistent with coupling of the unpaired electrons of the two Cu(II) atoms, giving rise to a singlet ground state ($S = 0$) with a triplet state ($S = 1$) lying only a few kJ mol^{-1} above it.^{13,57} The susceptibilities decrease from 300 K to a minimum value near 50 K. Below this, there is a rapid increase in magnetic susceptibility as the temperature is lowered further to 4.5 K.

The behaviour below 50 K is a consequence of small amounts of a monomeric Cu(II) ion ($< 9\%$, Table 3.5). Temperature dependence of the molar magnetic susceptibility of the dinuclear Cu complex of the NSAID Suprofen ($[\text{Cu}_2(\text{Sup})_4(\text{OH}_2)_2]$)²² also displays behaviour below 50 K consistent with small amounts of Cu(II) monomer.

The temperature dependence of the magnetic properties of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ i.e., **VI**, was significantly different. The magnetic moment decreased with decreasing temperature from 1.51 B.M. at 300 K to 0.63 B.M. at 50 K in a manner similar to the other dimers. At temperatures below 50 K, the value of μ_{eff} initially increases with decreasing temperature, reaching a maximum of 1.5 B.M. at 10 K, then it decreases again to ~ 0.88 B.M. at 4.5 K. This increase in magnetic moment with decreasing temperature from 50 K to 10 K is unusual and may suggest either interdimer ferromagnetic coupling, perhaps via hydrogen-bonding interactions involving the water molecules, or the presence of a magnetically active impurity.

3.7 Zero-Field and X-Band EPR Powder Spectroscopy

ZFEPR spectra were measured for all of the complexes at 90, 120 and 145 K to determine values for the symmetric and asymmetric crystal-field splitting parameters D and E , respectively, and the hyperfine spin-nucleus coupling constant A_{I} prior to simulation of the X-band EPR spectra. Despite the atypical magnetic susceptibilities of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ below 50 K, the magnetic properties for all of the studied complexes were similar at the temperatures relevant to the EPR studies. The results of the least-squares refinements for the values of D , E , g and A_{I} and best-fit X-band EPR parameters are collected in Table 3.6. A typical ZFEPR spectrum and X-band powder spectrum at RT and 110 K for the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes, along with the simulation of the 110 K spectrum, and are shown in Figures 3.8 and 3.9, respectively.

As illustrated for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ (Figure 3.9), the complexes exhibit seven-line ZFEPR spectra centred near 10 GHz. In all cases, the inner peaks were of similar intensity. The D and g parameters are typical of octahedral Cu(II) carboxylate complexes where the unpaired electron is in a $d_{x^2-y^2}$ orbital.^{2,31,32,34,58} The observed D values of 0.333 and 0.344 cm^{-1} are similar to those found for dinuclear Cu compounds with exclusively oxygen donors ($D = 0.34 \text{ cm}^{-1}$). The best-fit E values are all very small, in keeping with the strong axial symmetry observed in related X-band spectra.¹²

The distinctive resonances for Cu(II) dimers are illustrated in the X-band EPR spectrum (Figure 3.10) by the spin Hamiltonian parameters H_{z1} , H_{z2} and H_{L} .¹² The line shape and peak positions of the spectra were not substantially influenced by the axial ligands. All spectra exhibited features typical of dimeric complexes with axial symmetry and $g_{\text{I}} > g_{\text{L}}$,^{2,28,31,54,57,60} and a $d_{x^2-y^2}$ (or d_{xy}) ground state.¹

The X-band EPR spectra are better resolved than that of $[\text{Cu}_2(\text{Indo})_4(\text{DMSO})_2]$ described by Weser²¹ and in all cases they exhibit, at room temperature, a broad resonance at $g_{\text{eff}} \sim 2.1$ ($H_{\text{L}2} \sim 4720 \text{ G}$), with weak features at ~ 5980 (H_{z2}) and 500 G (H_{z1}) due to the spin-triplet state of the dimeric complex and a small resonance at 3300 G due to a Cu(II) monomer impurity. On cooling to 110 K, the broad resonance at $g_{\text{eff}} = 2.1$ increases in intensity due to the triplet state. At 110 K, the monomer feature becomes dominant and the powder spectrum exhibits a distinctive signal at 3300 G.

Table 3.6. X-band and Zero-field EPR Data for the [Cu₂(Indo)₄L₂] Complexes

Sample	<i>T</i> (K)	<i>g</i> values ^a (110 K)	<i>D</i> GHz (x 10 ⁻² cm ⁻¹) ^b	<i>E</i> GHz (cm ⁻¹) ^b	<i>A</i> ₁ MHz (x 10 ⁻⁴ cm ⁻¹) ^b
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^d	90	<i>g</i> ₁ 2.35, <i>g</i> ₁ 2.05, <i>g</i> _{eff} 2.15 ^c	9.975(1), (33.273(3))	0.028(2), (9.3(6) x 10 ⁻⁴)	230.5(2) (76.88(7))
[Cu ₂ (Indo) ₄ (OH ₂)]·1.5H ₂ O ^d	120	<i>g</i> ₁ 2.35, <i>g</i> ₁ 2.05, <i>g</i> _{eff} 2.15 ^c	10.003(1), (33.366(3))	0.017(3), (5.7(9) x 10 ⁻⁴)	229.0(5) (75.4(2))
[Cu ₂ (Indo) ₄ (DMF) ₂] ^d	90	<i>g</i> ₁ 2.36, <i>g</i> ₁ 2.06, <i>g</i> _{eff} 2.16 ^c	10.217(1), (34.080(3))	0.051(2) (1.70(6) x 10 ⁻³)	230.0(5) (76.7(2))
[Cu ₂ (Indo) ₄ (NMP) ₂] ^d	145	<i>g</i> ₁ 2.36, <i>g</i> ₁ 2.06, <i>g</i> _{eff} 2.16 ^c	10.317(1), (34.414(3))	0.085(1), (2.84(3) x 10 ⁻³)	219.2(5) (73.1(2))
[Cu ₂ (CH ₃ COO) ₄ (OH ₂) ₂]	-	<i>g</i> ₁ 2.42, <i>g</i> ₁ 2.08, <i>g</i> _{eff} 2.19 ^f	10.08(2) ^c , (33.623(6))	0.296 ^c , (0.01 ± 0.005) ^f	-

^a *g* value derived from X-band simulations; for an anisotropic system: *g*₁ = *g*_z, and *g*₁ = *g*_{xy}. ^b *D*, *E* and *A*₁ derived from ZFEPR simulations. ^c *g*_{eff} = 1/3(*g*₁ + 2 *g*₁).

^d Sample contained traces of monomer. ^e Taken from ref. 59. ^f Taken from ref. 8.

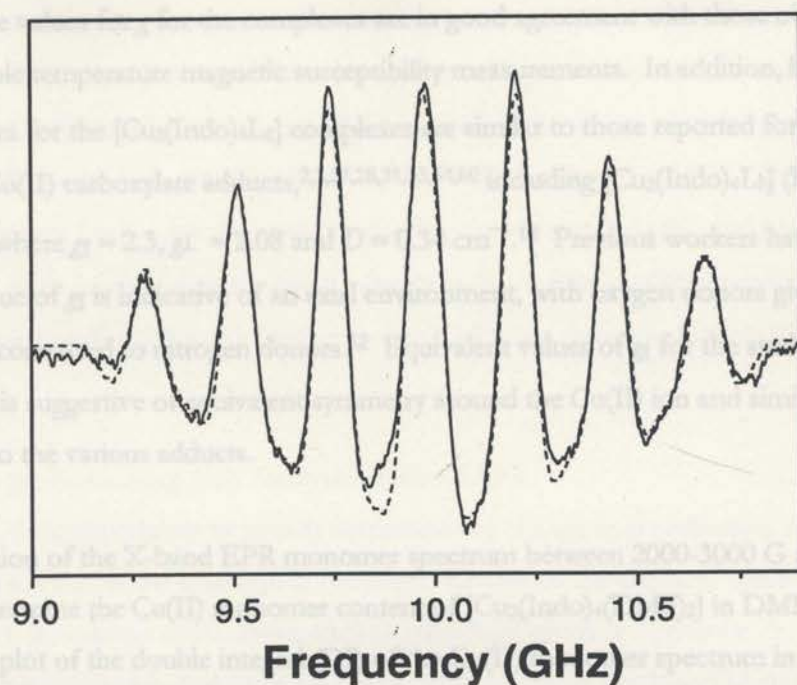


Figure 3.9. The experimental (—) and calculated (---) ZFEPR spectrum of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, $T = 90 \text{ K}$, $B \pm = 2 \text{ mT}$.

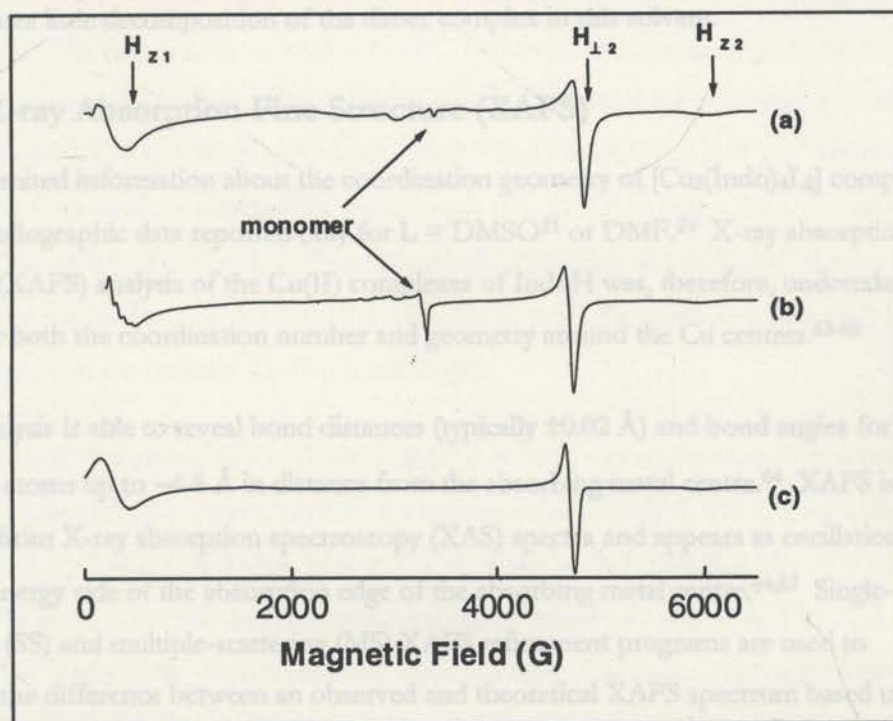


Figure 3.10. X-band powder EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (a) at RT, (b) 110 K and (c) simulation of the 110 K spectrum. The simulation does not include the Cu hyperfine interactions, which are discussed in detail in Chapter 4.

The average values for g for the complexes are in good agreement with those obtained from variable temperature magnetic susceptibility measurements. In addition, both the $g_{\parallel}$ and $g_{\perp}$ values for the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes are similar to those reported for other dinuclear Cu(II) carboxylate adducts,^{2,3,27,28,31,33,54,60} including $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{OH}_2$,⁶¹ DMSO ²¹) where $g_{\parallel} \approx 2.3$, $g_{\perp} \approx 2.08$ and $D \approx 0.34 \text{ cm}^{-1}$.¹² Previous workers have proposed that the value of $g_{\parallel}$ is indicative of an axial environment, with oxygen donors giving a lower value of $g_{\parallel}$ compared to nitrogen donors.⁶² Equivalent values of $g_{\parallel}$ for the studied complexes is suggestive of equivalent symmetry around the Cu(II) ion and similar ligand fields due to the various adducts.

Quantification of the X-band EPR monomer spectrum between 2000-3000 G at 4 K was used to determine the Cu(II) monomer content of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in DMF. A typical calibration plot of the double integral (DI) of the Cu(II) monomer spectrum in the 2000-4000 G region, using CuCl_2 standard solutions, along with the line of best fit and 1% error bars, are shown in Appendix 12. The Cu(II) monomer content of DMF solutions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ measured 3- and 18-hr post preparation was $\sim 2\%$ and 3% , respectively, and indicates little decomposition of the dimer complex in this solvent.

3.8 X-ray Absorption Fine Structure (XAFS)

There is limited information about the coordination geometry of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes, with crystallographic data reported only for $\text{L} = \text{DMSO}$ ²¹ or DMF .²⁴ X-ray absorption fine structure (XAFS) analysis of the Cu(II) complexes of IndoH was, therefore, undertaken to determine both the coordination number and geometry around the Cu centres.⁶³⁻⁶⁶

XAFS analysis is able to reveal bond distances (typically $\pm 0.02 \text{ \AA}$) and bond angles for back-scattering atoms up to $\sim 4\text{-}5 \text{ \AA}$ in distance from the absorbing metal centre.⁶⁴ XAFS is obtained from X-ray absorption spectroscopy (XAS) spectra and appears as oscillations on the high energy side of the absorption edge of the absorbing metal centre.^{64,67} Single-scattering (SS) and multiple-scattering (MS) XAFS refinement programs are used to minimise the difference between an observed and theoretical XAFS spectrum based upon a model of the absorber site to determine the coordination number, and geometry, respectively, around the absorber atom.⁶⁸⁻⁷¹ For complex chemical and biological molecules, an R_{xaf} value around 20% is considered a good fit to non-Fourier transformed data, whilst an R_{xaf} value $> 40\%$ is poor, with lower values of R_{xaf} achieved for model compounds.⁷²

In order to confirm the ability of XAFS analysis to accurately model the Cu_2O_{10} core in the dimeric Cu(II) complexes, solid samples of crystallographically characterised $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ were studied at room temperature and 10 K. A typical XAFS spectrum is given for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ collected at 10 K. The normalised X-ray absorption Cu K-edge, featureless pre-edge absorbance, XANES and post-edge XAFS oscillations, with insert plot of the pre-K edge peak due to the $1s \rightarrow 3d$ electron transition and the corresponding FT plot of the extracted XAFS, are shown in Figures 3.11 and 3.12, respectively.

3.8.1 Single Scattering (SS) Analyses of the XAFS

SS analyses were undertaken to initially determine any change in coordination around the Cu absorber as a result of the axial ligand exchange. Results of the two- and three-shell SS analyses and description of the seven-shell SS model are given in Appendix 13. The SS XAFS analysis using two- and three-shell SS models confirms that $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 10 K and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K and 293 K have similar six-coordinate dinuclear structures with Cu...Cu separations of $\approx 2.61 \text{ \AA}$. In addition, there is a decrease in the value of the Debye-Waller factors and goodness-of-fit parameters R_{xaf} at 10 K compared to room temperature. The two- and three-shell refined Cu...Cu vector distances (Appendix 13) are in excellent agreement with those obtained from room temperature crystallographic studies of $2.616(1) \text{ \AA}$ and $2.630(1) \text{ \AA}$ for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ¹⁸ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$,²⁴ respectively. The refined three-shell SS axial Cu-O_L bond length of $\sim 2.24 \text{ \AA}$ is longer than that reported for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ($2.156(4) \text{ \AA}$)¹⁸ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, ($2.143(5) \text{ \AA}$)²⁴ despite the resolution of the SS data, i.e., $\Delta R = 0.12 \text{ \AA}$, permitting the differentiation of the axial Cu-O_L bond length from the in-plane Cu-O_{Ac} bond length.

The seven-shell SS analyses of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K was undertaken to compare with the MS analyses and gave a dinuclear Cu...Cu separation of $2.64(2) \text{ \AA}$, Cu-O_{Ac} of $1.96(2) \text{ \AA}$ (for $N = 2, 5, 7, 8$) and $3.14 \text{ \AA}$ (for $N = 3, 4, 6, 9$), and Cu-C of $2.87(2) \text{ \AA}$ (for $N = 10, 11, 12, 13$). These distances are similar to crystallographic values of $2.630(1) \text{ \AA}$, $1.96 \text{ \AA}$, $3.11 \text{ \AA}$ and $2.84 \text{ \AA}$,²⁴ respectively. Vector distance restraints from Cu₀ to the axial oxygen atoms ($N = 14, 15$) of $2.14(2) \text{ \AA}$ and $4.77(2) \text{ \AA}$ and to the β -carbon atoms ($N = 20, 21, 22, 23$) of $4.24(2) \text{ \AA}$ are required, however, to model these distances to the crystal data of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.²⁴ The goodness-of-fit parameters for the seven-shell SS models, with and without vector distance restraints are unacceptably high, i.e., $R_{\text{xaf}} = 30.9\text{--}39.5\%$.

Furthermore, there is a significant residual spectrum in the XAFS and FT refinement data (Figure 3.13). This poor fit of the observed and calculated XAFS data is due to the exclusion of multiple back-scattering contributions from the seven-shell SS model (Section 3.8.2, Figure 3.15).

3.8.2 Multiple Scattering XAFS

The limitations of SS XAFS analysis with regard to the exclusion of multiple back-scattering contributions, were addressed in part by the MS analyses. A number of MS models of the crystallographically characterised $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ were investigated prior to the analyses of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMA}, \text{NMP}, \text{OH}_2$). The major differences between the various MS models can be summarised as inclusion/exclusion of the atomic XAFS (AXAFS) region, maximum number of legs per MS pathway, total distance traveled by the photoelectron (R_{eff}) and the starting distance for the Cu- O_L bond used in the model. The investigation of the various MS models highlighted the need to use the 130 pathway model in which no paths were filtered from the calculations ($R_{\text{eff}} = 10.2 \text{ \AA}$; maximum No. of legs = 6). Whilst a 65 path MS model ($R_{\text{eff}} = 10.0 \text{ \AA}$; maximum No. of legs = 5; CW and PW filters = 3% and 2%) resulted in refined bond distances for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 10 K and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT similar to crystallographic data, two refinement minima were found at 10 K for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (Table 3.11) depending upon the starting distance for the Cu- O_L bond used in the model. No such double refinement minima were found for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMA}, \text{NMP}$) at 10 K and RT. Descriptions of the most relevant 65 and 130 pathway MS models, i.e., k - and r -range, including starting distance for the Cu- O_L bond, maximum number of legs per MS path, data collection temperature, determinacy of the model and the value of the R_{xafs} parameter, along with their refinement results are given in Tables 3.7 and 3.8, respectively. Tables of some of the MS paths excluded from the 65 pathway MS model, but included in the 130 pathway MS model, and some of the MS paths incorporating the axial DMF/neighbouring dimer molecules, i.e., a 259 pathway model, but excluded from the 130 pathway model, are given in Tables 3.9 and 3.10, respectively. A diagram of the Cu core with the MS XAFS numbering and tables of all the paths and their importance relative to the strongest MS path of the 65 MS path model ($R_{\text{eff}} = 10.0 \text{ \AA}$; maximum number of legs = 5; CW and PW filters = 3% and 2%) and unfiltered 130 MS path model ($R_{\text{eff}} = 10.2 \text{ \AA}$; maximum number of legs = 6) are given in Figure 2.5 and Appendix 9, respectively. A summary of the structural relationships of $\text{CuO}_4\text{O}'$ and $\text{CuO}_4\text{N}'$ coordination environments in Cu(II) dimer complexes are given in Table 3.11.

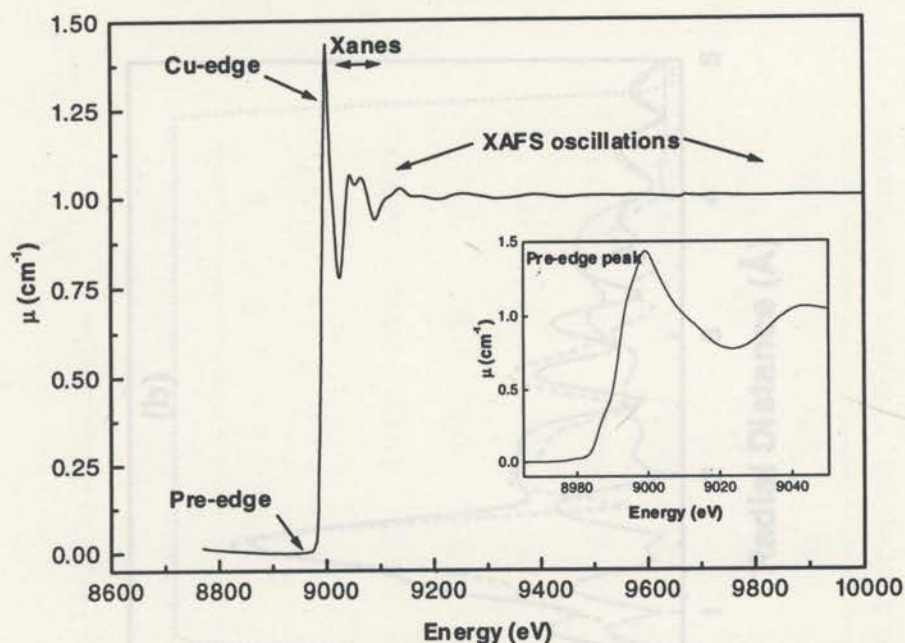


Figure 3.11. The normalised X-ray absorption Cu K-edge, featureless pre-edge absorbance, XANES and XAFS oscillations, with insert plot of the pre-K edge peak of solid $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K.

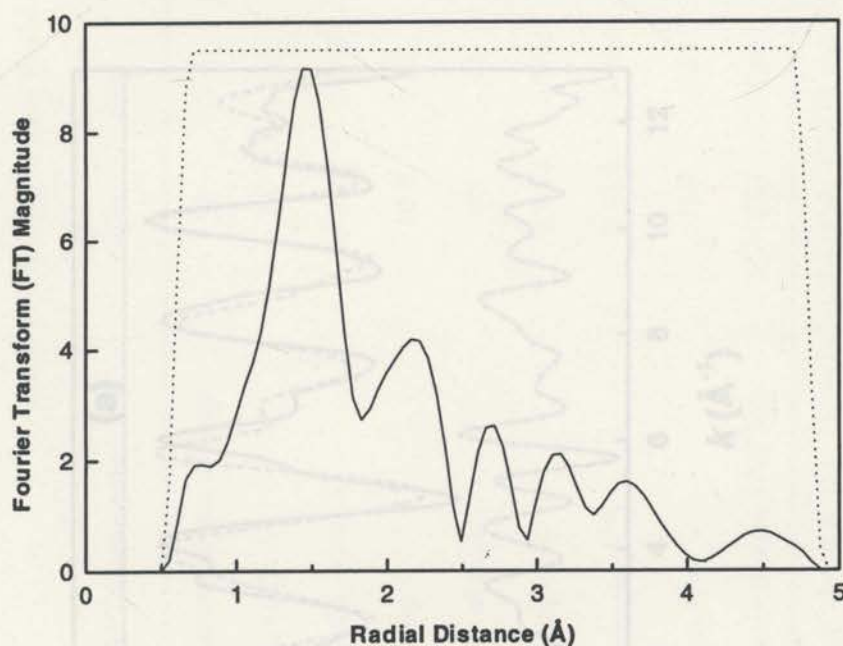


Figure 3.12. The Fourier transform (FT) of the observed XAFS of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K (k -range: 0.7-4.7 $\text{\AA}^{-1}$).

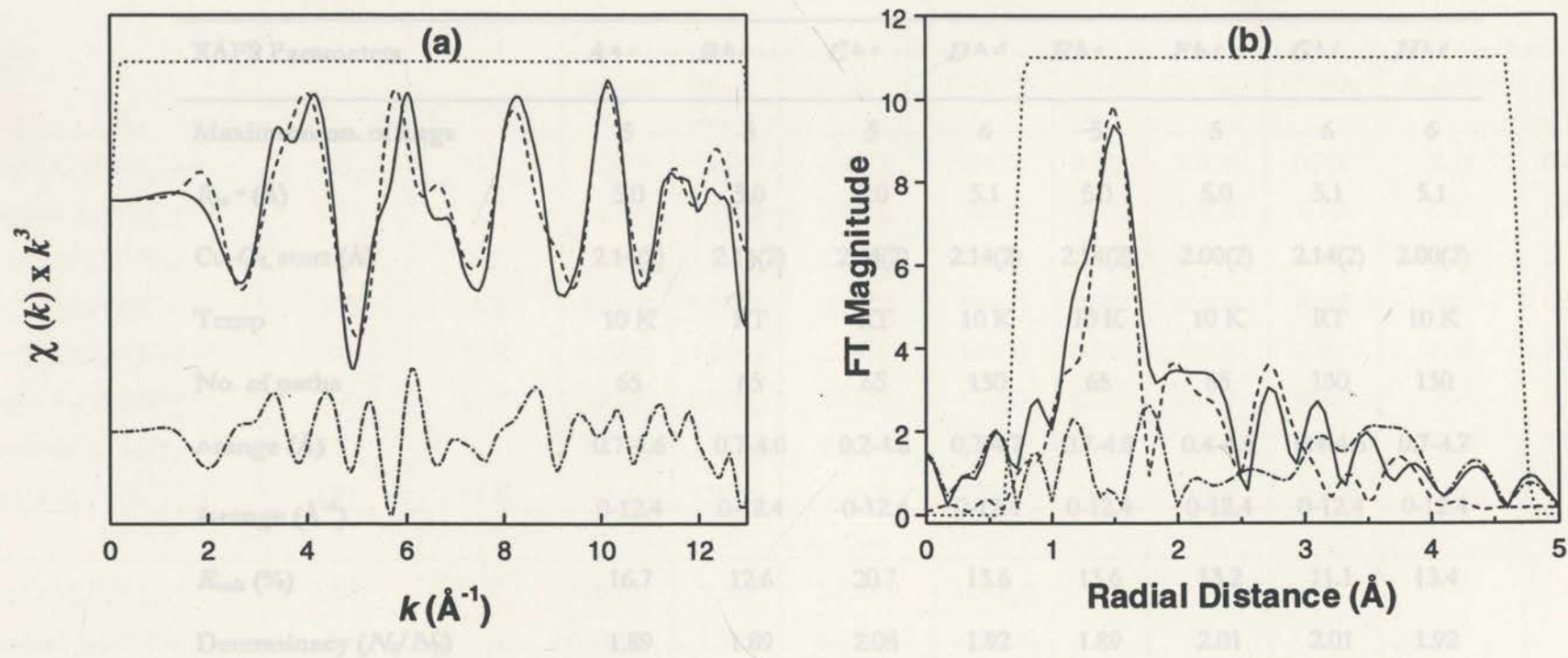


Figure 3.13. The seven-shell restrained Cu K-edge (a) SS XAFS and (b) corresponding FT observed (—), simulated (---) and residual (· · ·) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K ($R_{\text{XAF}} = 39.5\%$). The region shown by (· · ·) is the k window used in the Fourier filter.

Table 3.6. MS XAFS Refined Bond Distances (Å), Debye-Waller Factor (σ^2 (Å²)) and Bridging O-C-O Bond Angle (°) of [Cu₂(OAc)₄(OH₂)₂] and [Cu₂(Indo)₄(DMF)₂] Using Different Refinement Models, and Crystal Structure Data.

Complex	No. of paths	XAFS Parameters								XAFS parameter	
		<i>A</i> ^{a, c}	<i>B</i> ^{b, c}	<i>C</i> ^{b, c}	<i>D</i> ^{b, d}	<i>E</i> ^{b, c}	<i>F</i> ^{b, c}	<i>G</i> ^{b, d}	<i>H</i> ^{b, d}	σ^2	$\Delta\theta$
		Cu-O ₁	Cu-O ₁	Cu-O ₂	O-C-O	Cu-O ₁	Cu-O ₂	Cu-O ₁	Cu-O ₂		
Maximum no. of Legs		5	5	5	6	5	5	6	6		
<i>R</i> _{as} ^c (Å)		5.0	5.0	5.0	5.1	5.0	5.0	5.1	5.1		
Cu-O _L start (Å)		2.14(2)	2.14(2)	2.14(2)	2.14(2)	2.14(2)	2.00(2)	2.14(2)	2.00(2)		
Temp		10 K	RT	RT	10 K	10 K	10 K	RT	10 K		
No. of paths		65	65	65	130	65	65	130	130		
<i>r</i> -range (Å)		0.7-4.6	0.7-4.6	0.2-4.6	0.7-4.7	0.7-4.6	0.4-4.6	0.4-4.6	0.7-4.7		
<i>k</i> -range (Å ⁻¹)		0-12.4	0-12.4	0-12.4	0-12.4	0-12.4	0-12.4	0-12.4	0-12.4		
<i>R</i> _{xafs} (%)		16.7	12.6	20.7	13.6	13.6	13.2	11.1	13.4		
Determinacy (<i>N</i> _i / <i>N</i> _p)		1.89	1.89	2.08	1.92	1.89	2.01	2.01	1.92		

^a [Cu₂(OAc)₄(OH₂)₂]. ^b [Cu₂(Indo)₄(DMF)₂]. ^c %CW and %PW filters set at 3 and 2, respectively. ^d No paths were filtered in the refinement, i.e., %CW and %PW filters = 0. ^e The total distance travelled by the photoelectron *R*_{eff} is twice the value of *R*_{as}.

Table 3.8. MS XAFS Refined Bond Distances (Å), Debye-Waller Factors (σ^2 (Å²)) and Bridging O–C–O Bond Angle (°) of [Cu₂(OAc)₄(OH₂)] and [Cu₂(Indo)₄(DMF)₂] Using Different Refinement Models, and Crystal Structure Data.

Complex	No. of paths	Temp	Bond distance (Å)			Bond angle (°)	Debye-Waller factor (σ^2 (Å ²))			XAFS parameter		
			Cu–Cu	Cu–O _{Ac}	Cu–O _L		Cu–Cu	Cu–O _{Ac}	Cu–O _L	<i>E</i> ₀ (eV) ^h	<i>S</i> ₀ ² ⁱ	<i>R</i> _{xaf} (%) ^j
[Cu ₂ (OAc) ₄ (OH ₂)] ^{a, b}	65	10 K	2.61(2)	1.96(2)	2.16(2)	125(1)	0.003	0.006	0.008	-8.13	0.87	16.7
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, b}	65	RT	2.63(2)	1.95(2)	2.15(2)	125(1)	0.006	0.008	0.02	-9.97	0.91	12.6
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, b}	65	RT	2.63(2)	1.95(2)	2.15(2)	125(1)	0.006	0.009	0.02	-9.56	0.91	20.7
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, b}	130	10 K	2.63(2)	1.95(2)	2.14(2)	125(1)	0.003	0.004	0.008	-9.07	0.91	13.6
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, b}	65	10 K	2.62(2)	1.95(2)	2.19(2)	125(1)	0.003	0.004	0.008	-8.95	0.93	13.6
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, c}	65	10 K	2.62(2)	1.95(2)	2.15(2)	125(1)	0.003	0.004	0.007	-8.91	0.91	13.2
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, b}	130	RT	2.63(2)	1.95(2)	2.15(2)	125(1)	0.006	0.009	0.01	-9.58	0.91	11.1
[Cu ₂ (Indo) ₄ (DMF) ₂] ^{a, c}	130	10 K	2.62(2)	1.95(2)	2.14(2)	125(1)	0.003	0.004	0.009	-9.12	0.92	13.4
X-ray Data												
[Cu ₂ (OAc) ₄ (OH ₂)] ^d	RT		2.616(1)	1.969	2.156(4)	124.8(8)	–	–	–	–	–	–
[Cu ₂ (Indo) ₄ (DMF) ₂] ^e	RT		2.630(1)	1.959	2.143(5)	123.9(6)	–	–	–	–	–	–
[Cu ₂ (Indo) ₄ (DMSO) ₂] ^f	RT		2.632(6)	1.95	2.16(2)	NA	–	–	–	–	–	–
[Cu ₂ (Sup) ₄ (MeCN) ₂] ^g	RT		2.641(2)	1.95	2.182(7)	NA	–	–	–	–	–	–

^a The MS model is described in Table 3.7. ^b Cu–O_L refinement starting distance at 2.14 Å. ^c Cu–O_L refinement starting distance at 2.00 Å. ^d Taken from ref. 18. ^e Taken from ref. 24. ^f Taken from ref. 21. ^g Taken from ref. 22. ^h *E*₀ is relative to the conventional energy edge of 8980.3 eV.⁷³ ⁱ *S*₀² is the amplitude reduction factor. ^j Determinacy (*N_i/N_p*) of the models ~1.89, i.e., Δ*r* = 3.9 Å, Δ*k* = 12.4 Å⁻¹, *N_p* = 20, Σ*i* = 7.

Table 3.9. Some Paths of the 130-Pathway MS Model Excluded from the 65-Pathway MS Model.

Atoms in MS Path	No. of Legs	Degeneracy	R_{as} ^a (Å)	Importance Factor (%)
$Cu_0 \rightarrow O_8 \rightarrow C_{12} \rightarrow O_8 \rightarrow C_{12} \rightarrow O_8 \rightarrow Cu_0$	6	4	4.47	8.10%
$Cu_0 \rightarrow O_8 \rightarrow C_{12} \rightarrow O_9 \rightarrow C_{12} \rightarrow O_8 \rightarrow Cu_0$	6	4	4.47	4.25%
$Cu_0 \rightarrow O_7 \rightarrow C_{13} \rightarrow C_{23} \rightarrow C_{13} \rightarrow O_7 \rightarrow Cu_0$	6	4	4.71	4.05%
$Cu_0 \rightarrow C_{20} \rightarrow C_{10} \rightarrow O_4 \rightarrow Cu_0$	4	8	5.05	4.45%
$Cu_0 \rightarrow O_6 \rightarrow C_{13} \rightarrow C_{23} \rightarrow O_7 \rightarrow Cu_0$	5	8	5.09	4.64%

^a The total distance traveled by the photoelectron R_{eff} is twice the value of R_{as} .

Table 3.10. Some Paths of the 259-Pathway MS Model (Incorporating DMF and Neighbouring Dimer Molecules) Excluded from the 130-Pathway MS Model.

Atoms in MS Path	No. of Legs	Degeneracy	R_{as} ^c (Å)	Importance Factor (%)
$Cu_0 \rightarrow C_{16} \rightarrow Cu_0$ ^a	2	1	2.94	4.34%
$Cu_0 \rightarrow C_{16} \rightarrow O_{14} \rightarrow Cu_0$ ^a	3	2	3.26	2.29%
$Cu_0 \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$ ^a	4	2	3.82	1.70%
$Cu_0 \rightarrow N_{17} \rightarrow Cu_0$ ^a	2	1	4.14	3.60%
$Cu_0 \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$ ^a	3	2	4.17	4.18%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$ ^a	4	1	4.20	2.13%
$Cu_0 \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$ ^a	3	2	4.28	4.40%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$ ^a	4	2	4.31	2.27%
$Cu_0 \rightarrow C_{24} \rightarrow O_{14} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$ ^b	5	2	4.54	3.62%
$Cu_0 \rightarrow O_{14} \rightarrow C_{24} \rightarrow O_{14} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$ ^b	6	1	4.55	3.26%

^a DMF ligand atoms = C_{16} , N_{14} . ^b Indo atoms = C_{24} . ^c The total distance traveled by the photoelectron R_{eff} is twice the value of R_{as} .

Table 3.11. Summary of the Structural Relationships of the CuO_4O' and CuO_4N' Co-ordination Environments of Cu(II) dimers.

Coordination	Cu-O _{Ac} (Å)	Cu-O _L (Å)	Cu displacement ^a	O-C-O (°)	Bridging distance (Å) ^b	CuO ₅ (Å) ^c
CuO_4O' ^d	1.96	2.16	0.19-0.22	123.9	6.44	11.33
CuO_4N' ^d	1.97	2.11	0.22-0.32	125.6	6.42	11.42

^a Out-of-plane Cu displacement towards the axial ligand. ^b Sum of the bridging Cu-O-C-O-Cu bond lengths. ^c Sum of the Cu-O bond distances plus the half distance of the Cu...Cu vector. ^d Taken from refs. 2, 3, 5, 54.

The MS refined bond distances, Debye-Waller factors and bridging O–C–O bond angle for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) at RT and 10 K using the 130-path MS model are given in Table 3.12. The sum of the Cu–O–C–O–Cu bond distances, out-of-plane Cu displacement and sum of the Cu–O bond distances plus the half distance of the Cu...Cu vector of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$)^{2,3,5,54} using the 130-path MS model are given in Table 3.13. The observed, calculated and residual XAFS for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 10 K ($R_{\text{xaf}} = 16.7\%$) and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT ($R_{\text{xaf}} = 12.6\%$) and corresponding FTs using the 65 path MS model and Cu–O_L starting distance of 2.14 Å are shown in Figures 3.14 and 3.15, respectively.

The MS refined bond distances, Debye-Waller factors and bridging O–C–O bond angle for $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$) using 65 MS paths and $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{OH}_2$) using the 65 and 130 path MS models at 10 K and RT are given in Appendix 14. Figures of the observed, calculated and residual XAFS and the corresponding FT of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMA}, \text{NMP}$) using the 65 path MS model and Cu–O_L starting distance of 2.14 Å at RT, $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{OH}_2$) at RT using the 65 path MS model and Cu–O_L starting distance of 2.14 Å and $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{OH}_2$) at 10 K using the 65 path MS model and Cu–O_L bond distance restraints of 2.14 Å are given in Appendix 15.

The refined Cu...Cu, Cu–O_L and mean Cu–O_{Ac} bond lengths of 2.61 Å, 2.16 Å and 1.96 Å, respectively, for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 10 K using 65 unique MS pathways ($R_{\text{xaf}} = 16.7\%$) are in excellent agreement with the XRD-derived values of 2.616(1) Å, 2.156(4) Å and 1.969 Å.¹⁸ Likewise, the refined Cu...Cu, Cu–O_L and mean Cu–O_{Ac} bond lengths of 2.63(2) Å, 2.15(2) Å and 1.95(2) Å, respectively, for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT using 65 unique MS pathways ($R = 12.6\%$) are in excellent agreement with the crystallographic values of 2.630(1) Å, 2.143(5) Å and 1.959 Å.²⁴ Whilst the MS refinement of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K ($R_{\text{xaf}} = 13.6\%$) using 65 unique MS pathways, i.e., CW and PW filters set at 3% and 2%, respectively, results in an excellent agreement with values from XRD study for the Cu...Cu and Cu–O_{Ac} distances of 2.62(2) Å and 1.95(2) Å, respectively, the Cu–O_L is ~0.05 Å longer (i.e., 2.19 Å) than that reported in the crystal structure (2.143(5) Å).²⁴ MS refinement of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K with a starting Cu–O_L distance of 2.00(2) Å and 65 unique MS pathways results in an excellent agreement with XRD study, i.e., MS refined Cu...Cu, mean Cu–O_{Ac} and Cu–O_L bond lengths of 2.62(2) Å, 1.95(2) Å and 2.15(2) Å, respectively, and a slight improvement in the goodness-of-fit parameter (R_{xaf}) from 13.6% to 13.2%.

Table 3.12. MS XAFS Refined Bond Distances (Å), Debye-Waller Factors (σ^2 (Å²)) and Bridging O–C–O Bond Angle (°) of [Cu₂(Indo)₄L₂] (L = DMF, DMA, NMP, OH₂) at RT and 10 K Using 130 Paths.

Complex	No. of Paths	Temp	Bond distance (Å)			Bond angle (°)	Debye-Waller factor (σ^2 (Å ²))			XAFS parameter		
			Cu–Cu	Cu–O _{Ac}	Cu–O _L		Cu–Cu	Cu–O _{Ac}	Cu–O _L	<i>E</i> ₀ (eV) ^c	<i>S</i> ₀ ² ^d	<i>R</i> _{xaf} (%) ^e
[Cu ₂ (Indo) ₄ (DMF) ₂] ^a	130	10 K	2.63(2)	1.95(2)	2.14(2)	125(1)	0.003	0.005	0.01	-9.52	0.91	13.4
[Cu ₂ (Indo) ₄ (DMF) ₂] ^a	130	RT	2.62(2)	1.95(2)	2.15(2)	125(1)	0.006	0.009	0.01	-9.27	0.91	11.6
[Cu ₂ (Indo) ₄ (DMA) ₂] ^a	130	10 K	2.63(2)	1.95(2)	2.13(2)	125(1)	0.003	0.005	0.01	-9.48	0.91	13.4
[Cu ₂ (Indo) ₄ (DMA) ₂] ^a	130	RT	2.63(2)	1.95(2)	2.09(2)	125(1)	0.007	0.01	0.02	-10.07	0.90	12.5
[Cu ₂ (Indo) ₄ (NMP) ₂] ^a	130	10 K	2.63(2)	1.96(2)	2.14(2)	125(1)	0.003	0.005	0.01	-9.63	0.92	11.5
[Cu ₂ (Indo) ₄ (NMP) ₂] ^a	130	RT	2.63(2)	1.95(2)	2.10(2)	125(1)	0.006	0.008	0.01	-9.97	0.91	13.3
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^b	130	10 K	2.61(2)	1.94(2)	–	125(1)	0.002	0.003	–	-10.00	0.89	16.9
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^a	130	10 K	2.62(2)	1.95(2)	2.15(2)	125(1)	0.002	0.004	0.05	-9.50	0.91	17.2
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^a	130	RT	2.62(2)	1.95(2)	2.20(2)	125(1)	0.006	0.01	0.04	-9.18	0.91	10.5

^a Cu–O_L starting distance 2.00 Å. ^b Nil refinement of the Cu–O_L distance. ^c *E*₀ is relative to the conventional energy edge of 8980.3 eV.⁷³; ^d *S*₀² is the amplitude reduction factor. ^e Determinacy (*N_i/N_p*) of the models ~1.8, i.e., Δ*r* = 3.9 Å; Δ*k* = 12.5 Å⁻¹; *N_p* = 20; Σ*i* = 7.

Table 3.13. MS Refined Structural Data for the Bridging Groups of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) with a CuO_5 Coordination Geometry.

COMPLEX ^a	Temp	Cu Displacement (Å)	Cu—O—C—O—Cu bridge length (Å)	CuO ₅ (plus ½ Cu—Cu) distance (Å)
$[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$	10 K	0.18(2)	6.44(8)	11.31(12)
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$	10 K	0.19(2)	6.48(8)	11.23(12)
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$	RT	0.19(2)	6.42(8)	11.27(12)
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$	10 K	0.20(2)	6.42(8)	11.25(12)
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$	RT	0.20(2)	6.43(8)	11.21(12)
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$	10 K	0.20(2)	6.43(8)	11.29(12)
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$	RT	0.20(2)	6.42(8)	11.22(12)
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$	10 K	0.18(2)	6.41(8)	11.26(12)
$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$	RT	0.19(2)	6.41(8)	11.31(12)
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ^b	RT	0.20	6.435(16)	11.295(26)
$[\text{Cu}_2(\text{Indo})_4(\text{DMSO})_2]$ ^c	RT	NA ^f	6.38(10)	11.18(16)
$[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ^d	RT	0.19	6.458(18)	11.340(17)
$[\text{Cu}_2(\text{Sup})_4(\text{CH}_3\text{CN})_2]$ ^e	RT	0.15	6.39(28)	11.318(29)

^a Taken from Table 3.12. ($[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ data taken from Table 3.8). ^b Taken from ref 24. ^c Taken from ref. 21

^d Taken from ref. 18. ^e Taken from ref. 22. ^f Full details of the structure have not been published or deposited in the Cambridge Crystallographic Data Base.

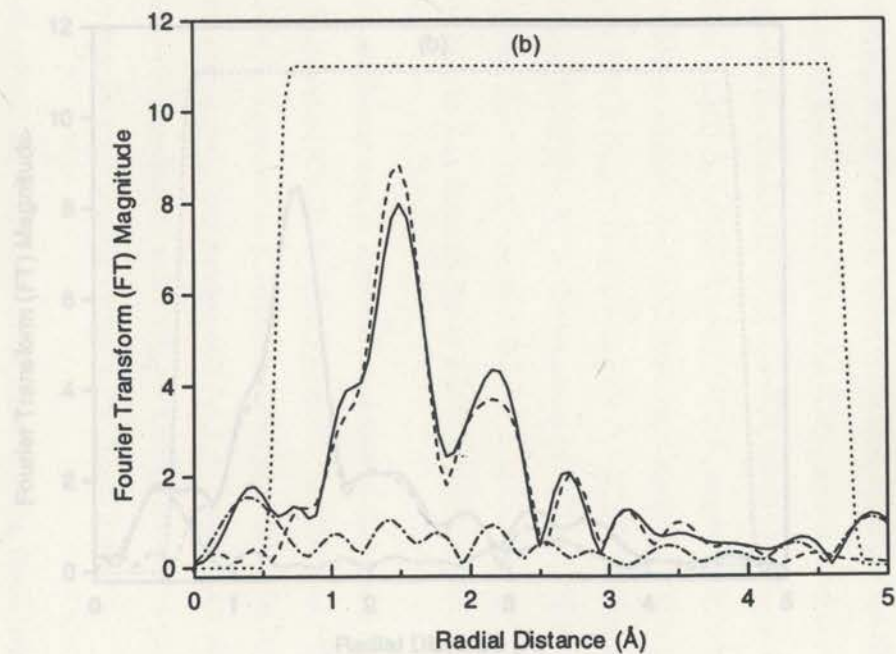
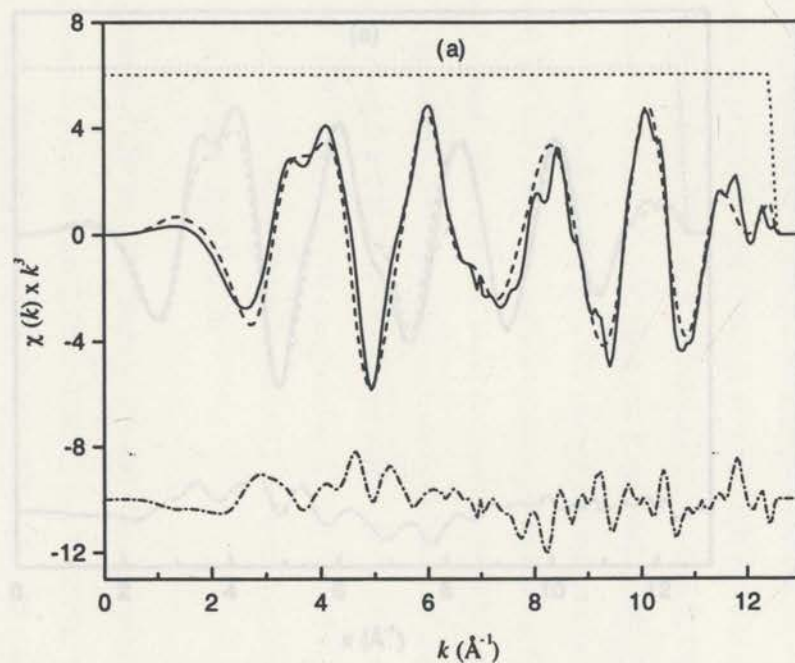


Figure 3.14. Cu K-edge (a) MS XAFS data and corresponding FT observed (—), simulated (···) and residual (---) and for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ at 10 K; ($R_{\text{xf}} = 16.7\%$); 65 unique MS pathways. The region shown by (···) is the k window used in the Fourier filter.

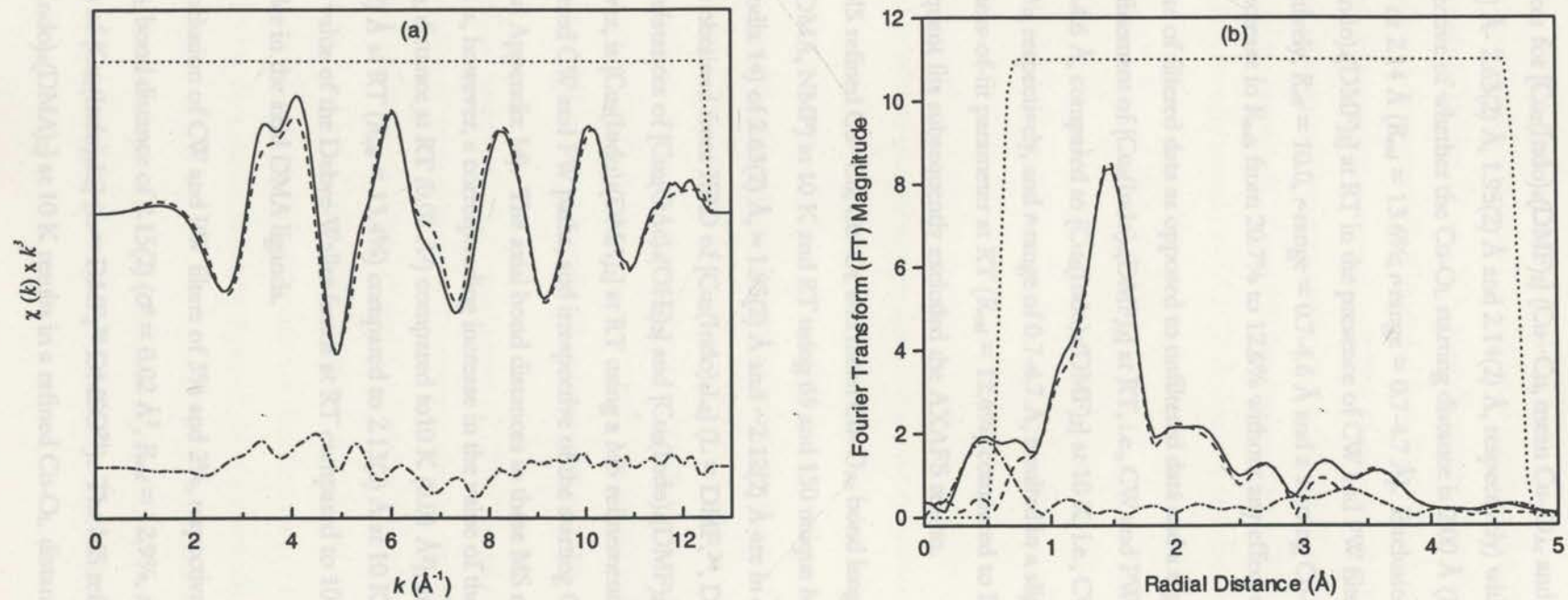


Figure 3.15. Cu K-edge (a) MS XAFS and corresponding FT observed (—), simulated (···) and residual (---) for solid $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT; $R_{\text{xaf}} = 12.6\%$; 65 unique MS pathways. The region shown by (···) is the k window used in the Fourier filter.

The removal of CW and PW filters from analysis of the XAFS data obtained at 10 K and an increase in R_{eff} from 10.0 Å to 10.2 Å also results in an excellent agreement of the fitted distances for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\text{Cu}\cdots\text{Cu}$, mean $\text{Cu}-\text{O}_{\text{Ac}}$ and $\text{Cu}-\text{O}_{\text{L}}$ bond lengths of 2.62(2) Å- 2.63(2) Å, 1.95(2) Å and 2.14(2) Å, respectively) with crystallographic value,²⁴ irrespective of whether the $\text{Cu}-\text{O}_{\text{L}}$ starting distance is 2.00 Å ($R_{\text{xaf}} = 13.4\%$; r -range = 0.7-4.7 Å) or 2.14 Å ($R_{\text{xaf}} = 13.6\%$; r -range = 0.7-4.7 Å). Exclusion of the AXAFS of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT in the presence of CW and PW filters set at 3% and 2%, respectively, $R_{\text{eff}} = 10.0$, r -range = 0.7-4.6 Å and a starting $\text{Cu}-\text{O}_{\text{L}}$ distance of 2.14 Å results in a decrease in R_{xafs} from 20.7% to 12.6% without any effect on the fitted parameters.

The use of filtered data as opposed to unfiltered data and a slightly smaller r -range used in the refinement of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT, i.e., CW and PW filters set at 0% and r -range of 0.7-4.6 Å, compared to $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K, i.e., CW and PW filters set at 3% and 2%, respectively, and r -range of 0.7-4.7 Å, resulted in a slight improvement in the goodness-of-fit parameter at RT ($R_{\text{xaf}} = 12.6\%$) compared to LT ($R_{\text{xaf}} = 13.6\%$). All subsequent fits subsequently excluded the AXAFS region.

The MS refined $\text{Cu}\cdots\text{Cu}$, $\text{Cu}-\text{O}_{\text{L}}$ and mean $\text{Cu}-\text{O}_{\text{Ac}}$ bond lengths of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMA}$, NMP) at 10 K and RT using 65 and 130 unique MS pathways (Table 3.12 and Appendix 14) of 2.63(2) Å, $\approx 1.95(2)$ Å and $\sim 2.12(2)$ Å are in excellent agreement to the values obtained from XRD of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}$,²⁴ DMSO ²¹) and the MS refined bond distances of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (Table 3.8). The exception, however, is $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ at RT using a MS refinement model of 130 paths, i.e., unfiltered CW and PW paths, and irrespective of the starting $\text{Cu}-\text{O}_{\text{L}}$ bond distances (Table 3.8 and Appendix 14). The axial bond distances in these MS models refine to 2.09(2) Å. There is, however, a corresponding increase in the value of the Debye-Waller factor of the $\text{Cu}-\text{O}_{\text{L}}$ distance at RT (0.02 Å²) compared to 10 K (0.01 Å²), and a shorter $\text{Cu}-\text{O}_{\text{L}}$ distance of 2.09(2) Å at RT ($R_{\text{xaf}} = 13.4\%$) compared to 2.13(2) Å at 10 K ($R_{\text{xaf}} = 12.5\%$). This increase in the value of the Debye-Waller factor at RT compared to 10 K may be due to greater disorder in the axial DMA ligands.

The inclusion of CW and PW filters of 3% and 2%, respectively, at RT result in a refined $\text{Cu}-\text{O}_{\text{L}}$ bond distance of 2.15(2) ($\sigma^2 = 0.02$ Å², $R_{\text{xaf}} = 12.9\%$, Appendix 14) similar to XRD values of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}$,²⁴ DMSO ²¹). The MS refinement of $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ at 10 K results in a refined $\text{Cu}-\text{O}_{\text{L}}$ distances of 2.12(2) Å similar to the

crystal structure of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]^{24}$ irrespective of the presence or absence of CW or PW filters (Table 3.12 and Appendix 14).

The refined $\text{Cu}\cdots\text{Cu}$ and mean $\text{Cu}-\text{O}_{\text{Ac}}$ bond lengths of 2.62(2) Å and 1.95(2) Å for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at RT and 10 K using 65 unique MS pathways are similar to the XAFS refined bond distances of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$). The $\text{Cu}-\text{O}_{\text{L}}$ distance of the aqua adduct refines, however, to 2.22 Å (Appendix 14). This longer $\text{Cu}-\text{O}_{\text{L}}$ bond length is obtained irrespective of temperature or the number of unique MS pathways, i.e., the presence or absence of CW and PW filters set at 3% and 2%, respectively. Axial bond distance symmetry restraints of 2.14(2) Å were required for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at RT and 10 K to simulate a similar $\text{Cu}-\text{O}_{\text{L}}$ bond distance to that of the other $\text{Cu}(\text{II})$ dimers. There is a significant concomitant increase in the values of R_{xaf} for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K and RT from 15.3% and 11.0%, respectively, to 16.8% and 11.8%, respectively, due to the inclusion of axial bond distance symmetry restraints (Appendix 14) for the 65 pathway model.

Exclusion of the two axial oxygen ligands from the refinement model of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K, using 130 MS pathways results in a marginal decrease in R_{xaf} from 17.2% to 16.9% (Table 3.12). An increase from 65 to 130 pathways and a starting $\text{Cu}-\text{O}_{\text{L}}$ distance of 2.00 Å results in a refined axial bond distance of 2.15(2) Å ($R_{\text{xaf}} = 17.2\%$) (Table 3.12) for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K but a value of 2.20(2) Å ($R_{\text{xaf}} = 10.5\%$) was obtained at RT (Appendix 14). Increasing the weight of the XAFS (w) from 1 to 8 also results in a longer than expected $\text{Cu}-\text{O}_{\text{L}}$ bond distance of 2.25(2) Å for $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K using the 65 pathway MS model (Appendix 14) despite using a $\text{Cu}-\text{O}_{\text{L}}$ starting distance of 2.00 Å.

The refined bridging $\text{O}-\text{C}-\text{O}$ bond angles of $125(1)^\circ$ for all the $\text{Cu}(\text{II})$ complexes were independent of the axial ligand and consistent with the crystal structure values for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($123.9(6)^\circ$)²⁴ and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ($124.8(8)^\circ$).¹⁸ Furthermore, the sum of the $\text{Cu}-\text{O}-\text{C}-\text{O}-\text{Cu}$ bridge lengths (~ 6.42 Å) and sum of the $\text{Cu}-\text{O}$ bond lengths around the Cu core (including the half $\text{Cu}\cdots\text{Cu}$ distance) (~ 11.26 Å) for all the complexes (Table 3.13) were similar to values reported elsewhere for dimeric $\text{Cu}(\text{II})$ carboxylate compounds (Table 3.13).^{2,3,5} The refined $\text{Cu}-\text{O}_{\text{L}}$ bond distances of $\sim 2.12(2)$ Å for the $\text{Cu}(\text{II})$ dimers of Indo are shorter than the reported $\text{Cu}-\text{N}_{\text{L}}$ distance of 2.182(7) Å for $[\text{Cu}_2(\text{Sup})_4(\text{MeCN})_2]$.²² The reported $\text{Cu}-\text{N}_{\text{L}}$ distance for $[\text{Cu}_2(\text{Sup})_4(\text{MeCN})_2]$,²² being

longer than the Cu-O_L distance for [Cu₂(Indo)₄(DMF)₂],²⁴ is contrary to that predicted by the structure relationships reviews.^{2,3,5,54}

MS refinements for [Cu₂(Indo)₄(DMF)₂] were also undertaken including all DMF, Indo and neighbouring dimer atoms less than 5.1 Å from the Cu absorber, i.e., Cu₀, (Appendix 8). The refinements were undertaken with and without bond distances and angle restraints on the atoms and occupancy numbers (*N*) for the DMF ligand atoms which were taken from XRD studies,²⁴ i.e., C₁₆ ~0.48, C₃₁ ~0.52, C₃₂ ~0.38, C₂₇ ~0.62 and N₁₇ ~1.0, and a starting Cu-O_L distance of 2.00 Å (Appendices 7 and 8). In all cases, errors of the refined positional parameters were unacceptably high and/or the value of the Debye-Waller factors were at the maximum values allowed by the refinement restraints.

Refinement of [Cu₂(Indo)₄(DMF)₂] at 10 K with bond distance and angle restraints on the DMF ligand atoms, and occupancies as described above, result in $R_{\text{xafs}} = 13.2\%$ ($N_i/N_p \sim 1.2$) and Cu...Cu, Cu-O_{Ac} and Cu-O_L distances of 2.62(2) Å, 1.95(2) Å, and 2.15(2) Å, respectively (Appendix 7). The values of the Debye-Waller factors and standard deviation of the refined positional coordinates for the refined DMF atoms, i.e., C₁₆, N₁₇, C₃₁, C₃₂ and C₂₇, are equivalent to the value of their restraints, and/or high, respectively. This indicates the low contributions to the XAFS of the outer atoms of the DMF ligands. In all other MS refinements in this work, the determinacy of the models (N_i/N_p) are ~2.0

3.9 Micellular Capillary Electrophoresis (MCE)

Whilst solid-state techniques were successfully applied to the characterisation of [Cu₂(Indo)₄L₂] (L = DMF, DMA, NMP, OH₂), attempts to separate IndoH from [Cu₂(Indo)₄(DMF)₂] proved less successful. The use of MEC to separate [Cu₂(Indo)₄(DMF)₂] from IndoH was, therefore, investigated. [Cu₂(Indo)₄(DMF)₂] was incorporated into micellular buffer solution to limit degradation of the complex. The resultant solution was buffered to ~pH 7.4 to ensure ionisation of IndoH ($pK_a = 4.5$).⁷⁴ MEC was undertaken using SDS (40 mM) in TES, triethanolamine, HEPES (100 mM), borate (50 mM) buffers or sodium nitrate (50 mM) in both the normal, i.e., inlet: positive and outlet: negative, and reverse polarity modes.

Elution profiles indicative of a separation of IndoH from [Cu₂(Indo)₄(DMF)₂] were observed only for SDS (40 mM) in TES (100 mM) in the normal polarity mode. A single

elution peak at ~ 6 -7 min was observed for the solution samples of IndoH. Degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to IndoH occurred during the MEC analysis as evidenced by two elution peaks at ~ 2.6 min and 4.7 min in the solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mg mL^{-1}). A reduction in the elution time and increase in the intensity of the elution peak of IndoH was observed with an increased concentration of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in solutions of IndoH plus $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Two elution peaks were observed at ~ 2.6 min and ~ 4.7 min, and 2.5 min and 4.5 min in the solutions of IndoH (0.5 mg mL^{-1}) plus $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.5 mg mL^{-1}) for the former and IndoH (0.2 mg mL^{-1}) plus $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.8 mg mL^{-1}) for the latter (Figure 3.16). The intensity of the peak at 2.6 min was only marginally enhanced when the concentration of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ was increased.

From EPR spectroscopy, the Cu(II) monomer content for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mg mL^{-1}) in SDS (40 mM) and TES (100 mM) 3- and 18-hr post preparation was less than 4% and 6%, respectively (Chapter 4). The Cu(II) monomer content of the eluent was not determined. Glycerol (20% v/v) was added to the buffer solution to ensure a vitrified sample suitable for low temperature EPR measurement. The use of glycerol (20% v/v) as a vitrifying agent had little effect on the Cu(II) monomer determination compared to DMSO (20% v/v). The Cu(II) monomer content for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mg mL^{-1}) in SDS (40 mM) and TES (100 mM) 1- and 3-hr post preparation using DMSO (20% v/v) was 9%. The estimated standard deviation in the Cu(II) monomer determination by EPR is $\sim 10\%$ (Chapter 4).

3.10 Electrospray Ionisation-Mass Spectroscopy (ESI-MS)

ESI-MS was also investigated as a technique to differentiate IndoH from $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. The negative-ion ESI-MS of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1.29 mM) and a 2:1 molar mixture of IndoH (1.56 mM): $[\text{Cu}(\text{CH}_3\text{COO})_2]$ (0.78 mM) in MeCN solvent with a 1:1 carrier mixture of methanol (MeOH) and water (0.1% acetic acid) along with selected m/z assignments and simulations are shown in Figures 3.17 and 3.18, respectively. A summary of the ESI-MS data for MeCN solution samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1.29 mM), IndoH (1.56 mM): $\text{Cu}(\text{CH}_3\text{COO})_2$ (0.78 mM), i.e., 2:1 molar ratio of IndoH:Cu(II), and IndoH (2.35 mM): $\text{Cu}(\text{CH}_3\text{COO})_2$ (0.31 mM), i.e., 7.5:1 molar ratio of IndoH:Cu(II), is given in Table 3.14.

Little differences, other than some variations in peak intensities, were observed between the scans of the ESI-MS of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, 2:1 and 7.5:1 molar ratio of IndoH:Cu(II). A more intense peak at m/z 715, corresponding to 2 mass units of $(\text{IndoH})_2^-$, was observed in the spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and Indo:Cu(II) (molar ratio 7.5:1) than in that of a 2:1 molar ratio of IndoH:Cu(II). Likewise, an m/z peak observed at 417, i.e., $\{\text{IndoH} + (\text{CH}_3\text{COO})\}^-$, is observed in the spectrum of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ but at a lower intensity in those of the 2:1 and 7.5:1 molar ratio solutions of Indo:Cu(II).

Peaks at ~ 417 , 834 and 1615 m/z , i.e., incorporating an acetate species, are due to the addition of acetic acid (0.1%) to the ESI-MS carrier solution. Aggregates of Cu+Indo+acetate were less pronounced in the spectra of the 7.5:1 molar ratio of IndoH:Cu(II) than in either those of the $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ or 2:1 molar ratio of IndoH:Cu(II) samples. However, three minor peaks at ~ 457 , 480 and 539 m/z were more pronounced in the ESI-MS scan of the 2:1 molar ratio mixture of Indo:Cu(II) than in the scans of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ or 7.5:1 molar ratio of IndoH:Cu(II).

No peaks were observed in any of the solution samples corresponding to the m/z assignment for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{MeCN})_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{MeOH})_2]$ or $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$. All samples exhibited a small peak at ~ 1554 m/z , which corresponded to a dimeric Cu species with four Indo units. Little differences, other than variations in peak intensities, were observed between the ESI-MS of the 2:1 and 7.5:1 molar ratio of Indo:Cu(II). No correlation was found between an increased amount of IndoH and the formation of large mass aggregates of IndoH, e.g., $\{\text{Cu} + 3(\text{Indo})\}^-$, $\{2(\text{Cu}) + 3(\text{Indo}) + (\text{IndoH})\}^-$. The ES-MS results indicate variable peak intensities of the -ve ion ESI-MS scan of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ irrespective of the IndoH or $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ concentration.

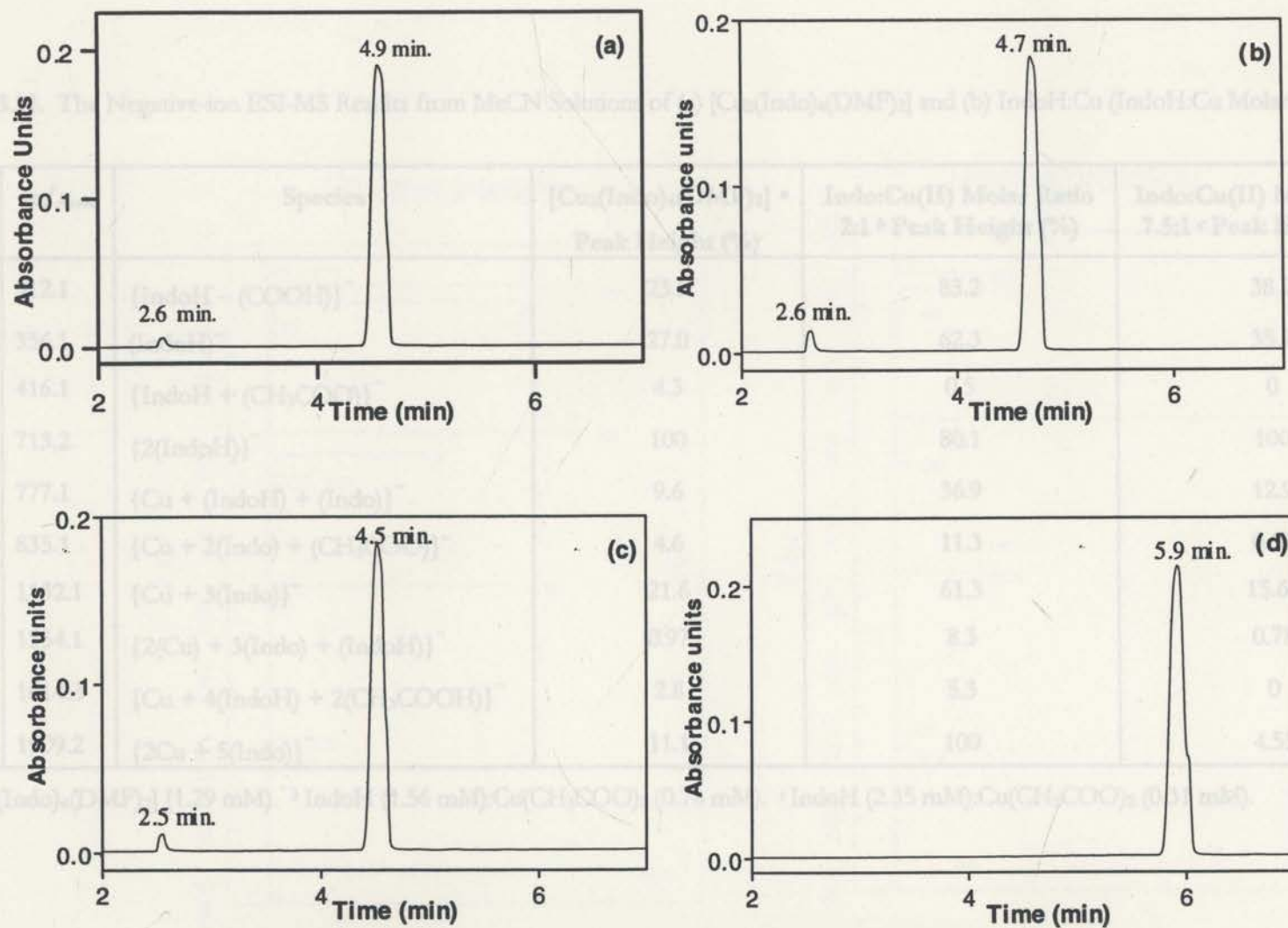


Figure 3.16. Elution times of (a) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.5 mg mL^{-1}) and IndoH (0.5 mg mL^{-1}), (b) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mg mL^{-1}) (c) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.8 mg mL^{-1}) and IndoH (0.2 mg mL^{-1}) and (d) IndoH (1 mg mL^{-1}) using SDS (100 mM) and TES (100 mM).

Table 3.14. The Negative-ion ESI-MS Results from MeCN Solutions of (a) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and (b) IndoH:Cu (IndoH:Cu Molar Ratio 2:1).

m/z_{calc}	m/z_{obs}	Species	$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]^a$ Peak Height (%)	Indo:Cu(II) Molar Ratio 2:1 ^b Peak Height (%)	Indo:Cu(II) Molar Ratio 7.5:1 ^c Peak Height (%)
312.8	312.1	$\{\text{IndoH} - (\text{COOH})\}^-$	23.5	83.2	38.7
356.8	356.1	$(\text{IndoH})^-$	27.0	62.3	35.7
416.8	416.1	$\{\text{IndoH} + (\text{CH}_3\text{COO})\}^-$	4.3	0.5	0
714.6	713.2	$\{2(\text{IndoH})\}^-$	100	80.1	100
777.1	777.1	$\{\text{Cu} + (\text{IndoH}) + (\text{Indo})\}^-$	9.6	36.9	12.9
835.2	835.1	$\{\text{Cu} + 2(\text{Indo}) + (\text{CH}_3\text{COO})\}^-$	4.6	11.3	0.38
1132.9	1132.1	$\{\text{Cu} + 3(\text{Indo})\}^-$	21.6	61.3	15.65
1554.2	1554.1	$\{2(\text{Cu}) + 3(\text{Indo}) + (\text{IndoH})\}^-$	0.97	8.3	0.78
1613.8	1614.3	$\{\text{Cu} + 4(\text{IndoH}) + 2(\text{CH}_3\text{COOH})\}^-$	2.8	5.3	0
1910.0	1909.2	$\{2\text{Cu} + 5(\text{Indo})\}^-$	11.1	100	4.55

^a $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1.29 mM). ^b IndoH (1.56 mM):Cu(CH₃COO)₂ (0.78 mM). ^c IndoH (2.35 mM):Cu(CH₃COO)₂ (0.31 mM).

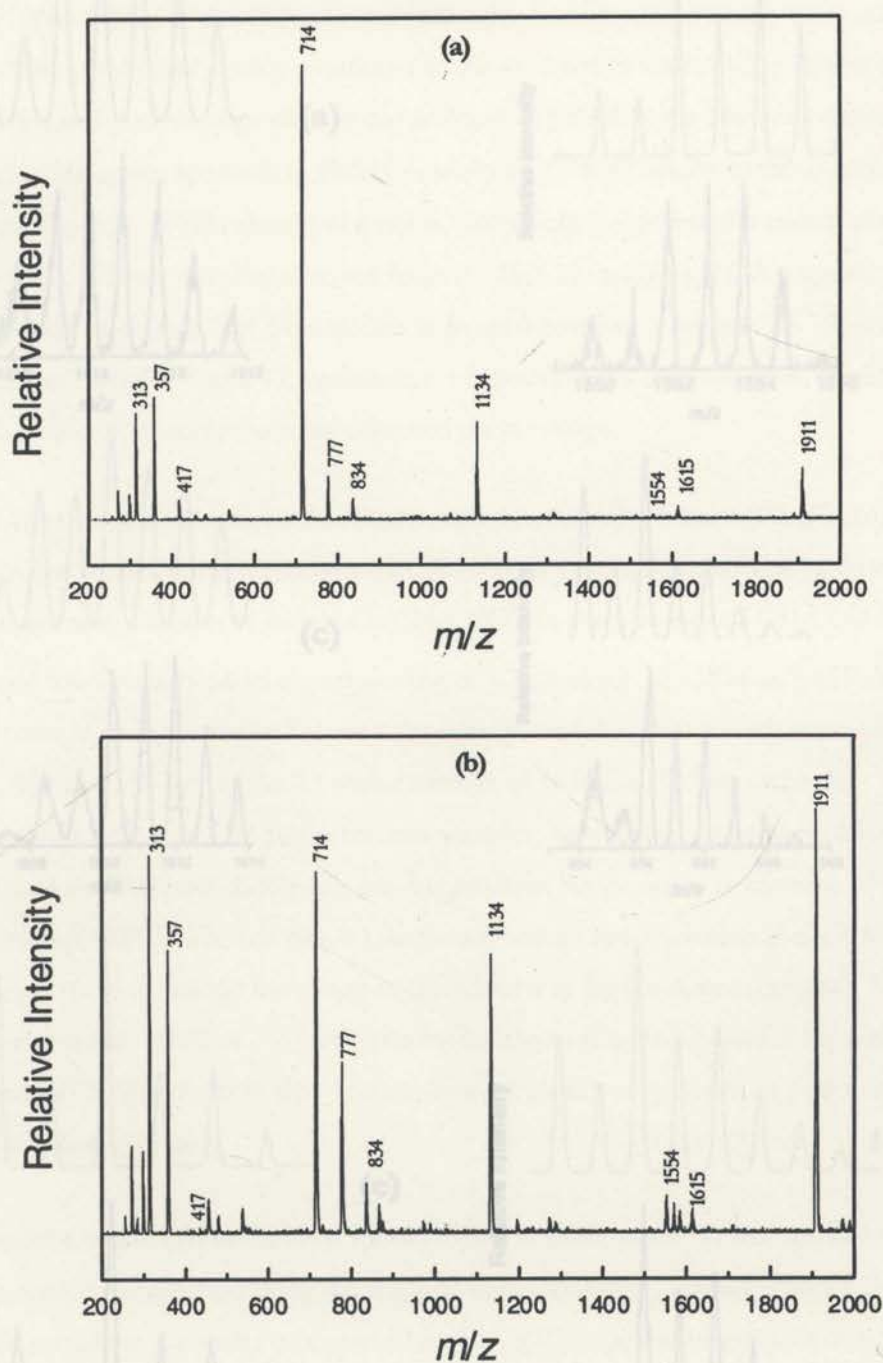


Figure 3.17. The negative-ion ESI-MS in MeCN of (a) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, (b) $\text{IndoH} + [\text{Cu}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ in a 4:1 molar mixture.

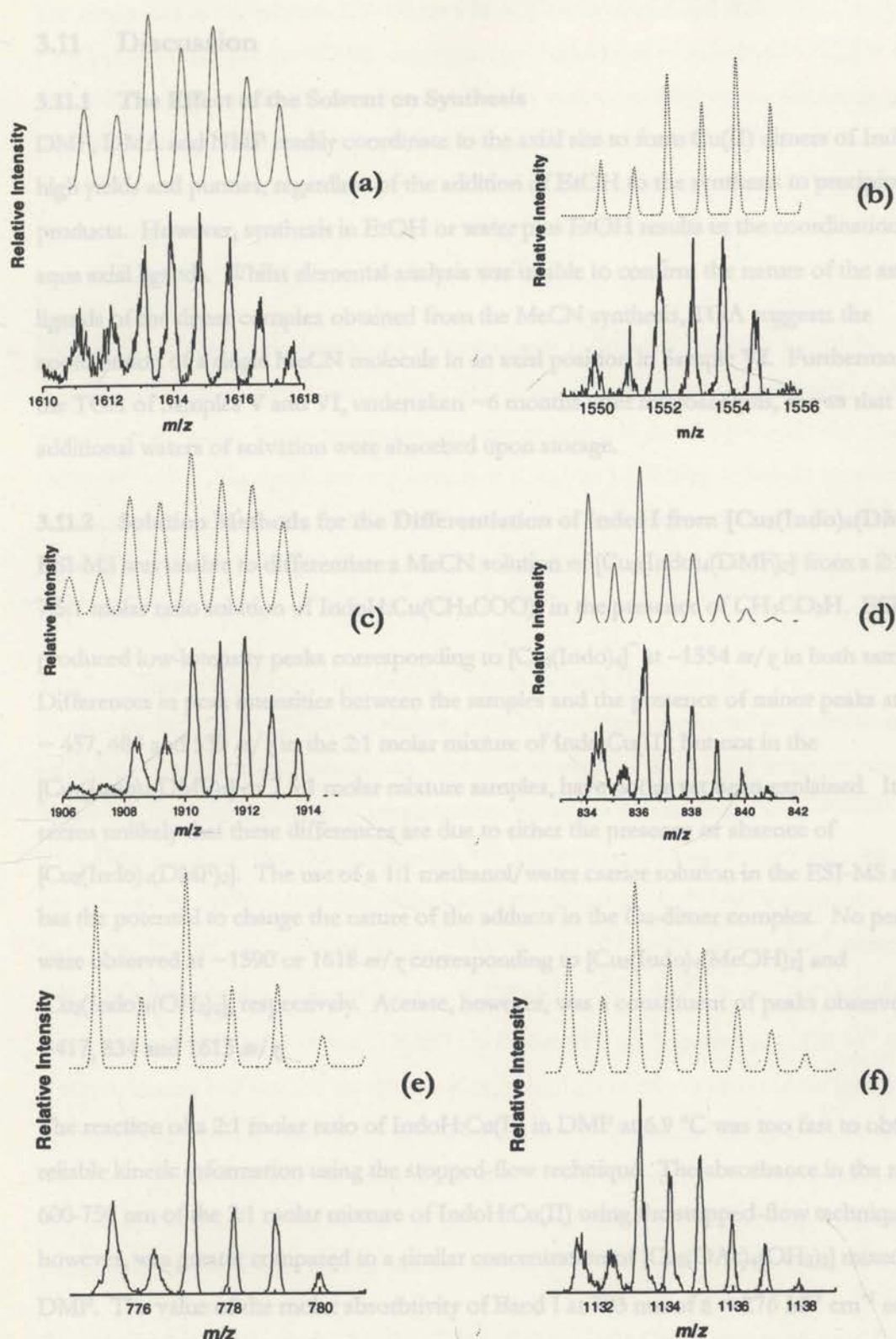


Figure 3.18. Selected electro-spray ionisation mass spectra of the observed (—) and simulated (····) assignments for (a) $1614\ m/z$, (b) $1554\ m/z$, (c) $1910\ m/z$, (d) $835\ m/z$, (e) $777\ m/z$ and (f) $1132\ m/z$.

3.11 Discussion

3.11.1 The Effect of the Solvent on Synthesis

DMF, DMA and NMP readily coordinate to the axial site to form Cu(II) dimers of Indo in high yields and purities, regardless of the addition of EtOH to the synthesis to precipitate products. However, synthesis in EtOH or water plus EtOH results in the coordination of aqua axial ligands. Whilst elemental analysis was unable to confirm the nature of the axial ligands of the dimer complex obtained from the MeCN synthesis, TGA suggests the coordination of a single MeCN molecule in an axial position in Sample VI. Furthermore, the TGA of Samples V and VI, undertaken ~6 months after microanalysis, shows that additional waters of solvation were absorbed upon storage.

3.11.2 Solution Methods for the Differentiation of IndoH from $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$

ESI-MS was unable to differentiate a MeCN solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ from a 2:1 or 7.5:1 molar ratio solution of IndoH:Cu(CH₃COO)₂ in the presence of CH₃CO₂H. ESI-MS produced low-intensity peaks corresponding to $[\text{Cu}_2(\text{Indo})_4]^-$ at $\sim 1554\text{ }m/z$ in both samples. Differences in peak intensities between the samples and the presence of minor peaks at $\sim 457, 480$ and $539\text{ }m/z$ in the 2:1 molar mixture of Indo:Cu(II), but not in the $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ or 7.5:1 molar mixture samples, have not as yet been explained. It seems unlikely that these differences are due to either the presence or absence of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. The use of a 1:1 methanol/water carrier solution in the ESI-MS set-up has the potential to change the nature of the adducts in the Cu-dimer complex. No peaks were observed at ~ 1590 or $1618\text{ }m/z$ corresponding to $[\text{Cu}_2(\text{Indo})_4(\text{MeOH})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, respectively. Acetate, however, was a constituent of peaks observed at $\sim 417, 834$ and $1615\text{ }m/z$.

The reaction of a 2:1 molar ratio of IndoH:Cu(II) in DMF at 6.9 °C was too fast to obtain reliable kinetic information using the stopped-flow technique. The absorbance in the range 600-750 nm of the 2:1 molar mixture of IndoH:Cu(II) using the stopped-flow technique, however, was greater compared to a similar concentration of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ mixed with DMF. The value of the molar absorptivity of Band I at 703 nm of $\epsilon = 276\text{ M}^{-1}\text{ cm}^{-1}$ and at 708 nm of $\epsilon_{708} = 318\text{ M}^{-1}\text{ cm}^{-1}$ in DMF for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the 2:1 molar mixture of IndoH:Cu(II) in DMF, respectively, suggests that the Cu(II) ions in the mixture are not coordinated in a similar manner to those of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ in DMF.

The similarities in the solution UV-Vis spectra of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_2(\text{DMF})_2]$ in the 500-800-nm region and IndoH and $[\text{Cu}_2(\text{Indo})_2(\text{DMF})_2]$ in the 250-400-nm region limits the usefulness of UV-Vis analysis to differentiate a solution of IndoH and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ from $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. In addition, the rapid equilibration that occurs between the dimeric Indo Cu-complex and its parent constituents has to date prevented speciation by MEC and ESI-MS methods of solution samples of IndoH, $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

Moreover, MEC proved unsuccessful in separating $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ from IndoH, despite numerous attempts using a variety of micelle solutions of biological buffers and experimental modes. Partial separation was only observed in an SDS (40 mM) and TES (100 mM) buffer, but significant degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to IndoH occurred under these conditions. Further work will be required to determine conditions for the separation of IndoH and $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ for quality control and studies of the pharmacology of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

3.11.3 Characterisation of the Cu(II) Dimers of IndoH

Despite the limitations of UV-Vis analysis, it has nevertheless, been used to characterise solid-state dinuclear Cu(II) complexes. Since the intensity of Band I can be appreciably higher for dinuclear carboxylate complexes than for mononuclear complexes,^{27,33,52,55,62,75,76} it has been suggested that this is diagnostic.^{33,75} However, the molar absorptivities are for the monomeric pyridine analogue of the Cu(II) complexes of the NSAIDs naprosyn, $\epsilon_{\text{dmf}} = 301 \text{ M}^{-1} \text{ cm}^{-1}$, and ibuprofen, $\epsilon_{\text{dmf}} = 263 \text{ M}^{-1} \text{ cm}^{-1}$,⁶⁰ similar to that of a dimeric DMSO Cu(II) complex of ibuprofen, $\epsilon_{\text{dmf}} = 398 \text{ M}^{-1} \text{ cm}^{-1}$.⁶⁰ The reported value of the molar absorptivity for the dimeric DMSO Cu(II) complex of ibuprofen ($\epsilon = 178 \text{ M}^{-1} \text{ cm}^{-1}$)²⁷ is approximately half that for the dimeric $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes under investigation. It is unclear if this reported value of ϵ is per dimer or per Cu. Notwithstanding the confusion over the reported values of the molar absorptivity of the dimeric DMSO Cu(II) complex of ibuprofen, it is clear that the intensity of Band I cannot be used to definitively determine the presence of monomeric or dimeric units.

Characterisation of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, DMA, NMP, OH_2) using solid-state FT IR and normal Raman spectroscopy is also problematic. Difficulties in assigning the $\nu_{\text{sym}}(\text{COO})$ and $\nu_{\text{asym}}(\text{COO})$ stretching frequencies limits their usefulness in conclusively characterising the mode of ligand coordination around the Cu centre. The loss of intensity

in the IR spectrum of the $\nu(\text{C-O})$ assignment at 1307 cm^{-1} and corresponding increase in a strong peak at 1401 cm^{-1} (ν_{sym}), (shoulder peak at 1445 cm^{-1}) and 1630 cm^{-1} (ν_{asym}) compared to IndoH is, however, suggestive of bidentate carboxylate coordination. Both the solid-state Raman and IR spectra of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) suggest the assignments of the $\nu_{\text{sym}}(\text{COO})$ and $\nu_{\text{asym}}(\text{COO})$ stretching frequencies are not due to pure fundamental vibrations of carboxylates. IR and Raman studies using isotopically labelled IndoH, which would be of assistance in assigning the $\nu_{\text{sym}}(\text{COO})$ and $\nu_{\text{asym}}(\text{COO})$ stretching frequencies are outside the current emphasis of the research, since XAFS, EPR and magnetic measurements have been used to give structural information.

Similarly, isotopic labelling studies may assist in confirming the $\nu(\text{Cu-O})$ assignments of the Cu(II) complexes of IndoH and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. Whilst two broad $\nu(\text{Cu-O})$ bands at $\sim 330\text{ cm}^{-1}$ and $370\text{--}380\text{ cm}^{-1}$ are assigned in the IR spectra of the Cu(II) complexes of IndoH and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ based upon IR assignments of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ reported elsewhere,^{43,44,47} only the $\sim 330\text{ cm}^{-1}$ band is observed in the Raman spectra of the Cu(II) Indo complexes and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$. UV-Vis and IR spectroscopies, together with elemental analysis, of the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes are suggestive, rather than definitive, of a dimeric Cu site in all the complexes investigated. Thus, magnetic susceptibility and EPR measurements were undertaken to determine both the nature and extent of any $\text{Cu}\cdots\text{Cu}$ interactions in the complexes.

3.11.4 Magneto-structural Characteristics of the Cu(II) Dimers

The room-temperature magnetic susceptibilities confirm the dimeric nature of all of the complexes under investigation. The large negative J values obtained from the variable-temperature magnetic susceptibility studies show that the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes have a singlet ground state with a triplet excited state, which is $284\text{--}304\text{ cm}^{-1}$ ($2J$) higher in energy. The observed values of J are similar to those of other dinuclear Cu(II) complexes,^{31,56} including $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ ($J = -142\text{ cm}^{-1}$).³¹ Dimeric Cu(II) arylcarboxylates have a stronger coupling ($J \sim -150\text{ cm}^{-1}$, compared to polymeric materials, $J \sim -50\text{ cm}^{-1}$)^{54,56,77} further confirming the dimeric nature of the present complexes.

Whilst the fine details remain obscure, it is clear that dinuclear compounds with either a $\text{CuO}_4\text{N}'$ or $\text{CuO}_4\text{O}'$ coordination have noticeably different average values of $-2J$ of 348 cm^{-1} and 316 cm^{-1} , respectively.³ This observation is consistent with the results for

$[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$, $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, where the average value of $-2J$ is 294 cm^{-1} .^{3,24} The strength of the anti-ferromagnetic coupling was independent of the nature of the axial ligand. Conflicting evidence exists in the literature concerning the influence of the nature of the terminal ligand on the value of J . Some workers have proposed that the value of J is only weakly dependent on the nature of the axial donor ligand and is not a simple function of the base strength of the bridging carboxylate ligand.⁵⁴ Others have concluded that the strength of the anti-ferromagnetic interaction tends to increase as either the axial ligand, L , or the carboxylate constituent becomes a stronger electron donor,⁷⁸ and the $\text{p}K_a$ value of L increases.^{79,80} In addition, larger J values have been attributed to a weaker σ donation by the axial ligand, with the suggestion that when there is a corresponding increase in the ligand field of the four carboxylato-oxygen atoms around the metal ion, larger splitting of the $d-d$ energy levels and a blue shift of Band I results.^{28,30} However, it is well established that the magnitude of the magnetic coupling is sensitive to the geometry of the Cu-O-C-O-Cu moiety² and the similarity in J and λ_{max} amongst the complexes indicates they all retain a similar Cu_2O_8 core.^{3,36}

The only measurable difference found amongst the complexes is in the value of D , as measured by ZFEPR spectroscopy. The DMF and NMP ligands contribute to a greater extent to the exchange between the ground state of one Cu and the excited state of the other Cu, compared to the aqua ligand. The magnitude of the axial splitting, D , is a function of two terms,^{15,58,81,82} a dipolar contribution D^{dip} , which is related to the Cu-Cu distance, and the exchange contribution, D^{exch} . The latter term is more critical, with weaker-field donors that have excited states nearer in energy to the ground state, yielding larger values of D^{exch} and hence D .⁸² Conversely, for a strong-field donor, the energy gap to the excited state is larger, leading to a smaller value for D^{exch} and hence, D . Assuming that the Cu-Cu separation, and hence D^{dip} , is similar for the various complexes under investigation, it would appear that the $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ complexes have a weaker ligand-field contribution from their respective axial ligands (as indicated by a higher value for D) when compared to those of the aqua complexes. These very small differences in the value of D do not influence either the UV-Vis spectra (the energies of Band I and Band II are not sensitive indicators to small differences in bonding) or the strength of the anti-ferromagnetic exchange (J).

The important conclusion that flows from the electronic and magnetic studies of the complexes is their relative insensitivity to changes in the axial ligands. Since the axial ligands are likely to exchange during drug metabolism, these results show that the physical properties and hence, reactivities and efficacies of the drug are not likely to be sensitive to the nature of the axial ligands. This is consistent with the lack of variation in the pharmaceutical activities of the Cu-Indo drugs with changes in the nature of the axial ligand.^{83,84}

The other important aspect of the magnetic and EPR measurements is that they are extremely sensitive to mononuclear Cu(II) at low temperatures as a result of the diamagnetic ground states of the dinuclear species and paramagnetic state of the monomer. Thus, both techniques are valuable in quality control of pharmaceutical preparations, since even small amounts of mononuclear Cu(II) can be quantified readily by such techniques. EPR spectroscopy, however, is a more convenient technique for this application. The results reported here establish that the concentrations of such impurities are very low (< 1 %) in the complexes used for pharmaceutical preparations.

3.11.5 XAFS Analysis

Whilst the magnetic data confirm the dimeric nature of the Cu(II) complexes of IndoH, XAFS was used to verify a similar geometry coordination of the Cu_2O_8 core irrespective of the nature of the axial ligand. The determination of the geometry of the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes is important as the axial ligands may exchange upon incorporation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ into pharmaceutical preparations or in biological media. XAFS has previously been used to determine the coordination of Cu(II) complexes with a variety of dinucleating ligands involving carboxylates,⁸⁵ sulfides and benzimidazoles,⁸⁶ and metallothioneins.⁸⁷ The excellent agreement of the refined bond distances of the XAFS of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ with crystallographic data and the low values of R_{xaf} confirmed the validity of the MS model to simulate the XAFS of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMA}, \text{NMP}, \text{OH}_2$). MS XAFS confirms that $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ have a similar Cu_2O_{10} core to other Cu(II)-acetate-type dimers.^{3,5,31,54} This result is not surprising given the similar magnetic and spectroscopic data for all the complexes.²⁴

The advantage of MS over SS XAFS analyses is validated in the current work. Despite the k range of the three-shell SS XAFS data being sufficient to distinguish the distances of the

three shells, the refined axial Cu–O_L bond length of ~ 2.25 Å was dissimilar to values obtained from XRD for [Cu₂(OAc)₄(OH₂)₂] and [Cu₂(Indo)₄(DMF)₂]. This shows the importance of the inclusion of MS pathways in XAFS analyses. Similarly, the refined Cu...Cu, Cu–O_L and Cu–O_{Ac} and Cu–C distances of the Cu₂O₁₀ core for the seven-shell SS analysis of the XAFS of [Cu₂(Indo)₄(DMF)₂] at 10 K were not consistent with those obtained from XRD. The large residuals, particularly in the *r*-spectra range > 3 Å, and high value of *R*_{xaf} (30–40%) show significant back-scattering contributions from unmodelled pathways in the seven-shell SS model. The limitation of the seven-shell SS model is highlighted by its inability to satisfactorily refine the β -carbon and axial oxygen positional parameters to the crystal structure values without vector distance symmetry restraints.

The MS refined bond lengths and bridging O–C–O angle of the XAFS of [Cu₂(OAc)₄(OH₂)₂] and [Cu₂(Indo)₄L₂] (L = DMF, DMA, NMP) are similar to values obtained from XRD for [Cu₂(OAc)₄(OH₂)₂]¹⁸ and [Cu₂(Indo)₄(DMF)₂].²⁴ Two refinement minima were found for the Cu–O_L bond distance of the 10 K XAFS of [Cu₂(Indo)₄(DMF)₂] at 10 K using 65 unique MS pathways and [Cu₂(Indo)₄(OH₂)₂] using 130 MS paths, depending upon the starting positional parameters of the axial oxygen ligand. The observation of false minima in XAFS refinements is reported elsewhere^{88,89} and shows the need to explore different starting distances in MS analyses. A slight increase in *R*_{eff} from 10.0 Å to 10.2 Å and the absence of CW and PW filters, i.e., giving ~ 130 unique MS pathways, result in an excellent agreement of the refined distances to crystallographic values for [Cu₂(Indo)₄(DMF)₂] at 10 K. An increase from 65 to 130 unique MS pathways yields additional 5- and 6-leg MS paths, which may be important in the refinement model at low temperature. This also highlights the need to investigate different maximum numbers of legs per MS path during the analyses. Refinements of the XAFS of [Cu₂(Indo)₄L₂] (L = DMA, NMP) at 10 K did not yield two refinement minima for the Cu–O_L bond distances.

Higher values of *R*_{xafs} were found for all the complexes at 10 K compared to RT. This is attributed to increased noise in the collected data due to the absorption of the windows of the cryostat and more important contributions from unmodelled MS pathways at the lower temperatures. There was a slight reduction in the value of the Debye-Waller factors at low temperature compared to RT for all the complexes. This reflects a decrease in both the thermal motion and errors of the refined atomic positional parameters. There is a noticeable increase in the intensity of the FT shells at 10 K compared to RT – particularly in the *r*-range

greater than 4.0 Å. Increased contributions from unmodelled back-scattering contributions of neighbouring dimers and Indo atoms will, therefore, be more evident at low temperature than RT. This increased contribution, unless correctly modelled, at low temperature may have contributed to the two refinement minima for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K. This is observed in the XAFS of $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$.

Attempts were made to incorporate into the MS dimer model all the non-hydrogen atoms of the axial DMF, Indo groups and neighbouring dimer molecules at a distance of less than or equal to 5.0 Å from Cu_0 (Appendix 6). This proved unsuccessful, i.e., values of $R_{\text{xaf}} > 20\%$, despite bond distances and angles restraints on these atoms consistent with XRD values (Appendix 6). High values of R_{xaf} , Debye-Waller factors and/or positional parameter errors were obtained. The r -spectra range greater than 4.0 Å corresponds to scattering contributions from unmodelled Indo and neighbouring dimer units (Appendix 8).

Unmodelled atoms of entrapped solvent and water molecules²⁴ may also have contributed to the residual spectrum in this region.

The difficulty in modelling the disordered DMF ligand and Indo groups of adjacent molecules within 5 Å is not surprising given the disorder of the ligands, irregular packing of the Indo (R) groups in the crystal structure (Appendix 8) and the low determinacy for this model. The irregular packing of the Indo groups is evident by different distances of the β carbon atom from the 2-methyl carbon atom, i.e., 3.21 Å and 3.15 Å, in each symmetric Indo group (Appendix 8).

In all refinement models, the fitting procedure was well over-determined, with the value of the determinacy (N_i/N_p) much greater than 1. This shows that the high symmetry of the models enable reliable predictions of structure.

3.11.6 XAFS of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$

Whilst there is no published literature on the XAFS of Cu(II) dimers of NSAIDs, SS analysis of XAFS of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ has been reported.⁹⁰ However, it gave poor fits of the calculated to the observed XAFS data, whereas the current MS analysis accurately reproduces the XRD results. Despite the limitations of the published work, the longer reported refined Cu–O_L distance in a liquid ethanolic (2.37 Å) and vitrified water plus glycerol sample (2.28 Å) is similar to the results of the three-shell SS analyses (Appendix 13). The current and published SS XAFS analyses of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ are consistent with

Hathaway's review of the XAFS of Cu(II) structures.¹ Hathaway recommends caution in accepting the long Cu–L distances suggested for Cu(II) compounds, until the ability of the XAFS technique to predict both long and short Cu–L distances on model complexes of known crystal structures has been confirmed.¹

3.11.7 XAFS Analysis and Structural Relationships

The use of XAFS to determine small changes in the coordination geometry is limited by the conservative systematic error of 0.02 Å⁶³ although the Cu–O_{Ac} values may be more precise. Several reviews of the relationship between structural and magnetic properties of dimeric Cu(II) carboxylate complexes have been published,^{2,3,5,54} with differences of less than or equal to 0.02 Å reported in the Cu–O_{Ac} and bridging Cu–O–C–O–Cu distances of Cu(II) acetate-type dimers containing either CuO₄O or CuO₄N coordination environments (Table 3.11),^{2,3,5} which would not be determined with certainty by XAFS.

It is proposed that the nature of the axial ligand plays a role in determining the sum of the Cu–O bond lengths and all the inter-atomic distances around the Cu core (including the half distance of the Cu···Cu separations), bridging Cu–O–C–O–Cu distance and the displacement of the Cu atom from its basal plane (Table 3.11).²⁻⁴ An almost constant Cu–O_{Ac} distance of 1.96 Å and 1.97 Å for CuO₄O' and CuO₄N', respectively, and longer O–C and Cu–L distances in CuO₄O' than in the CuO₄N' of 1.26 Å and 2.16 Å for the former, and 1.24 Å and 2.11 Å for the latter are reported.³ The sum of all inter-atomic distances around the Cu(II) core, including the half value of the Cu···Cu distances, is reported to be almost constant in CuO₄O' and CuO₄N' at 11.33 Å and 11.42 Å, respectively.²

Furthermore, the bridging Cu–O–C–O–Cu distance shows a small but constant difference at 6.44 Å for CuO₅ environments and 6.42 Å for CuO₄N' environments³ and a displacement of the Cu atom from the basal plane towards the apical ligand of 0.22–0.32 Å for CuO₄N' and 0.19–0.22 Å for CuO₄O' environments.³ The O–C–O bridging angles are reportedly larger in CuO₄N' (125.6°) than CuO₄O' (123.9°) environments.³ Whilst numerous acetate structures have been determined, Hathaway reports limited occurrence of the acetate type structure with nitrogen ligands.¹ Furthermore, because of the restricted bite of the carboxylate oxygen atoms, there is little variation in the Cu···Cu separation of ~ 0.3 Å.¹

The rigid geometry of acetate-type Cu(II) dimers is confirmed by the XAFS analyses of the Cu(II) dimers of Indo. Attempts to obtain structures of a MeCN adduct of Cu–Indo (i.e.

$\text{CuO}_4\text{N}'$) for comparison was unsuccessful due to decomposition of the crystal structure during data collection. Crystal structure data of $[\text{Cu}_2(\text{Sup})_4(\text{CH}_3\text{CN})_2]$,²² i.e., $\text{CuO}_4\text{N}'$ coordination geometry, gives a Cu—O—C—O—Cu bridge length, sum of interatomic distances around the Cu(II) atom and out-of-plane Cu(II) displacement of 6.390(28) Å, 11.318(29) Å and 0.15 Å, respectively, compared to 6.42 Å, 11.42 Å and 0.22–0.32 Å expected from the structural reviews of $\text{CuO}_4\text{N}'$.^{2,3,5,54}

An increase in the Cu...Cu separation with increasing acid strength of the bridging carboxylate group of Cu(II) dimers is also reported.^{2,3,5,54} The most significant structural change accompanying this increase in the Cu...Cu separation is the movement of the Cu atom out of the basal plane of its square pyramidal coordination polyhedron toward the axial ligand. The refined Cu...Cu separation determined from the XAFS of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, DMA, NMP, OH_2) are similar (2.62(2)–2.63(2) Å) to the crystal structure and XAFS determined Cu...Cu separation for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ of 2.616(1) Å¹⁸ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ of 2.630(1) Å,²⁴ respectively. While the differences are within systematic errors, such errors are likely to be constant for a series of complexes with the same XAFS model. The slightly longer crystallographic determined Cu...Cu separation for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ of 2.630(1) Å²⁴ is consistent with a somewhat greater acid strength of IndoH (pK_a 4.5)⁷⁴ compared to acetic acid of 2.616(1) Å¹⁸ (pK_a 4.8).⁷⁴ There is no significant difference between the out-of-plane displacement of the Cu(II) atom of 0.20 Å for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ²⁴ compared to 0.19 Å for $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$.³ All the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, DMA, NMP, OH_2) complexes displayed a similar out-of-plane displacement of the Cu(II) atom.

3.12 Conclusion

The important conclusion that flows from the electronic, magnetic and XAFS studies of the complexes is that they are relatively insensitive of their properties to changes in the axial ligation. Since the axial ligands are likely to exchange during drug metabolism, these results show that the physical properties and hence, reactivities and efficacies of the drug are likely to be insensitive to the nature of the axial ligands. This is consistent with the lack of dependence in the pharmaceutical activities of the Cu-Indo drugs on the nature of the axial ligand.^{83,84}

Low temperature and RT MS analyses of the XAFS of $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, respectively, show excellent agreement of the refined bond distances with those obtained from crystallography, with low R_{xaf} values. A model comprising a Cu_2O_{10} core plus the β -carbon atom of the coordinated Indo group is an excellent model for the MS analysis $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$) and $[\text{Cu}_2(\text{OAc})_4(\text{OH}_2)_2]$ containing low percentages of Cu(II) monomer impurities.

Refinements models of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ regularly resulted in longer than expected $\text{Cu}-\text{O}_\text{L}$ distances. These results are inconsistent with expected values based on the similar magnetic and spectroscopic data of the other $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}$). It may, however, be a useful indicator of aqua ligand coordination in the Cu(II) complexes of IndoH or partial occupancy of the axial ligand sites. Only the MS refinement of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ at 10 K using the 130 pathway model and a starting $\text{Cu}-\text{O}_\text{L}$ distance of 2.00 Å resulted in refined $\text{Cu}\cdots\text{Cu}$, $\text{Cu}-\text{O}_\text{Ac}$ and $\text{Cu}-\text{O}_\text{L}$ distance of 2.61(2) Å, 1.94(2) Å and 2.15(2) Å, respectively, similar to crystallographic values for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.²⁴ The value of the Debye-Waller factor of the axial ligand of 0.05 Å² is considerably higher than for all other refinements. Furthermore, the values of R_{xaf} (17.2%), $\text{Cu}\cdots\text{Cu}$ (2.62(2) Å), $\text{Cu}-\text{O}_\text{Ac}$ (1.95(2) Å) and Debye-Waller factors ($\sigma^2(\text{Cu}\cdots\text{Cu}) = 0.002 \text{ Å}^2$, $\sigma^2(\text{Cu}\cdots\text{O}_\text{Ac}) = 0.004 \text{ Å}^2$) are similar to the refinement model excluding the $\text{Cu}-\text{O}_\text{L}$ distance ($R_{\text{xaf}} = 16.9\%$, $\text{Cu}\cdots\text{Cu} = 2.61(2) \text{ Å}$, $\text{Cu}-\text{O}_\text{Ac} = 1.95(2) \text{ Å}$, $\sigma^2(\text{Cu}\cdots\text{Cu}) = 0.002 \text{ Å}^2$, $\sigma^2(\text{Cu}-\text{O}_\text{Ac}) = 0.003 \text{ Å}^2$) (Table 3.12).

The magnetic and EPR measurements allow quantification of monomeric Cu(II) impurities of the Cu(II) dimers of IndoH. The application of EPR measurements and MS XAFS analysis, therefore, has important applications in the quantification, quality control and characterisation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in patented veterinary pharmaceutical preparations and biological samples. Application of these techniques will be discussed in Chapter 4.

3.13 References

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CHAPTER FOUR

PHARMACEUTICAL & BIOLOGICAL SAMPLES

4.1 Introduction

In order for pharmaceuticals to enter human clinical trials and be registered in the Northern Hemisphere for veterinary applications, appropriate analytical methods need to be developed. The nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in a variety of veterinary pharmaceutical formulations was investigated to establish whether its dimeric structure is retained. High pressure liquid chromatography (HPLC) and other quality control analytical techniques, e.g., MEC and ESI-MS, attempted by BVR and as a part of the research reported here, respectively, have not been successful to date. Therefore, X-band EPR spectroscopy and MS XAFS analyses were used to investigate the integrity of the dimer (including quantification of the Cu(II) monomer content) and coordination geometry, respectively, of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in a number of pharmaceutical preparations. The Cu(II) monomer contaminant is reported as a percentage of the total initial Cu content in the dimer of the formulation, i.e., Cu(II) monomer content (% w/w Cu). The MS XAFS analyses of selected pharmaceutical formulations were undertaken using the 130-unique-MS-pathways model (Chapter 3) and a starting Cu-O_L distance of 2.00 Å, unless otherwise stated.

The pharmaceutical formulations investigated included *Cu-Algesic* tablets, granules, gelatin capsules and carbopol (hydroxypropyl methylcellulose) (1% w/w) paste, *Avigesic Forte* intravenous solution, and MCT and paraffin pastes of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Their formulations are given in Chapter 2 (Table 2.1). *Cu-Algesic* tablets are used to treat inflammatory conditions in dogs, with *Cu-Algesic* granules and carbopol (1% w/w) paste administered orally to horses. MCT and paraffin pastes of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ were investigated as alternatives to the carbopol (1% w/w) formulation. *Avigesic Forte* was manufactured by BVR for use as an intravenous anti-inflammatory drug in ostriches, but is no longer in production. Gelatin capsules of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ were formulated by BVR for use in dogs as single-use ophthalmic eye drops. The micelle “pre-solution” and “pre-fill” solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (48 mg mL⁻¹) and (1.25 mg mL⁻¹), respectively, used in the manufacture of the capsules were also investigated.

In addition, carbopol (1% w/w) pastes and intravenous formulations of $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]$ were studied, as BVR propose to use this adduct in future product

development. Carbopol (1% w/w) preparations of $[\text{Cu}_2(\text{Indo})_4(\text{OH}_2)_2]$ were prepared using sample V (Chapter 2). Preservative-free carbopol (1% w/w) pastes and carbopol (1% w/w) pastes of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$) using benzalkonium chloride (0.1% or 1.1% w/w), instead of Nippasol M as preservative, were analysed. Nippasol M (0.5% w/w) is used in the proprietary *Cu-Algesic* paste formulation. Benzalkonium chloride (0.1% w/w) and Nippasol (0.5% w/w) are typically used as antibacterial agents in pharmaceutical products.¹ A detailed discussion of the compositions of the formulations and their effects on the chemistry is given in the Discussion section of this chapter. The Cu(II) monomer content and UV-Vis spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the micellar biological buffer solutions, TES (100 mM) + SDS (40 mM) and HEPES (100 mM) + SDS (40 mM) are reported along with the results of some preliminary investigations of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in artificial “gastric juice” and MOPS (100 mM) + SDS (40 mM) solutions. The UV-Vis spectra of equine serum following the addition of *Avigesic-Forte* and results of the determination of the Cu content of equine whole blood are also presented.

4.2 X-Band EPR Spectra of Pharmaceutical Formulations

X-Band EPR spectroscopy of the veterinary formulations *Cu-Algesic* tablets, *Cu-Algesic* granules and carbopol (1% w/w), MCT and paraffin paste in the 100-7000 G region were undertaken to check for the presence of the dimer drug and paramagnetic impurities (Figure 4.1). The X-band EPR spectra of the *Cu-Algesic* formulations and paraffin at ~100 K and powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT are shown in Figure 4.1. The X-band EPR spectra of powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at RT and 110 K, with inset plots of the Cu-hyperfine resonances at ~500 G and ~5980 G are shown in Figure 4.2. The X-band EPR spectra of *Avigesic Forte*, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (200 mg (5 g)⁻¹) in MCT paste and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mg mL⁻¹) in neat DMF in the 100-7000 G region are shown in Appendix 16.

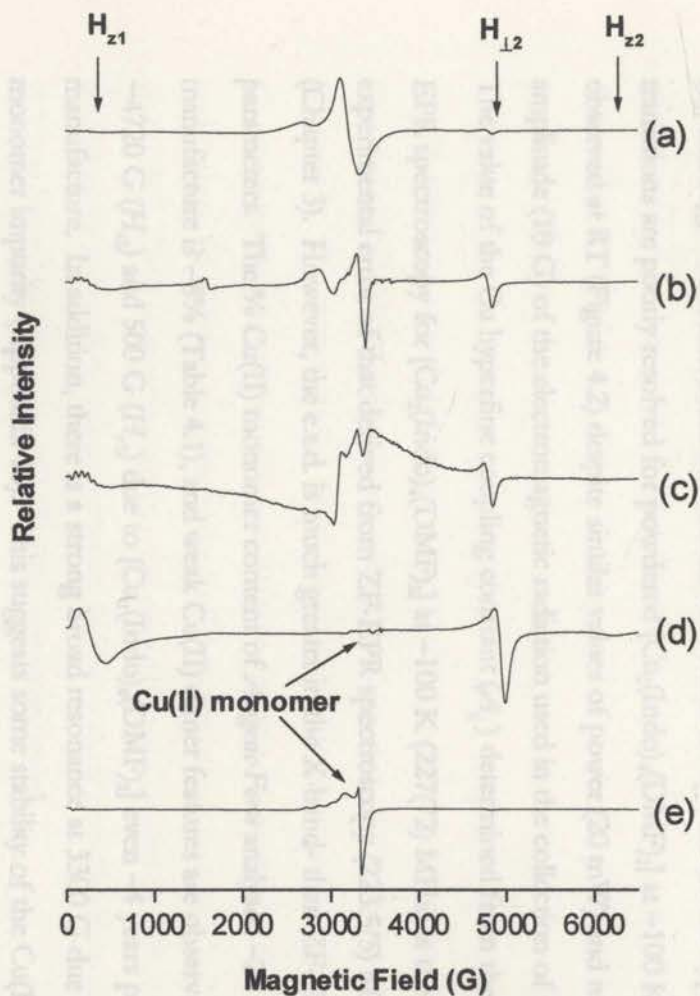


Figure 4.1. X-band EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in (a) paraffin at 100 K, (b) *Cu-Algesic* tablets at 98 K, (c) *Cu-Algesic* granules at 98 K, (d) neat solid-state sample at RT and (e) *Cu-Algesic* paste at 98 K.

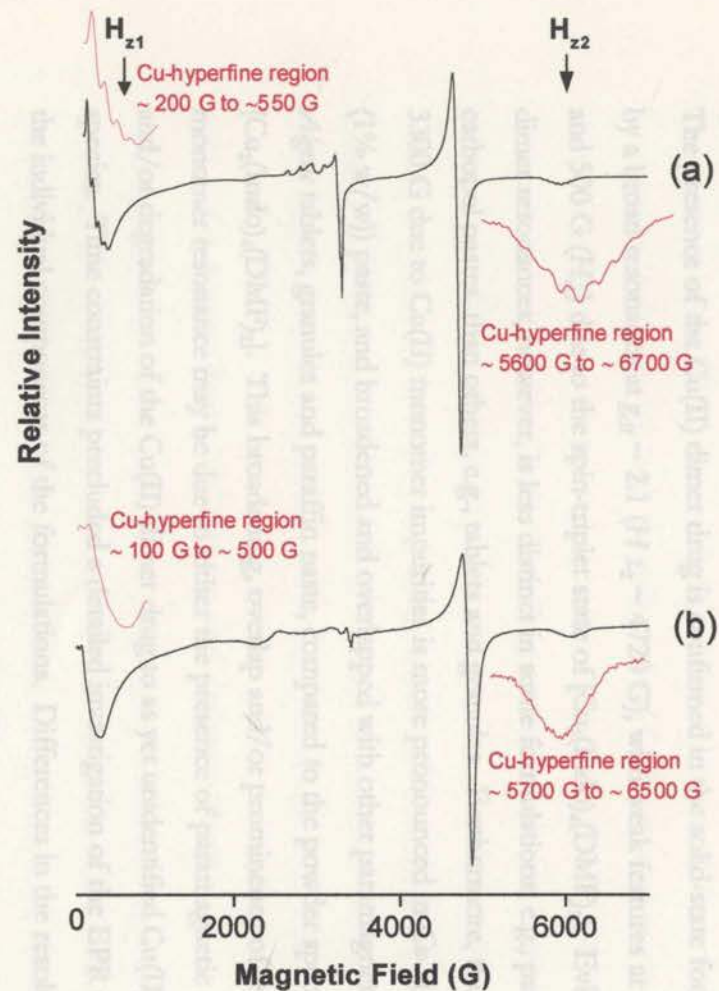


Figure 4.2. X-band EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at (a) 110 K and (b) RT with inset plots of the Cu-hyperfine coupling transitions (—).

The presence of the Cu(II) dimer drug is confirmed in the solid-state formulations by a broad resonance at $g_{\text{eff}} \sim 2.1$ ($H_{\perp 2} \sim 4720$ G), with weak features at ~ 5980 (H_{22}) and 500 G (H_{21}) due to the spin-triplet state of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Evidence of the dimer resonances, however, is less distinct in some formulations, e.g., paraffin and carbopol pastes, than others, e.g., tablets and granules. Furthermore, the resonance at 3300 G due to Cu(II) monomer impurities is more pronounced in *Cu-Algesic* (carbopol (1% w/w)) paste, and broadened and overlapped with other paramagnetic species in *Cu-Algesic* tablets, granules and paraffin paste, compared to the powder spectrum of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. This broadening, overlap and/or prominence of the Cu(II) monomer resonance may be due to either the presence of paramagnetic excipients and/or degradation of the Cu(II) dimer drug to as yet unidentified Cu(II) monomer species. Time constraints precluded a detailed investigation of the EPR spectroscopy of the individual components of the formulations. Differences in the resolution of the Cu hyperfine couplings at ~ 500 G (H_{21}) and ~ 5980 (H_{22}) in the tablets and granules spectra at 100 K are evident compared to the powder spectrum at RT (Figures 4.1 and 4.2). Each unpaired electron in the Cu(II) dimer interacts equivalently with both Cu(II) centres, where the ground state nuclear spin quantum number (I) of Cu(II) is $3/2$.² This leads to an expected seven-line Cu hyperfine structure on each of the two $g_{\parallel}$ signals, i.e., H_{21} and H_{22} ,^{3,4} with intensities in the ratio 1:2:3:4:3:2:1.² Whilst the Cu hyperfine transitions are poorly resolved for powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at ~ 100 K, none are observed at RT (Figure 4.2) despite similar values of power (20 mW) and modulation amplitude (10 G) of the electromagnetic radiation used in the collection of the spectra. The value of the Cu hyperfine coupling constant ($A_{\parallel}$) determined from the X-band EPR spectroscopy for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at ~ 100 K (227(72) MHz) is the same within experimental error of that derived from ZF-EPR spectroscopy (223.5(5) MHz) (Chapter 3). However, the e.s.d. is much greater in the X-band- than ZF-EPR-derived parameters. The % Cu(II) monomer content of *Avigesic-Forte* analysed ~ 24 months after manufacture is $\sim 8\%$ (Table 4.1), and weak Cu(II) dimer features are observed at ~ 4720 G (H_{22}) and 500 G (H_{21}) due to $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ even ~ 4 years post manufacture. In addition, there is a strong broad resonance at 3300 G due to a Cu(II) monomer impurity (Appendix 16). This suggests some stability of the Cu(II) dimer in the micellular *Avigesic Forte* formulation over a prolonged period. Similarly, X-band EPR

spectroscopy of the MCT paste confirms the dimeric nature of the complex in this formulation (Appendix 16), with the well-resolved Cu(II) monomer impurity signal observed at 4 K ~2 months post preparation consistent with only 6% of Cu(II) monomer impurities. The Cu(II) dimer drug structure is also confirmed in neat DMF at 100 K (Appendix 16) as evident by a broad resonance at ~ 4720 G ($H_{\perp 2}$) and features at ~ 500 G (H_{z1}) and ~ 5980 G (H_{z2}) due to the spin-triplet state, despite poor resolution of this spectrum. The strong resonance at ~ 3300 G in neat DMF sample of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ represents only 2-4% Cu(II) monomer impurities (Table 4.4).

4.2.1 *Cu-Algesic Paste, MCT Paste and Avigesic-Forte*

All the carbopol (1% w/w) pastes were prepared by first dissolving carbopol in distilled or Milli-Q water, adding the preservative benzalkonium chloride (0.1% or 1.1% w/w) or Nippasol M (*n*-propyl-4-hydroxybenzoate, 0.5% w/w), if required, and heating the mixture to achieve dissolution. The pH values of the pastes were then adjusted to ~ 7.0 with a NaOH (8.5% w/v) aqueous solution. The addition of the NaOH causes the thickness and viscosity of the formulation to increase to the extent that a paste is formed.⁵ $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$) was then mixed mechanically with the paste to form a homogeneous smooth green-blue composition. The MCT paste was prepared by dissolving $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($200 \text{ mg } (5 \text{ g})^{-1}$) in tetraglycol (40% v/w) + Span 80 (20% w/w) prior to the addition of the flow-enhancing agent Aerosil (7% w/w)⁶ and dilution in MCT (Table 2.1). The paraffin paste was prepared by adding $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($200 \text{ mg } (5 \text{ g})^{-1}$) to white soft paraffin. *Avigesic-Forte* (40 mg mL^{-1}) was prepared by dissolving $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$) in *N*-methyl-2-pyrrolidone (NMP) (4% v/v), cremophor (4% v/v) and Milli-Q water to produce an o/w emulsion. Dissolution in NMP is expected to result in the formation of $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ (Chapter 3). The determination of the Cu(II) monomer content (%) in three batches of *Cu-Algesic* pastes prepared by different BVR personnel ~ 1 -2 years post manufacture was undertaken. In addition, analysis of the Cu(II) monomer content of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$) in preservative-free carbopol (1% w/w) pastes or with the preservatives *n*-propyl-4-hydroxybenzoate (0.5% w/v), or benzalkonium chloride (1.1% w/w) ~ 2 months post manufacture, was investigated.

Table 4.1. X-Band EPR Spectroscopic Results of the *Cu-Algesic* Pastes and *Avigesic-Forte*.

Sample ^a	Colour of Formulation	Cu(II) monomer (%)	pH _{before} ^j	pH _{after} ^k
[Cu ₂ (Indo) ₄ (DMF) ₂] + Nippasol M (0.5% w/w) + carbopol (1% w/w) paste ^b	olive green	59, ^d 50 ^f	unknown	unknown
[Cu ₂ (Indo) ₄ (DMF) ₂] + Nippasol M (0.5% w/w) + carbopol (1% w/w) paste ^b	olive green	83, ^d 72 ^f	unknown	unknown
[Cu ₂ (Indo) ₄ (DMF) ₂] + Nippasol M (0.5% w/w) + carbopol (1% w/w) paste ^{b, d}	olive green	22	~7.0	not determined
[Cu ₂ (Indo) ₄ (DMF) ₂] + preservative-free carbopol (1% w/w) paste ^{c, e}	olive green	40	7.1	6.9
[Cu ₂ (Indo) ₄ (DMF) ₂] + benzalkonium chloride (0.1% w/w) + carbopol (1% w/w) paste ^{c, e}	olive green	36	7.0	6.6
[Cu ₂ (Indo) ₄ (OH ₂) ₂] + Nippasol M (0.5% w/w) + carbopol paste (1% w/w) ^{c, e}	olive green	26	7.2	7.3
[Cu ₂ (Indo) ₄ (OH ₂) ₂] + benzalkonium chloride (0.11%) + carbopol (1% w/w) paste ^{c, e}	olive green	23	7.0	6.6
[Cu ₂ (Indo) ₄ (OH ₂) ₂] + benzalkonium chloride (1.1% w/w) + carbopol (1% w/w) paste ^{c, e}	olive green	31	7.0	6.4
[Cu ₂ (Indo) ₄ (OH ₂) ₂] + preservative-free carbopol (1% w/w) paste ^{c, e}	olive green	35	7.13	6.9
[Cu ₂ (Indo) ₄ (DMF) ₂] + MCT paste ^{b, e, g}	bright green	1.5, 3, 6	— ^b	— ^b
[Cu ₂ (Indo) ₄ (DMF) ₂] + white soft paraffin paste ^{b, e}	bright green	unknown ⁱ	— ^b	— ^b
[Cu ₂ (Indo) ₄ (DMF) ₂] <i>Avigesic-Forte</i> ^{b, f}	bright green	8.0	not determined	5.4
[Cu ₂ (Indo) ₄ (OH ₂) ₂] <i>Avigesic-Forte</i> ^{c, e}	bright green	5.6	not determined	5.4

^a [Cu₂(Indo)₄L₂] (L = DMF, OH₂) (200 mg (5 g⁻¹) in carbopol (1% w/w) paste; density ~1.05 g mL⁻¹; [Cu₂(Indo)₄L₂] (L = DMF, OH₂) (40 mg mL⁻¹) in *Avigesic Forte*.

^b Manufactured by BVR. ^c Prepared in this work. ^d Analysed ~12 months after manufacture. ^e Analysed ~2 months after manufacture. ^f Analysed ~24 months after manufacture. ^g Triplicate determination following separate insertions into the EPR cavity. ^h pH value of oil cannot be determined. ⁱ Cu(II) monomer signal in the paraffin paste was obscured by as yet unknown paramagnetic impurities. ^j pH_{before} = pH value before the addition of [Cu₂(Indo)₄L₂] (L = DMF, OH₂) to the formulation. ^k pH_{after} = pH value after the addition of [Cu₂(Indo)₄L₂] (L = DMF, OH₂) to the formulation.

Approximately 32(7)% of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ decomposed ~2 months after preparation into, as yet, unidentified Cu(II) monomer species in the carbopol (1% w/w) pastes formulated in this work. The extent of this degradation of the Cu(II) dimer to monomeric species was similar irrespective of the presence or absence of preservatives, or the nature of the adduct. As the pastes formulated in this work were analysed within 2 months of their preparation, the results indicate a significant and rapid degradation of the Cu(II) dimer complexes by carbopol (1% w/w).

Further decomposition of the dimer complex to monomeric Cu(II) over a 1-2 year period appears to be minimal, given the similar Cu(II) monomer contents of the carbopol (1% w/w) paste prepared by BVR, i.e., Cu(II) monomer content ~22%, and that prepared during the course of this research, i.e., Cu(II) monomer content 26%, analysed ~12 and 2 months, respectively, post manufacture. (The pH values of these two pastes were adjusted to ~7 prior to the addition of the Cu(II) dimer complex). The $\text{pH}_{\text{before}}$ values of the carbopol pastes containing 60-80% Cu(II) monomer were not recorded by BVR. However, there was no significant increase in the Cu(II) monomer content of these pastes over a 12-month study period.

The only visible differences noted between the pastes were the loss of viscosity of the carbopol (1% w/w) paste containing 60-80% Cu(II) monomer, compared to the other carbopol pastes, and the bright green colour of the MCT and paraffin pastes compared to the olive-green colour of all of the carbopol pastes. The Cu(II) monomer content of the paraffin paste could not be determined due to a broad resonance at ~3300 G, which is attributed to as yet unidentified paramagnetic species in the formulation. However, the bright green colour of the paraffin paste was similar to the micellar MCT paste and *Avigesic Forte* formulations that have ~6-8% Cu(II) monomer content with respect to total initial Cu content. There was no major change in the pH values of the carbopol (1% w/w) pastes following the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

No notable differences were apparent between the Cu(II) monomer content of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, OH_2) prepared with benzalkonium chloride (0.1% or

1.1% w/w) or *n*-propyl-4-hydroxybenzoate (0.5% w/w). The *Avigesic-Forte* formulations contained < 8% Cu(II) monomer, irrespective of the age of formulations and nature of the axial ligand. The bright green colour of the micelle *Avigesic Forte* solutions suggest that the NMP (4% v/v) + cremophor (4% v/v) micelles protect the Cu(II) dimer complex from degradation to monomers, with the presence of a dimer observed by EPR spectroscopy ~4 years post manufacture (Appendix 16).

The values of the e.s.d.'s for the X-band EPR spectroscopy determinations of the Cu(II) monomer content are unknown, however, an e.s.d of ~10% is proposed given the results for the analyses of the BVR carbopol pastes over a 2-year testing period. The position of the sample in the EPR cavity may affect the Cu(II) monomer determination. Analysis of the MCT paste following triplicate, yet separate insertions of the sample into the EPR cavity, resulted in values of the mean Cu(II) monomer content and e.s.d. of 3.5% and 1.9%, respectively. The correlation coefficient of the Cu(II) monomer calibration curves were typically greater than 0.98 indicating an excellent goodness-of-fit for the standard curve for all the Cu(II) monomer determinations of the pastes and intravenous formulations (Appendix 12). These factors taken together indicate that analysis of the Cu(II) monomer content have a high reproducibility.

4.2.2 *Cu-Algesic Capsules*

A gradual colour change from green to yellow in the liquid contents of gelatin capsules of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($0.5 \text{ mg } (0.4 \text{ mL})^{-1}$) containing α -tocopherol (α -TocH) (0.025% w/v) was observed following storage of the capsules for ~6 months at RT. The rate of this colour change was accelerated by light. In addition, BVR report a colour change from green to yellow over a 24-hr period of the "pre-solution" of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (48 mg mL^{-1}) following the addition of empty gelatin capsules. The formulae of the *Cu Algesic* capsules and the "pre-solution" and "pre-fill" solution are given in Chapter 2 (Table 2.1).

The gelatin capsules were manufactured for BVR from gelatin sheets, which were heated and molded for filling with the "pre-fill" solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($1.25 \text{ mg complex g}^{-1}$, density 0.944 g mL^{-1}) and then heat-sealed. The gelatin had been extracted from collagen by heating with glycerol, followed by the addition of glycerol and MCT to

produce the gelatin sheets.⁶ $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ was mixed with Span 80 (20% w/v) + tetraglycol (40% v/v) to produce a dark green “pre-solution”. The “pre-solution” was subsequently mixed with MCT plus α -TocH (0.025% w/v) to produce the “pre-fill” solution. The liquid filled gelatin capsules were prepared from this “pre-fill” solution.

Concerns by BVR over the reason for the colour change in the liquid filled gelatin capsules during storage resulted in the determination of their Cu(II) monomer content (%) and those of the “pre-solution” and “pre-fill” solution ($\pm \alpha$ -TocH (0.025% w/v)), by low temperature X-band EPR spectroscopy. The bright green colour of the “pre-solution” and “pre-fill” solution and yellow colour of the liquid-filled capsule contents suggests a loss of the dimeric nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the capsule compared to its retention in the “pre-solution” and “pre-fill” solution. The results of the total Cu content of the liquid filled and empty gelatin capsules and Cu(II) monomer content (%) as determined by AAS and low temperature EPR spectroscopy, respectively, are given in Table 4.2.

While 100% of the total Cu content of the whole liquid-filled capsules was detected by AAS, only 12.5% was present within the capsule contents following ~6 month storage at R.T. This showed substantial sequestering of the Cu from the capsule contents into the soft gelatin shell over this period. Of the total calculated Cu(II) dimer content in the bright green “pre-solution” (analysed 6-12 months post-preparation) and “pre-fill” solution (analysed 9 months post-manufacture), less than 2% was detected as Cu(II) monomer. No significant change in the colour of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ was observed in the “pre-solution” and “pre-fill” solution ($\pm \alpha$ -TocH), however, addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to neat MCT plus α -TocH (0.025% w/v) results in a colour change from green to olive-green. Only 6-9% of the total initial Cu content was detected as Cu(II) monomer in the yellow liquid-filled capsule contents, irrespective of the presence or absence of α -TocH (0.025% w/v). Whilst a Cu(II) monomer signal was observed in the liquid-filled capsule contents ($\pm \alpha$ -TocH (0.025% w/v)) and α -TocH-free “pre-fill” solution, it was obscured in the “pre-fill” solution containing α -TocH (0.025% w/v) due to the formation of a radical. The *Cu-Algesic* capsules are being re-formulated by BVR as a result of this research.

Table 4.2. AAS and X-Band EPR Spectroscopic Analyses of the Gelatin Capsules and Manufacturing Solutions.

Sample ^a	α -TocH (% w/v)	Colour of Solution/Capsule Contents	Cu _{calc} (ppm) ^b	Cu _{obs} (ppm) ^c	Cu(II) monomer content (%) ^d	Cu loss from capsule contents (%) ^e
Whole capsule ^f	0.025	yellow	1.20	1.25	—	—
Empty gelatin capsule	unknown	NA	Unknown	< 0.05 ^b	—	—
Capsule contents ^f	0.025	yellow	1.20	0.15	6.1	87.5
Capsule contents ^f	nil	yellow	1.20	0.11	9.3	90.8
“pre-solution” ^g	nil	bright green	—	—	2.3	—
“pre-solution” ^h	nil	bright green	—	—	1.0	—
“pre-fill” solution ^b	0.025	bright green	—	—	Signal obscured	—
“pre-fill” solution ^b	nil	bright green	—	—	2.1	—

^a Density of the “pre-fill” solution $\sim 0.94 \text{ g mL}^{-1}$. ^b Calculated from the total dimer content of *Cu-Algesic* capsules and assuming a negligible Cu content of the empty gelatin capsule shell. ^c Determined by AAS. ^d Determined at 4 K using a Bruker ESP 300 X-band EPR spectrometer. ^e $\text{Cu}_{\text{obs}} - \text{Cu}_{\text{calc}}$. ^f Analysed ~ 6 months post preparation. ^g Analysed ~ 9 months post preparation. ^h Analysed ~ 1 month post preparation.

4.2.3 Solution Samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$

As a prelude to investigations of the nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in aqueous solutions and samples used in the MEC analysis (Chapter 3), the Cu(II) monomer contents of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 1 \text{ mg mL}^{-1}$) in TES (100 mM) + SDS (40 mM), HEPES (100 mM) + SDS (40 mM), neat DMSO, neat DMF, and artificial “gastric juice” (HCl (pH 1.6)) were determined using low-temperature EPR spectroscopy. An investigation of a micelle environment for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ was of interest given the potential for the complex to be “solubilised” in the GI tract by bile salts.⁷ Enhanced GI absorption of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is reported in rats compared to IndoH in the presence of the bile salt sodium deoxycholate.⁷ The non-carboxylate-type biological buffers TES, HEPES and MOPS were investigated as $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 1 \text{ mg mL}^{-1}$) in borate (100 mM) + SDS (40 mM) results in a colour change of the sample from green to colourless over $\sim 5 \text{ min}$.

Aqueous solutions were analysed at $\sim 4 \text{ K}$ using either glycerol (20% v/v) or DMSO (20% v/v) to produce a vitrified sample suitable for analysis. The solutions were analysed $\sim 30 \text{ min}$ post preparation, unless otherwise stated, to determine the Cu(II) monomer content ($\% \text{Cu}_{\text{mon}}$) (Table 4.3). All buffer solutions were protected from light prior to their EPR and UV-Vis spectroscopic analyses.

Due to concerns over the possible binding of Cu(II) to glycerol (20% v/v) in the vitrified sample, DMSO (20% v/v) was used in the low-temperature EPR spectroscopy in measurements in artificial “gastric juice” sample. The artificial “gastric juice” was analysed following sonication of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.08 mg mL^{-1}) in HCl (pH 1.6) for $\sim 5 \text{ min}$ prior to freezing in liquid nitrogen. Duplicate determinations of the Cu(II) monomer content of the resultant filtrate of the artificial “gastric juice” sample was undertaken by X-band EPR spectroscopy $\sim 20 \text{ min}$ post sample preparation. The solid residue obtained from the artificial “gastric juice” sample of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.08 mg mL^{-1}) was retained for solid-state IR spectroscopic analysis and the spectrum is compared with that of the solid residue obtained from IndoH (0.25 mg mL^{-1}) in artificial “gastric acid” (Figure 4.3). The concentrations were chosen to simulate the administration of the drugs to dogs⁸ and humans,¹ respectively.

Table 4.3. The Cu(II) Monomer Content of Solution Samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

Sample	Time	Sample Colour	Cu(II) monomer (%Cu _{tot})
DMF ^a	6 hr	green	2.0 ^e
DMF ^a	18 hr	green	4.3
DMF ^a	10 days	green	2.0
DMSO ^a	6 hr	green	< 0.4
TES (100 mM) + SDS (40 mM) ^{a, b}	1 hr	green	7.5
TES (100 mM) + SDS (40 mM) ^{a, b}	3 days	olive green	22.3
TES (100 mM) + SDS (40 mM) ^{a, b}	7 days	olive green	75
TES (100 mM) + SDS (40 mM) ^{a, b}	5 days	olive green	65
TES (100 mM) + SDS (40 mM) ^{a, b}	8 days	green	85
TES (100 mM) + SDS (40 mM) ^{a, c}	1 hr	green	9.2
TES (100 mM) + SDS (40 mM) ^{a, c}	3 hr	olive green	9.9
HEPES (100mM) + SDS (40 mM) ^{a, b}	1 hr	green	4.2
HEPES (100mM) + SDS (40 mM) ^{a, b}	18 hr	green	6.1
HEPES (100mM) + SDS (40 mM) ^{a, b}	4 days	olive green	57
Artificial "gastric juice (filtrate)" ^{c, d}	20 min	clear	42

^a $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 1 \text{ mg mL}^{-1}$). ^b Contains glycerol (20% v/v). ^c Contains DMSO (20% v/v). ^d $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 0.08 \text{ mg mL}^{-1}$). ^e The presence of the Cu(II) dimer drug is evident in neat DMF at 100 K (Appendix 16).

Whilst the dimer is retained (EPR) in the pharmaceutical formulations, addition of the complex to artificial “gastric juice” resulted in decomposition of the Cu complex to ~42% of a Cu(II) monomer, which is yet to be characterised. The $\nu_{\text{asym}}(\text{COO})$ and $\nu(\text{C}=\text{O})$ IR bands at 1717 cm^{-1} and 1404 cm^{-1} , respectively, of powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ were not observed in the IR spectra of the residue from $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in artificial “gastric juice”, whilst the $\nu(\text{C}=\text{O})$ amide band is observed in both of these spectra (Appendix 17). The IR spectrum of the solid residue obtained from $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in artificial “gastric juice” is different from that of the solid residue obtained from IndoH in artificial “gastric juice”, which was unchanged from that of powdered IndoH.

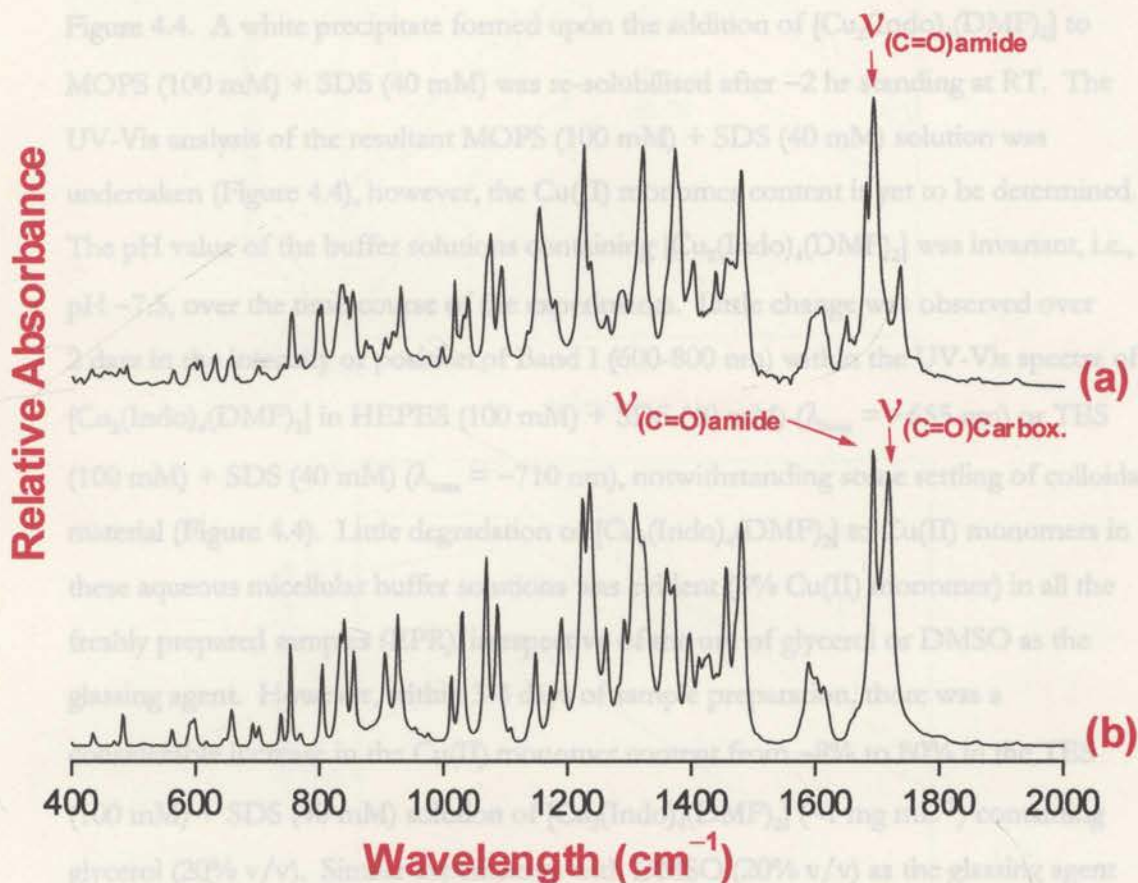


Figure 4.3. The IR spectra of the solid residue obtained from artificial “gastric juice” of: (a) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.08 mg mL^{-1}); and (b) IndoH (0.25 mg mL^{-1}).

Whilst there is significant degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in HCl (pH 1.6) to Cu(II) monomer species, there is variable degradation in the other solution samples. The presence of the Cu(II) dimer is evident in neat DMF at 100 K (Appendix 16) by weak features at ~ 5980 (H_{22}) and 500 G (H_{z1}). Whilst, the signal at 3300 G in neat DMF at 100 K is clearly evident, it corresponds to only 2% Cu(II) monomer contaminants, and little variation was found in its intensity over a 10 days. Similarly, $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ dissolved in neat DMSO after 6-hr period contains a low Cu(II) monomer content.

The UV-Vis spectra in the 300-800-nm region of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in TES (100 mM) + SDS (40 mM), HEPES (100 mM) + SDS (40 mM) and MOPS (100 mM) + SDS (40 mM) (glycerol-free) recorded over a 2-day period are given in Figure 4.4. A white precipitate formed upon the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to MOPS (100 mM) + SDS (40 mM) was re-solubilised after ~ 2 hr standing at RT. The UV-Vis analysis of the resultant MOPS (100 mM) + SDS (40 mM) solution was undertaken (Figure 4.4), however, the Cu(II) monomer content is yet to be determined. The pH value of the buffer solutions containing $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ was invariant, i.e., pH ~ 7.5 , over the time-course of the experiments. Little change was observed over 2 days in the intensity or position of Band I (600-800 nm) within the UV-Vis spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in HEPES (100 mM) + SDS (40 mM) ($\lambda_{\text{max}} = \sim 655$ nm) or TES (100 mM) + SDS (40 mM) ($\lambda_{\text{max}} = \sim 710$ nm), notwithstanding some settling of colloidal material (Figure 4.4). Little degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to Cu(II) monomers in these aqueous micellar buffer solutions was evident (8% Cu(II) monomer) in all the freshly prepared samples (EPR), irrespective of the use of glycerol or DMSO as the glassing agent. However, within 3-4 days of sample preparation, there was a considerable increase in the Cu(II) monomer content from $\sim 8\%$ to 80% in the TES (100 mM) + SDS (40 mM) solution of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (~ 1 mg mL $^{-1}$) containing glycerol (20% v/v). Similar experiments with DMSO (20% v/v) as the glassing agent have yet to be performed. There is a decrease and slight shift to a higher energy of Band I in MOPS (100 mM)/SDS (40 mM) by 2 hr and 6 days, respectively. This indicates not only some settling of colloidal material but also possible degradation of the dimer.

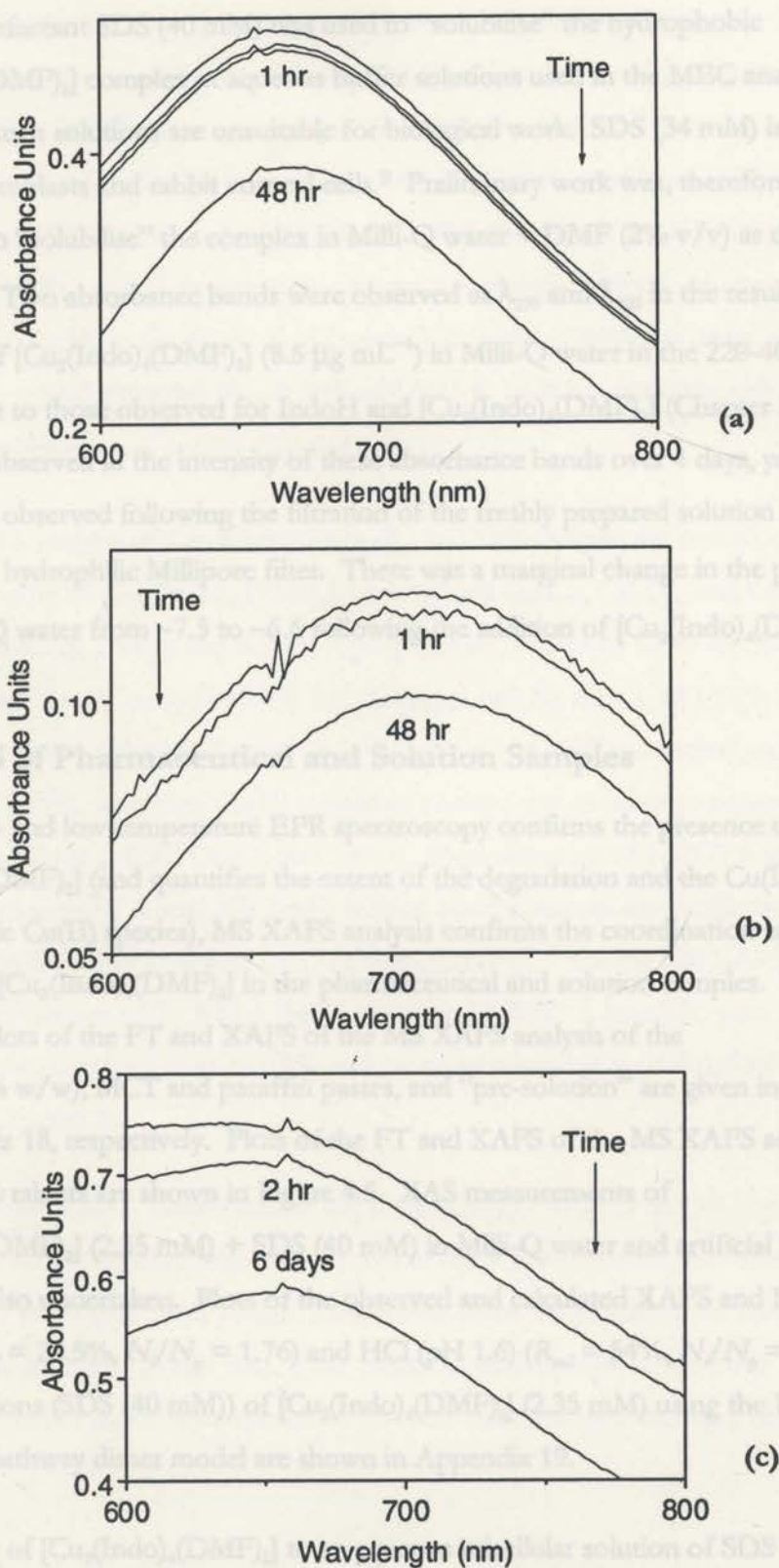


Figure 4.4. Solution UV-Vis spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 1 \text{ mg mL}^{-1}$) in (a) HEPES (100 mM)/SDS (40 mM), (b) TES (100 mM)/SDS (40 mM), and (c) MOPS (100 mM)/SDS (40 mM).

Whilst the surfactant SDS (40 mM) was used to “solubilise” the hydrophobic $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ complex in aqueous buffer solutions used in the MEC analyses (Chapter 3), such solutions are unsuitable for biological work. SDS (34 mM) is cytotoxic to human fibroblasts and rabbit corneal cells.⁹ Preliminary work was, therefore, undertaken to “solubilise” the complex in Milli-Q water + DMF (2% v/v) as described elsewhere.¹⁰ Two absorbance bands were observed at λ_{270} and λ_{320} in the resultant UV-Vis spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($8.6 \mu\text{g mL}^{-1}$) in Milli-Q water in the 220–400 nm region similar to those observed for IndoH and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (Chapter 2). Little change was observed in the intensity of these absorbance bands over 4 days, yet a decrease was observed following the filtration of the freshly prepared solution with a 45 μm nylon hydrophilic Millipore filter. There was a marginal change in the pH value of the Milli-Q water from ~ 7.5 to ~ 6.6 following the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

4.3 XAS of Pharmaceutical and Solution Samples

Whilst room- and low-temperature EPR spectroscopy confirms the presence of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (and quantifies the extent of the degradation and the Cu(II) dimer to monomeric Cu(II) species), MS XAFS analysis confirms the coordination and geometry of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the pharmaceutical and solution samples. The results and plots of the FT and XAFS of the MS XAFS analysis of the carbopol (1% w/w), MCT and paraffin pastes, and “pre-solution” are given in Table 4.4 and Appendix 18, respectively. Plots of the FT and XAFS of the MS XAFS analysis of the *Cu-Algesic* tablets are shown in Figure 4.5. XAS measurements of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (2.35 mM) + SDS (40 mM) in Milli-Q water and artificial “gastric juice” were also undertaken. Plots of the observed and calculated XAFS and FT of the aqueous ($R_{\text{xf}} = 20.5\%$, $N_i/N_p = 1.76$) and HCl (pH 1.6) ($R_{\text{xf}} = 54\%$, $N_i/N_p = 1.67$) micelle solutions (SDS (40 mM)) of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (2.35 mM) using the 130-unique-MS-pathway dimer model are shown in Appendix 19.

The addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to an aqueous micellular solution of SDS (40 mM) results in refined Cu...Cu, Cu-O_{Ac} and Cu-O_L distances of 2.62(2), 1.95(2) and 2.10(2) Å ($R_{\text{xf}} = 20.5\%$), respectively, which are within experimental error of those obtained for powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at 10 K (Cu...Cu (2.63 Å), Cu- 1.95(2) Å) and Cu-

O_L (2.14(2) Å) (Table 3.12). Despite the small k -range studied in this sample due to noise in the data, the determinacy of this model ($N_i/N_p = 1.76$) and goodness-of-fit parameter ($R_{\text{xaf}} = 20.5\%$) were acceptable. The retention of the dimer structure in the aqueous SDS (40 mM) micelles is consistent with the detection of low Cu(II) monomer contaminants (EPR) and little change in Band I (600-800 nm) of the UV-Vis spectra of $[Cu_2(Indo)_4(DMF)_2]$ over 2 days in such aqueous micellar solutions. However, the poor fit of the XAFS to the dimer model when $[Cu_2(Indo)_4(DMF)_2]$ is added to HCl (pH 1.6) + SDS (40 mM) ($R_{\text{xaf}} = 54\%$, $(N_i/N_p) = 1.67$) is consistent with its degradation to monomers ($\sim 42\%$ (Table 4.3)).

The MS XAFS analysis of *Cu-Algesic* tablets ($R_{\text{xaf}} = 17.4\%$) shows the retention of the Cu(II) dimer core of $[Cu_2(Indo)_4(DMF)_2]$, with refined Cu-Cu, Cu- O_{Ac} and Cu- O_L bond distances of 2.62(2), 1.94(2) and 2.15(5) Å, respectively, similar to those reported from crystallographic data (Cu-Cu (2.630(1) Å), Cu- O_{Ac} (1.969 Å), Cu- O_L (2.143(5) Å)).¹¹ The MS XAFS analyses of the MCT paste ($R_{\text{xaf}} = 14.4\%$, 6% Cu(II) monomer) and paraffin paste ($R_{\text{xaf}} = 13.5\%$, unknown Cu(II) monomer content due to other paramagnetic impurities) results in refined Cu-Cu and Cu- O_{Ac} bond distances of 2.62(2) Å and 1.94(2)-1.97(2) Å, respectively. These MS XAFS refined bond distances are similar to those obtained from the X-ray structure of $[Cu_2(Indo)_4(DMF)_2]$,¹¹ but the MS XAFS refined Cu- O_L bond distances of 2.23(8) Å (MCT paste) and 2.22(2) Å (paraffin paste) are greater than that obtained from the MS analysis of powdered $[Cu_2(Indo)_4(DMF)_2]$ (2.14(2) Å, Chapter 3).

While the refined Cu- O_L bond distance for $[Cu_2(Indo)_4(DMF)_2]$ in the MCT paste (2.23(8) Å) is within experimental error of that in the XRD structure, the value in paraffin paste was significantly longer, irrespective of the weighting of the XAFS data to the MS model restraints (Table 4.4). This points to an exchange of the DMF adduct for H_2O in the paraffin paste, since similar results were obtained with the aqua complex (Chapter 3).

Table 4.4. Refined XAS Parameters for the Fitting of the $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ MS Model in Pharmaceutical Formulations.

Formulation	Refined Distance (Å)			Debye-Waller Factors (σ^2 (Å ²))			Refined XAFS Parameters			
	Cu–Cu	Cu–O _{Ac}	Cu–O _L	Cu–Cu	Cu–O _{Ac}	Cu–O _L	E_0 (eV)	S_0^2	R_{xaf} (%)	Determinacy of Model
<i>Cu-Algesic</i> tablets ^a	2.62	1.94	2.15	0.02	0.02	0.05	-13.4	0.91	17.4	1.67
Paraffin paste ^a	2.62	1.97	2.22	0.02	0.02	0.02	-9.5	0.89	13.5	1.88
Paraffin paste ^b	2.61	1.95	2.18	0.02	0.02	0.02	-9.4	0.89	11.0	1.88
MCT paste ^a	2.62	1.94	2.23	0.02	0.02	0.08	-13.5	0.89	14.4	1.65
<i>Cu-Algesic</i> paste ^a	2.59	1.95	2.28	0.02	0.02	0.08	-11.7	0.89	32.9 ^c	1.88
<i>Avigesic Forte</i>	2.60	1.95	2.15	0.02	0.02	0.02	-10.9	0.91	25.8 ^d	1.63
“pre-solution” ^a	2.62	1.94	2.07	0.02	0.02	0.05	-13.8	0.86	26.4 ^d	1.88

^a XAFS weighting factor = 1. ^b XAFS weighting factor = 8. ^c These high R_{xaf} values are related to Cu monomer formation. *Cu-Algesic* paste contains ~30% Cu(II) monomer (Table 4.1) and gives a poor fit of the observed and calculated XAFS data in the FT shells corresponding to Cu–O and Cu···Cu MS back-scattering contributions, i.e., FT radial distances in the 2–3 Å region. ^d Whilst *Avigesic-Forte* and the “pre-solution” contain only ~6% and ~2% Cu(II) monomer contaminants (Table 4.2), respectively, the values of the R_{xaf} are high, despite good agreement of the refined bond Cu···Cu and Cu–O_{Ac} bond distances to crystallographic data.¹¹ This reflects the poorer quality of the XAFS data.

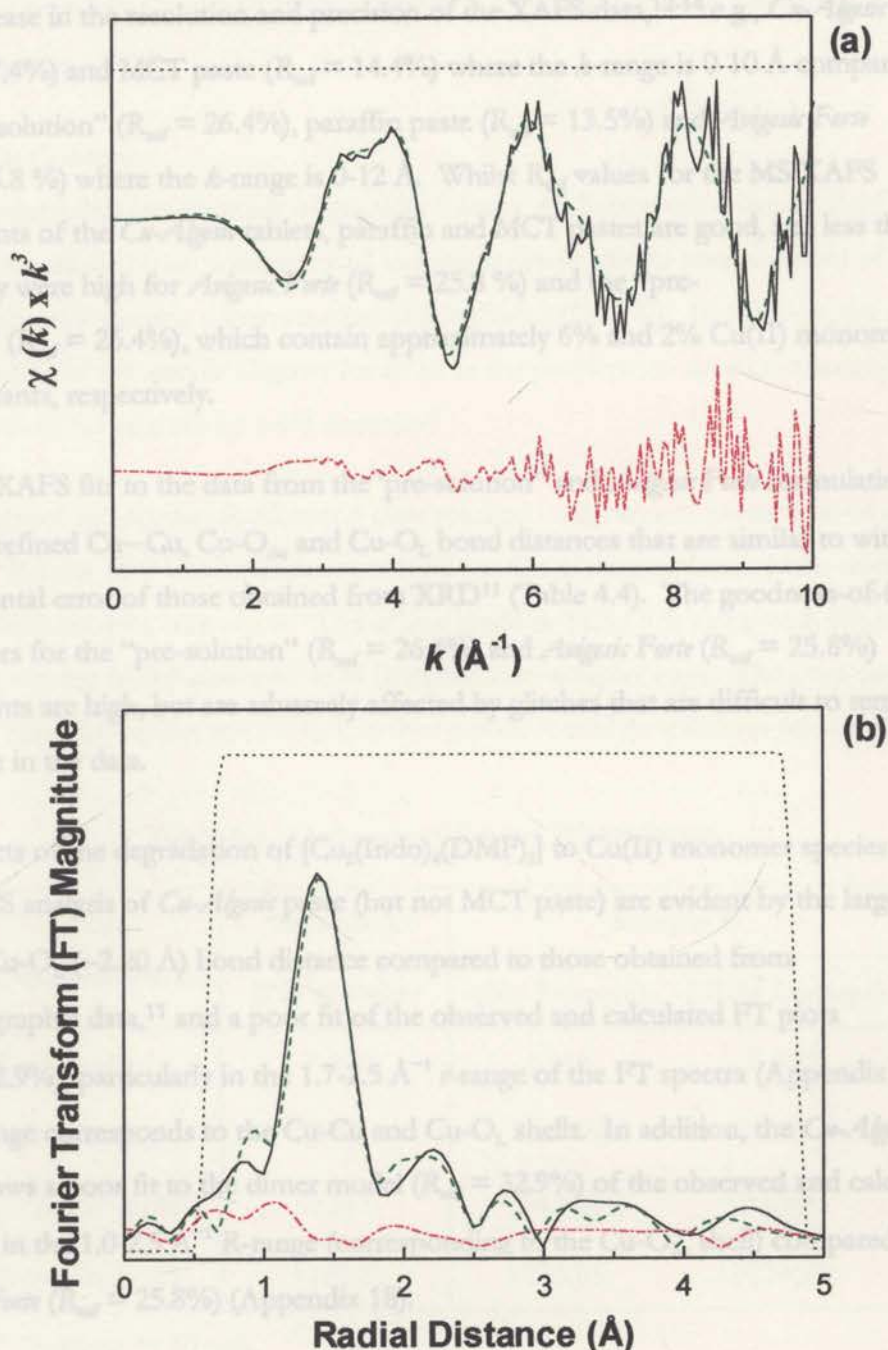


Figure 4.5. Cu-K edge (a) MS XAFS data and (b) corresponding Fourier transform observed (—), simulated (---) and residual (· · ·) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the *Cu-Algesic* tablets, $R_{\text{xaf}} = 17.4\%$. The region shown by (· · ·) is the k window used in the Fourier filter. Excessive noise in the XAFS data in the region greater than $10 \text{ \AA}^{-1}$ prevented the use of a larger k -range.

Small changes are expected in the refined bond lengths as the k range decreases due to a decrease in the resolution and precision of the XAFS data,¹²⁻¹⁴ e.g., *Cu-Algesic* tablets ($R_{\text{xaf}} = 17.4\%$) and MCT paste ($R_{\text{xaf}} = 14.4\%$) where the k -range is 0-10 Å compared to the “pre-solution” ($R_{\text{xaf}} = 26.4\%$), paraffin paste ($R_{\text{xaf}} = 13.5\%$) and *Avigesic Forte* ($R_{\text{xaf}} = 25.8\%$) where the k -range is 0-12 Å. Whilst R_{xaf} values for the MS XAFS refinements of the *Cu-Algesic* tablets, paraffin and MCT pastes are good, i.e., less than 20%, they were high for *Avigesic Forte* ($R_{\text{xaf}} = 25.8\%$) and the “pre-solution” ($R_{\text{xaf}} = 26.4\%$), which contain approximately 6% and 2% Cu(II) monomer contaminants, respectively.

The MS XAFS fits to the data from the “pre-solution” and *Avigesic Forte* formulation result in refined Cu...Cu, Cu-O_{Ac} and Cu-O_L bond distances that are similar to within experimental error of those obtained from XRD¹¹ (Table 4.4). The goodness-of-fit parameters for the “pre-solution” ($R_{\text{xaf}} = 26.4\%$) and *Avigesic Forte* ($R_{\text{xaf}} = 25.8\%$) refinements are high, but are adversely affected by glitches that are difficult to remove, and noise in the data.

The effects of the degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to Cu(II) monomer species on the MS XAFS analysis of *Cu-Algesic* paste (but not MCT paste) are evident by the larger refined Cu-O_L (~2.20 Å) bond distance compared to those obtained from crystallographic data,¹¹ and a poor fit of the observed and calculated FT plots ($R_{\text{xaf}} = 32.9\%$), particularly in the 1.7-2.5 Å⁻¹ r -range of the FT spectra (Appendix 18). This r -range corresponds to the Cu-Cu and Cu-O_L shells. In addition, the *Cu-Algesic* paste shows a poor fit to the dimer model ($R_{\text{xaf}} = 32.9\%$) of the observed and calculated FT plots in the 1.0-2.5 Å⁻¹ R -range (corresponding to the Cu-O_{Ac} shell) compared to the *Avigesic Forte* ($R_{\text{xaf}} = 25.8\%$) (Appendix 18).

4.4 Equine Blood

As a prelude to future investigations by BVR of the effects of inflammation and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ on Cu levels in animals and the fate of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in biological media, various analytical measurements were required. Graphite furnace AAS and UV-Vis analyses were used to determine the whole blood Cu level and spectra of

$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in equine plasma, respectively, of a healthy horse ("Denis") maintained on a low Cu diet by BVR. "Denis" is a 15-year-old male Australian quarter horse weighing ~390 kg.

4.4.1 Cu Content

The reproducibility and accuracy of graphite furnace AAS determinations of the Cu levels within whole blood were assessed as a requirement for the measurement of Cu levels in horses following administration of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. In addition, the effects of the storage of the sample aliquots for 24 hr in the polystyrene auto-sampler cups used in the graphite furnace set-up were examined.

The storage of the equine blood over 5 days resulted in significant haemolysis of the samples. Results of five independent but triplicate Cu determinations of two whole blood equine samples taken from "Denis" are given in Table 4.5, along with the results following storage of the analytes for 24 hr in the auto-sampler cups. The auto-sampler cups had been rinsed, but not soaked, in the respective analyte solutions prior to use.

Table 4.5. Cu Levels of Equine Whole Blood.

Cu Determination	Sample A (No storage)	Sample B	
		(No Storage)	(24-hr Storage)
Blank (ppb)	7.5(4)	5.6(4)	6.8(1)
Unknown (ppb)	28.5(4)	24.1(15)	25.5(1)
Standard ^a (ppb)	48.6(7)	50.5(8)	55.9(4)
Blood Cu (ppb)	1168(5)	1024(85)	1039(23)

^a Standard = 50 ppb.

The graphite furnace AAS analysis of two separate samples of equine whole blood resulted in values of the mean Cu content of 1096(90) ppb ($17(1.5) \mu\text{mol Cu L}^{-1}$). This result is consistent with a moderately low to normal Cu diet,¹⁵ where Cu in equine whole blood is reported to range from 832 ppb¹⁶ to 2794 ppb¹⁵ and with mean whole blood Cu levels of 1384 ppb to 1416 ppb.¹⁵

No change in the value of the whole blood Cu levels was observed upon storage of the analyte solutions in polystyrene sample holders over 24 hr. However, a slight increase in the Cu level of individual analyte solution (blank, unknown, standard), was observed over 24-hr compared to those solutions that were freshly prepared and analysed. This indicates the polystyrene sample cups may contain trace amounts of Cu, which are subsequently leached into the analyte solutions over time. This highlights the need to adequately presoak the auto-sampler cups prior to use.

Neither spiking of the samples with a Cu standard, nor analysis of a serum or blood standard of known Cu content was used to determine the accuracy of the AAS technique. However, five independent analyses of the 50-ppb reference standard resulted in a concentration of 50(1) ppb.

The therapeutic dose of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in horses of $200 \text{ mg } (430 \text{ kg})^{-1}$ mean body weight, i.e., 5 g of *Cu-Algesic* paste for "Denis", results in peak IndoH plasma levels of $\sim 130 \text{ ng mL}^{-1}$.¹⁷ This is equivalent to equine plasma IndoH and Cu levels (as a result of the administration of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ of $\sim 7.6 \times 10^{-2} \text{ nmol mL}^{-1}$ and $\sim 3.86 \times 10^{-2} \text{ nmol mL}^{-1}$, respectively. This administered Cu dose corresponds to $\sim 0.2\%$ of mean equine plasma Cu ($1416 \mu\text{g mL}^{-1}$).¹⁵ Consequently, a dose of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in horses of $\sim 10 \text{ g } (430 \text{ kg})^{-1}$ mean body weight would be required to detect a 10% increase in equine plasma Cu levels using the graphite furnace AAS technique described in this work. This is 50 times the recommended therapeutic dose.¹⁷ Therefore, AAS cannot be used to monitor Cu uptake of the drug into the plasma. However, it does highlight the low additional Cu load placed on the animal at therapeutic levels and hence the negligible risk of Cu toxicity.

4.4.2 UV-Vis Analysis of *Avigesic Forte* in Equine Serum

UV-Vis analysis of an *Avigesic-Forte* ($[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (8 mg mL^{-1})) formulation prepared in this work and dissolved in equine serum was undertaken. This enabled a preliminary investigation of the effect of serum (collected from "Denis") on the UV-Vis characteristics of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the 400-600-nm region (Band I). The equine serum samples were collected by BVR in sterile CORVAC Integrated serum separator tubes without anti-coagulant. The samples were immediately centrifuged at 1000 rpm

for ~20 s and *Avigesic-Forte* ($[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (8 mg mL^{-1}) was added to the serum in a 1:5 dilution, i.e., equine serum + $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ($\sim 1.6 \text{ mg mL}^{-1}$) ~1 hr post venipuncture. UV-Vis analysis of the sample over ~40 min was undertaken using an equine serum sample blank. The UV-Vis analysis of the equine serum over 40 min using a Milli-Q water sample blank at ~2 hr post venipuncture and a 1:5 dilution of *Avigesic Forte* (drug-free):equine serum using an equine serum blank at ~1.5 hr, ~2 hr and ~3 hr post venipuncture are shown in Figure 4.6. All serum samples were analysed within ~3 hr of venipuncture.

Addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to serum resulted in a strong visible absorbance in the 600-800-nm region (Figure 4.6(b)) corresponding to the $d_{xy,yz} \rightarrow d_{x^2-y^2}$ transition (Band I), with a subsequent shift to higher energy, i.e., increased octahedral crystal field splitting (Δ_o) energy of the d orbitals, and loss of intensity of the band occurring over ~40 min. This showed a loss in the dimeric nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, rather than simply axial ligand exchange, in the serum given the negligible differences in the electronic absorption spectra of the $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ complexes (Chapter 2).¹¹ Furthermore, the change in dimeric nature was rapid, as evidenced by a major shift and loss of intensity of Band I after ~3 min following the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to the serum.

No major change was noted in the UV-Vis spectra of the equine serum alone in the 400-900-nm region over 40 min at ~1.5 hr post venipuncture, although some precipitation of colloidal material was observed. This is evident by the approximate uniform loss of intensity in absorbance over the 400-900-nm region. However, the UV-Vis characteristics of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in equine serum as a result of a 1:5 dilution of the *Avigesic Forte* formulation with equine serum may substantially differ from that found *in vivo*. This was confirmed by the significant deviation from the zero baseline in the 500-600-nm region of the spectra in the equine serum following the addition of drug-free *Avigesic Forte* to the equine serum. The small but uniform loss in intensity of absorbance at wavelengths greater than 600 nm is attributed to precipitation of serum constituents. Analysis at RT (rather than body temperature) and oxidation of serum constituents, e.g., albumin,¹⁸ may also affect the Cu(II) binding and/or redox potentials of the serum constituents.

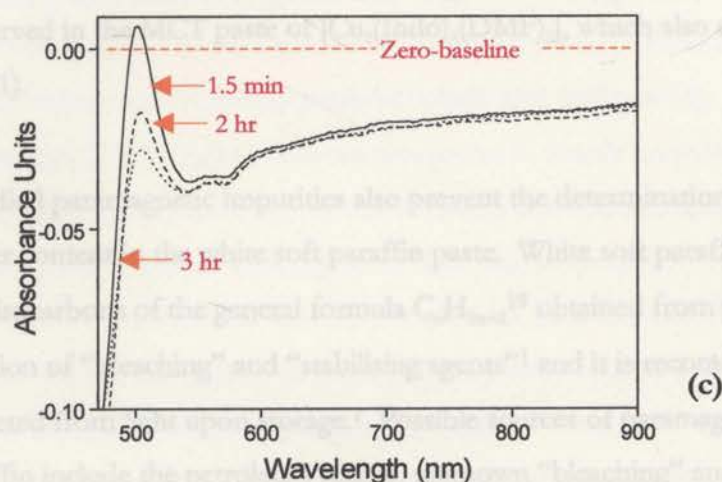
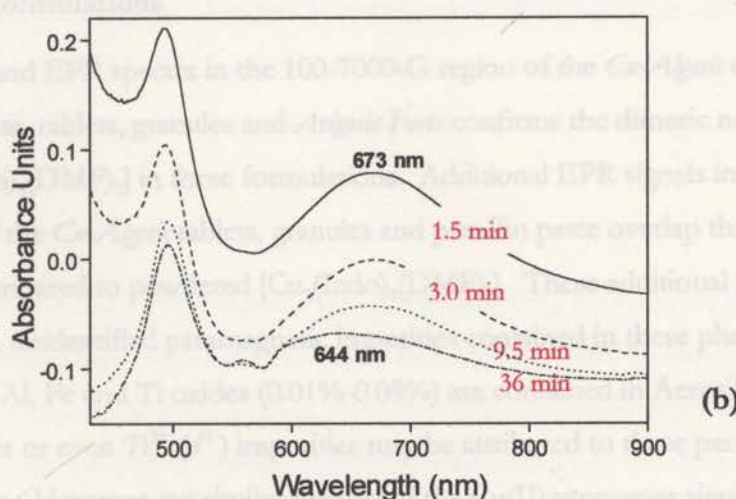
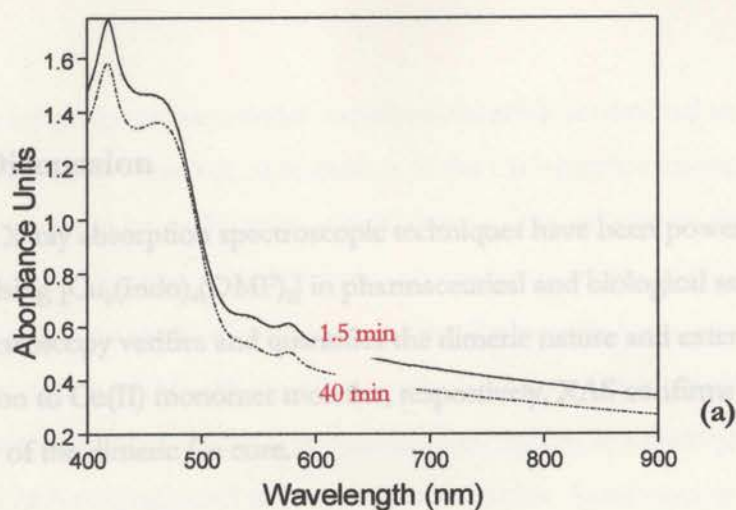


Figure 4.6. UV-Vis spectra of of: (a) equine serum over 40 min at 2-hr post venipuncture;

(b) 1:5 dilution of *Avigesic-Forte* $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (8 mg mL^{-1}) + equine serum over

40 min at 1-hr post venipuncture and (c) 1:5 dilution of *Avigesic-Forte* (drug-free) + equine serum at 1.5 hr, 2 hr and 3 hr post venipuncture.

4.5 Discussion

EPR and X-ray absorption spectroscopic techniques have been powerful in characterising $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in pharmaceutical and biological samples. Whilst EPR spectroscopy verifies and quantifies the dimeric nature and extent of any degradation to $\text{Cu}(\text{II})$ monomer moieties, respectively, XAS confirms the coordination geometry of the dimeric Cu core.

4.5.1 Formulations

The X-band EPR spectra in the 100-7000-G region of the *Cu-Algesic* carbopol (1% w/w), MCT paste, tablets, granules and *Avigesic Forte* confirms the dimeric nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in these formulations. Additional EPR signals in the 2500-3500-G region of the *Cu-Algesic* tablets, granules and paraffin paste overlap the $\text{Cu}(\text{II})$ monomer signal compared to powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. These additional signals correspond to, as yet, unidentified paramagnetic impurities contained in these pharmaceuticals. Since traces of Al, Fe and Ti oxides (0.01%-0.08%) are contained in Aerosil (Table 2.1), Fe^{3+} (d^5) oxide or even Ti^{3+} (d^1) impurities may be attributed to these paramagnetic impurities. However, no similar overlap of the $\text{Cu}(\text{II})$ monomer signal with other signals was observed in the MCT paste of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, which also contains Aerosil (Table 2.1).

Unidentified paramagnetic impurities also prevent the determination of the $\text{Cu}(\text{II})$ monomer content in the white soft paraffin paste. White soft paraffin is a mixture of solid hydrocarbons of the general formula $\text{C}_n\text{H}_{2n+2}$ ¹⁹ obtained from petroleum following the addition of "bleaching" and "stabilising agents"¹ and it is recommended that paraffin be protected from light upon storage.¹ Possible sources of paramagnetic impurities in the paraffin include the petroleum source, unknown "bleaching" and "stabilising agents" and/or contaminated manufacturing equipment or storage containers. The paraffin paste had been stored by BVR in a plastic storage container prior to EPR spectroscopic analysis.

The X-band Cu -hyperfine signals from powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ are better observed at 100 K than RT. While the resolution is still poor, the same value of the Cu -

hyperfine coupling constant, within experimental error, is obtained as that from ZF-EPR spectroscopy. The loss of resolution of the Cu-hyperfine transitions in the X-band EPR spectrum can be attributed to a breakdown of the spin-lattice relaxation conditions in the solid-state samples at the temperatures necessary for the production of a sharp EPR signal.²⁰ As a result, the resolution of the Cu hyperfine resonances will depend on the concentration of the sample, since narrow EPR lines require low concentrations of the paramagnetic ions, typically 1% paramagnetic doping in a diamagnetic environment and 0.001 M for complexes,² rather than neat samples. Consequently, better resolution of the Cu hyperfine was observed in the magnetically dilute pastes than powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. The better resolution at low temperature was because of the line width of the EPR spectra (σ_r), which depends on the temperature according to $\sigma_r \propto T_1^{-1}$.²

Whilst the X-band EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ confirm the presence of the dimer in neat DMF, with minimal Cu(II) monomer species, addition of the dimer drug to some pharmaceutical formulations, aqueous buffer solutions and artificial “gastric juice” results in variable and sometimes significant degradation of the drug to, as yet, unidentified Cu(II) monomer species with unknown biological activities. Despite the report of the complete decomposition of the NSAID Cu(II) dimer complex of niflumic acid (2-[3[(trifluoromethyl)phenyl]aminonicotinic acid (niflumathH); $[\text{Cu}_2(\text{niflumato})_4(\text{DMSO})_2]$) to monomeric species in freshly prepared DMF samples,²¹ only 10% Cu(II) monomer was observed in neat DMF solution samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in this work, up to 10 days post preparation. The presence of small amounts of water in the DMF solution sample of $[\text{Cu}_2(\text{niflumato})_4(\text{DMSO})_2]$ may be an explanation for the discrepancy in the Cu(II) monomer content (%) reported for $[\text{Cu}_2(\text{niflumato})_4(\text{DMSO})_2]$ compared to this work. Future work should include an investigation of the effects of varying amounts of water on the degradation of dimeric Cu(II) complexes to Cu(II) monomeric species.

Analyses of the carbopol (1% w/w) paste and gelatin capsules show significant degradation of the dimer to Cu(II) monomers compared to other formulations, e.g., the MCT paste, *Avigesic Forte*, “pre-solution” and “pre-fill” solution. The degradation of

$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to monomeric Cu(II) and perhaps Cu(I) in carbopol (1% w/w) paste and gelatin capsules was significant. The micelles of cremophor (4% v/v) and NMP (4% v/v) in *Avigesic-Forte* appear to protect the dimer from degradation to Cu(II) monomers, as does the hydrophobic environment of the "pre-solution", "pre-fill" solution and MCT paste. This is confirmed by EPR and X-ray absorption spectroscopy.

In order to understand the potential reasons for the stabilisation or degradation of the Cu(II) drug in the pharmaceutical preparations, it is important that the properties of these constituents are analysed. MCT, a mixture of triacylglycerols not dissimilar from tetraglycol, contains mainly C_8 and C_{10} fatty acid triglycerides, i.e., fatty acid esters of glycerol.¹ Tetraglycol, a component of the MCT paste, is a water-miscible glycerol-like vehicle used in pharmaceutical preparations for solubilising compounds that are either insoluble or unstable in water.¹ Span 80, likewise used in the MCT paste, is a non-ionic solubilising agent consisting of partial esters of sorbitol, mono- and di-anhydrides of sorbitol and oleic acid (*cis*-9-octadecenoic acid).¹ Cremophor, used in *Avigesic Forte*, is a water-soluble non-ionic surfactant consisting of a mixture of monosterate and disterate esters of mixed polyoxyethylene glycols and is used to solubilise hydrophobic drugs in oil-in-water (o/w) emulsions.¹ The pharmaceutical constituents of *Avigesic Forte* and the "pre-solution" may, therefore, have the potential to complex Cu(II) and so alter the dimeric nature of these drugs in these formulations.

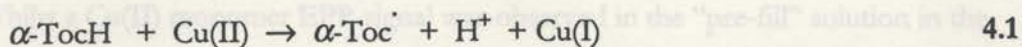
Carbopol has a significant number of carboxylate donors (~68%)¹ that could replace Indo. The exact nature of the Cu(II) monomer in the carbopol formulations and mechanism of degradation are unknown but may involve an interaction of the carboxylate residues of carbopol with the Cu ions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Whilst there is the potential for the Cu(II) dimer complex to interact with the preservatives, the nature of the gelling agent appears to be a far more important consideration. Carbopol is highly ionic and slightly acidic and is used to produce thick and stable o/w emulsions.¹ An aqueous dispersion of carbopol (1% w/w) has a pH value of ~3,¹ but neutralised aqueous gels of carbopol are most viscous between pH 6 and 11. The viscosity is considerably reduced if the pH value is less or greater than 3 and 12, respectively, if it contains strong electrolytes, or is exposed to light.¹ The increased viscosity of the carbopol (1% w/w)

paste containing ~60-80% compared to those containing 30-40% Cu(II) monomer species may, therefore, be due to the significant decomposition of the neutral Cu(II) dimer drug to charged Cu(II) monomers. There are other features of the formulations that must be considered. Carbopol is incompatible with the carboxylic drugs benzoic and salicylic acid¹ and is used with basic drugs, e.g., ephedrine, to form derivatives used in prolonged-release preparations.¹ Benzalkonium chloride, which is a mixture of alkylbenzyltrimethylammonium chlorides of the general formula $[C_6H_5CH_2N(CH_3)_3R]Cl$, ($R = C_8H_{17}$ to $C_{18}H_{37}$) decreases the viscosity of carbopol solutions.¹ In addition, alkaline aqueous solutions of benzalkonium chloride are incompatible with carboxylic acid, e.g., tartrates and salicylates, and anionic surfactants.¹ The anti-bacterial activity of *n*-propyl-4-hydroxybenzoate is reduced in the presence of nonionic surfactants, e.g., Span 80, and is incompatible with methylcellulose.¹ An interaction between *n*-propyl-4-hydroxybenzoate and sucrose esters is reportedly due to hydrogen and hydrophobic bonding.¹ Both preservatives, therefore, have the potential to interact with the anionic molecules of carbopol at near neutral pH values. Likewise, the preservative may interact with the carboxylate groups of IndoH and $[Cu_2(Indo)_4(DMF)_2]$. Despite these potential complications, no significant increase in Cu(II) monomer content (%) was observed in the carbopol (1% w/w) pastes due the different preservatives investigated.

The large variation in the Cu(II) monomer content (%) of the carbopol pastes supplied by BVR compared to those manufactured during the course of this work is unexplained. However, it may be due to different preparative methods, e.g., addition by BVR of $[Cu_2(Indo)_4(DMF)_2]$ to the carbopol paste in the factory before rather than after adjusting the paste to pH ~7. Incorporation of the drug into either paraffin or MCT paste results in significantly less degradation to Cu(II) monomer species, whilst still retaining the bright green colour and X-band EPR dimer characteristics of $[Cu_2(Indo)_4(DMF)_2]$. Therefore, such formulations may be more appropriate for pharmaceutical preparations.

Gelatin, like carbopol, has a significant number of carboxylate residues. The exact nature of the degradation of $[Cu_2(Indo)_4(DMF)_2]$ with gelatin and α -ToCH in the liquid-filled gelatin capsules in the presence of light has not been elucidated. Gelatin is extracted from animal collagen,¹ and contains a heterogeneous mixture of water soluble proteins.²²

Approximately 20 different amino acids have been identified in gelatin, with the molar percent content of some of these amino acids given as glycine (27%), proline (18%), serine (4%), threonine (2%), hydroxyproline (14%) and lysine (6%), as well as the disulfide amino acid cystine (0.2%).¹⁹ Cystine is capable of being converted to cysteine (a thiol) by mild reducing agents.²³ Furthermore, Cu(II) binds to lysine in collagen and other amino acids and peptides, so forming radical species *in vitro*.²⁴ The many carboxylate and polyol donors of gelatin, e.g., serine and threonine, may replace Indo in $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Whilst α -TocH is an antioxidant capable of acting as a radical trap and inhibiting radical reactions that cause cell damage,²³ it has also been shown to act as a reductant for Cu(II):²⁵



so triggering the initiation of the oxidation of lipoproteins in humans.²⁵ This reaction appears to diminish or totally eliminate the antioxidant activity of α -TocH in the course of the lipoprotein oxidation.²⁵ α -TocH, therefore, is capable of reducing $\text{Cu(II)}_{\text{aq}}$ to Cu(I)_{aq} and cystine of gelatin to cysteine. Preliminary investigations of the electrochemistry of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mM) in DMF (0.1 M tetrabutylammonium tetrafluoroborate) (Appendix 20) indicates a quasi-reversible redox potential which has a much more negative redox potential ($E_{\text{pc}} = -855$ mV and $E_{\text{pa}} = -733$ mV versus Ag/AgCl/(6 M NaCl)) than either $\text{Cu(II)}/(\text{I})_{\text{aq}}$ ²⁶ or α -TocH.²⁷ This decreases the likelihood of α -TocH contributing to the initial degradation of the Cu(II) dimer complex. Whilst the mechanism of the degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in the gelatin capsules has not been elucidated, any resulting Cu(I) could be stabilised by thiol donors, which are powerful nucleophiles,²³ e.g., cysteine, or could disproportionate to Cu(II) and Cu(0).²⁶ Furthermore, photolysis of the lipids in the formulation may occur. This will form powerful oxidising species, e.g., alkoxyl radicals (standard redox potential (E^0) = ~ 1600 mV),²⁷ which may further interact with thiol groups, e.g. cysteine, to generate an equally powerful cystine disulfide reducing species $\text{RSSR}^{\bullet -}$ ($E_0 = -1500$ mV)²⁷ (Thiols such as glutathione have a standard redox potential of ~ 240 mV),²⁷ whereas $\text{RSSR}^{\bullet -}$ will reduce metals such as Cu(I) and Cu(II):²⁷



and no doubt $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Whatever the mechanism of the degradation of the Cu(II) dimer complex, a significant proportion of the total Cu(II) dimer content of the gelatin capsules is eventually sequestered into the soft gelatin shell. Only a small proportion of the total initial Cu content in the liquid-filled gelatin capsules is present as Cu(II) monomer. The EPR spectroscopy of the α -TocH-free “pre-solution” used in the manufacture of the gelatin capsules shows little degradation of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to Cu(II) monomer species or colour change over a 9-month period following incorporation into micelles.

Whilst a Cu(II) monomer EPR signal was observed in the “pre-fill” solution in the absence of α -TocH, the signal was obscured in the “pre-fill” solution containing α -TocH (0.025% w/v). α -TocH does not have an EPR signal, however, it could generate an EPR resonance following oxidation to produce Cu(I) and an α -TocH radical (Equation 4.1). The observation of a Cu(II) monomer signal in the capsules contents (were α -TocH is an additive of the “pre-fill” solution) suggests the lipid soluble α -TocH may be sequestered into the gelatin capsule or degraded in the gelatin capsules. Nonetheless, the greater than 90% loss of Cu from the capsule contents, irrespective of the addition of α -TocH, suggests gelatin is more important for the decomposition of the Cu(II) dimer than is α -TocH. Furthermore, the loss of the green colour of the capsule contents suggests eventual reduction of the Cu(II) dimer to a colourless Cu(I) ion²⁶ during the decomposition process. The exact role of UV-Vis radiation and potentiation of the degradation process of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ is unknown. However, UV radiation induces photo-oxidation and auto-oxidation of lipids,^{28,29} i.e., triacylglycerols such as MCT. Furthermore, UV radiation has been reported to decompose IndoH in aqueous anaerobic conditions by the oxidation of the drug in the presence of other oxidants.³⁰ The *Cu-Algesic* capsules is being re-formulated by BVR as a consequence of this research.

MS XAFS analysis was used successfully to determine the coordination of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in powdered and pharmaceutical formulations using the transmission

and fluorescence modes, respectively. Most importantly, the XAFS experiments show the power of this technique to characterise the geometry of the Cu(II) dimer drug that cannot be obtained by other methods in pharmaceutical preparations. As expected, XAFS is sensitive to the Cu(II) monomer content and nature of the formulation such that a high Cu(II) monomer content, e.g. in the carbopol (1%) paste, results in a poor fit of the observed XAFS to that calculated using the dimer MS model. The development of additional MS models incorporating both Cu dimer and monomer absorbing species is yet to be undertaken. We were unable to adequately refine the axial bond distance in the MCT and paraffin paste using the MS model, possible due to an exchange of the DMF ligands for aqua ligands. The technique is, therefore, particularly useful in analysing preparations containing low concentrations of Cu(II) monomer impurities and may also predict exchange of the DMF ligand for H₂O in some pharmaceutical formulations. The XAFS analyses also establish the retention of the dimer core of [Cu₂(Indo)₄(DMF)₂] in an aqueous micellular environment, e.g., SDS (40 mM) and *Avigesic Forte*, and oily “pre-solution” but not artificial “gastric juice”.

4.5.2 Biological Samples and Biological Buffers

Addition of *Avigesic Forte* micelles of [Cu₂(Indo)₄(DMF)₂] to equine serum results in rapid degradation of the Cu(II) dimer structure to as yet undefined Cu species. Whilst there is a possible change in the dimeric nature of Cu(II) in the *Avigesic Forte* formulation, the degradation of micelles of [Cu₂(Indo)₄(DMF)₂] in equine serum is faster (~3 min) compared to aqueous micelle buffer solutions of HEPES (100 mM) + SDS (40 mM) and TES (100 mM) + SDS (40 mM) as observed by UV-Vis analyses (1-2 d). Dilution of the micellular *Avigesic Forte* formulation in serum most probably results in the initial decomposition of the micelle followed by degradation of the Cu(II) dimer. Addition of the drug-free *Avigesic Forte* formulation causes changes in the UV-Vis characteristics of the serum and is, therefore, not a good *in vivo* model. Nonetheless, the results in this work indicate the potential for the decomposition of [Cu₂(Indo)₄(DMF)₂] and its Cu(II) byproducts in biological media. Blood contains a number of complexing and reducing agents, e.g., ceruloplasmin, albumin, ascorbic acid and α-TocH,^{25,31} which may be responsible for the rapid degradation of Cu(II). Whether the fate of [Cu₂(Indo)₄(DMF)₂] in biological media mimics that observed in this preliminary UV-Vis analysis of equine serum remains to be investigated.

The addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to artificial "gastric juice" results in a significant increase in the Cu(II) monomer content within the acid sample. However, the IR spectrum of the insoluble organic residue obtained from artificial "gastric juice" was dissimilar from powdered $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Significantly though, the IR spectrum of the solid residue from IndoH dissolved in artificial "gastric juice" was closer to that of powdered IndoH than the residue obtained from dissolution of the complex in the same medium. This indicates limited hydrolysis of free IndoH in the artificial "gastric juice" compared to that complexed in $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and suggests a possible difference between the fate of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and IndoH in the GI tract. This may be important in explaining the lack of GI toxicity in dogs⁸ and rats³² of the Cu(II) dimer drug compared to IndoH. Future research should include the characterisation and investigation of the GI toxicity and anti-inflammatory effects of the metabolites of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$, including the application of mass and NMR spectroscopy to the characterisation of these products. This may lead onto the development of new anti-inflammatory drugs.

X-ray absorption and EPR spectroscopy has been used to characterise $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and/or quantify the degradation of the dimeric complex to Cu(II) monomer species, respectively, in a number of pharmaceutical formulations and biological samples. These studies also highlight the need to differentiate the role of the Cu(II) dimer from Cu(II) monomer moieties of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in explaining biological activity.

Preliminary investigations of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in aqueous micelle solutions and the pharmaceutical preparations shows variable decomposition of the dimer complex to Cu(II) monomer species, depending upon the media. For example, UV-Vis and EPR spectral analysis of the TES (100 mM) + SDS (40 mM) and HEPES (100 mM) + SDS (40 mM) buffer solutions of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ suggest that the micelle environment offers some protection to the Cu(II) dimer complex from degradation to Cu(II) monomer species and possibly EPR-silent Cu(I) species. However, addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ to HCl (pH 1.6) or MOPS (100 mM) + SDS (40 mM) results in a change in the dimer structure despite the micellular environment. Time limitations on this work unfortunately prevent an analysis of the EPR spectra of the Cu(II) monomer species observed in the solid-state,

pharmaceutical and biological/aqueous samples of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. This will be important in determining the biological significant species of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. Further work is required to confirm the nature of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and its breakdown products in Milli-Q water and artificial "gastric juice".

The aqua as well as the DMF adduct should be investigated as BVR are reformulating their proprietary *Cu-Algesic* formulations with $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]$ as a result of this work. Investigations could include stopped-flow UV-Vis analysis, EPR, NMR, electrochemistry and X-ray absorption and mass spectroscopy of the aqueous and biological samples of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$). The development of analytical techniques to measure $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{OH}_2$) and its metabolites in biological samples will, therefore, be of utmost importance.

Interest in "pro-drugs" of IndoH and other NSAIDs with significantly less GI toxicity yet potent anti-inflammatory activity has also led to the synthesis and evaluation of esters of IndoH.³³⁻³⁵ Whilst variable hydrolysis of these "pro-drugs" is reported in simulated gastric acid,^{33,36} esters of IndoH show potent anti-inflammatory activity similar to IndoH,³⁶ with significantly less GI toxicity,^{33,34,36} in a manner reminiscent of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. The morpholinoalkyl ester of IndoH displays enhanced lipophilic properties³⁴ and rapid hydrolysis in rat plasma (half-life in the range 1-30 min).^{33,34} Investigations of the rates of alkaline and neutral hydrolysis of the 2-formylphenyl ester of IndoH point to an intra-molecular catalytic route for this potent anti-inflammatory "pro-drug" of IndoH.³⁵

Whether Cu-NSAIDs, with their potent anti-inflammatory effects yet lack of GI toxicity, exhibit a bioavailability and mode of action similar to the esters of IndoH remains to be investigated. In any event, it opens up an array of potentially new drugs for the treatment of inflammation. The hydrophobic nature of the exterior of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (and the ester of IndoH) may, however, be the most important factor in facilitating their rapid dissolution in micelles in the GI tract and transport through biological membranes, despite their hydrolysis in artificial "gastric juice". Furthermore, acidic, hydrolytic and/or catalytic byproducts of these complexes may be the biological active moieties rather than the parent drug. An explanation for the biological activity of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ remains, therefore, to be elucidated.

4.6 References

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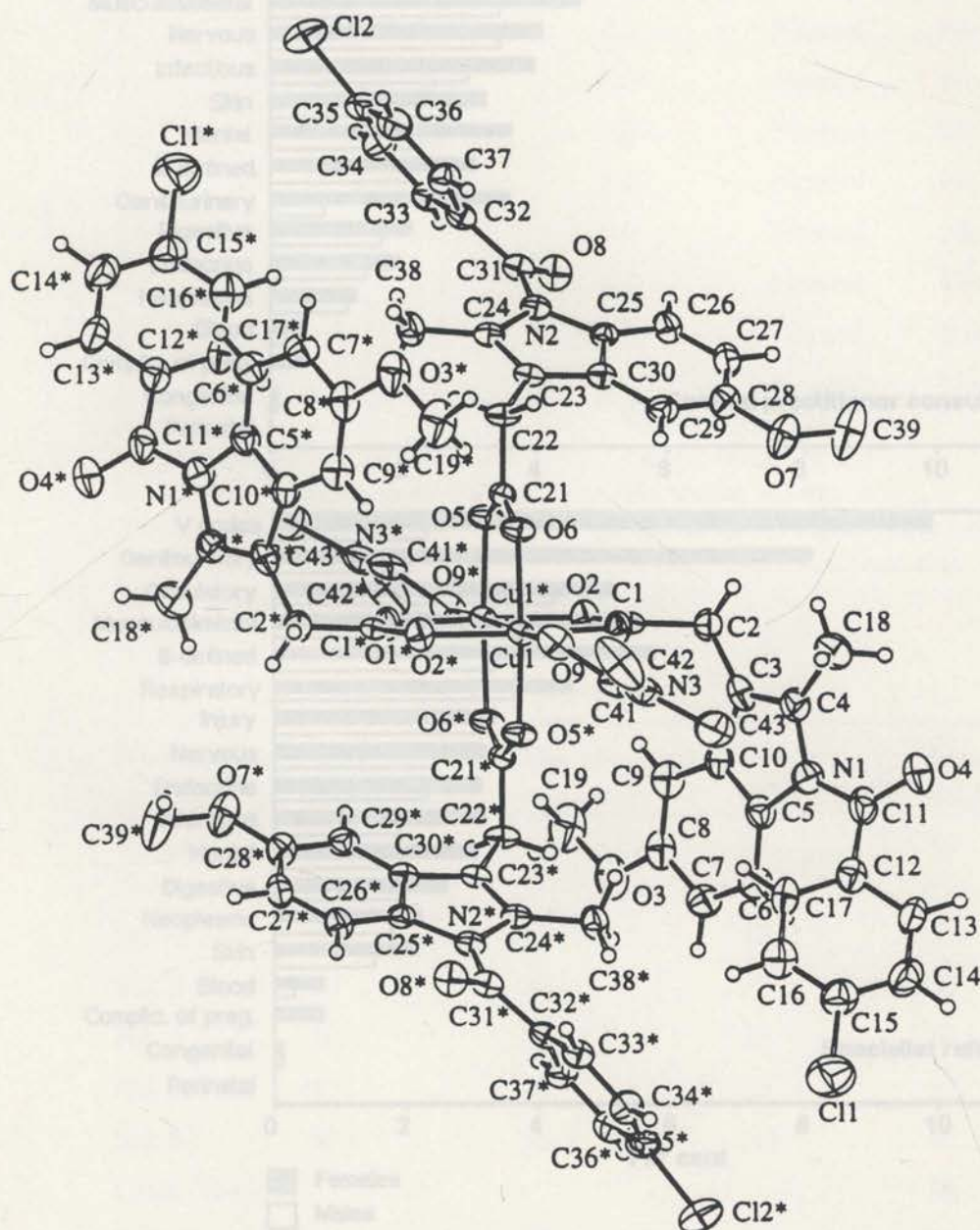
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APPENDICES

APPENDICES

APPENDIX 1.

ORTEP OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ IN THE CRYSTAL STRUCTURE OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot 1.6\text{DMF}^1$ WITH 30% ELLIPSOIDS.

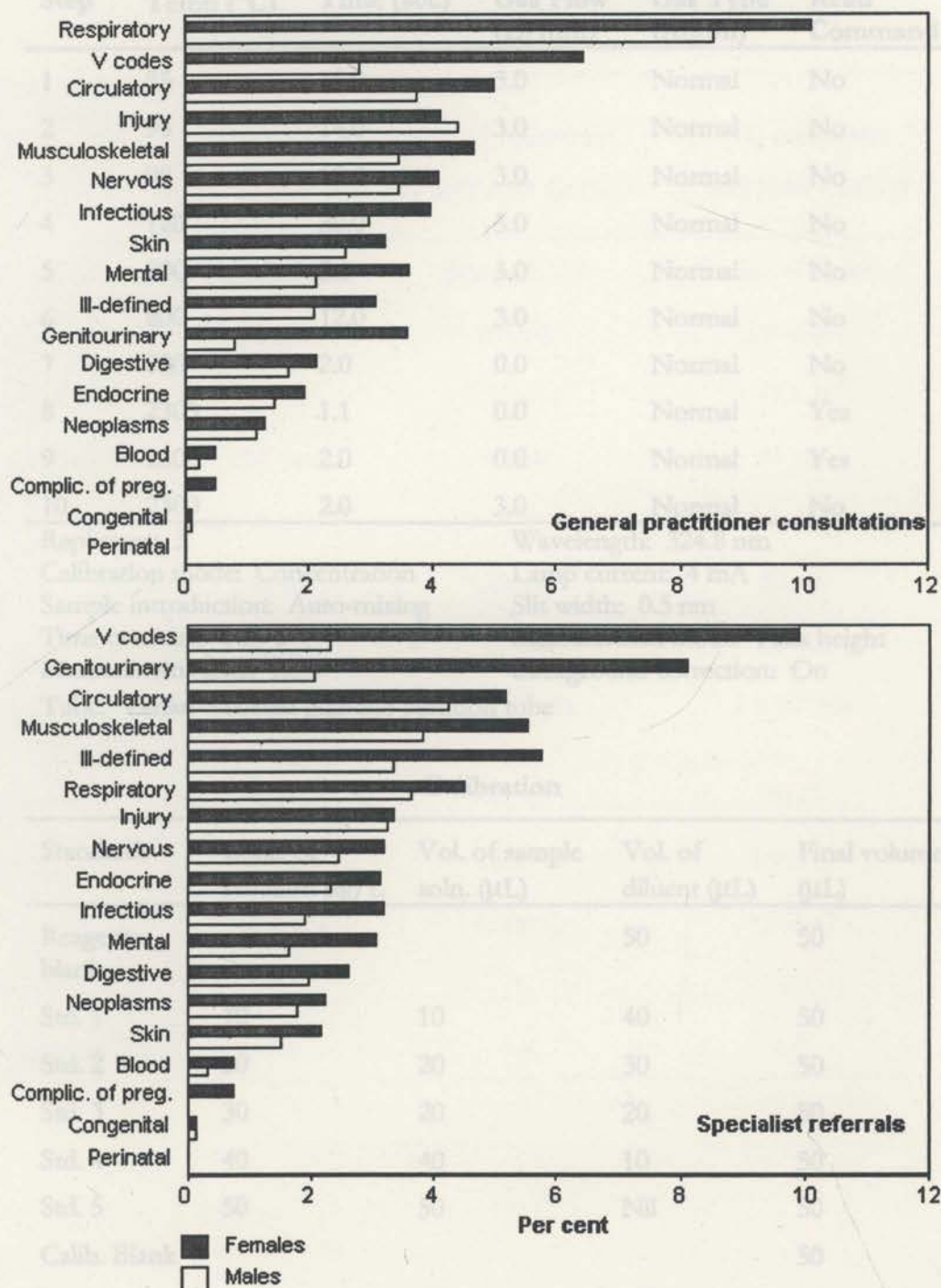


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APPENDIX 2.

GENERAL PRACTITIONER & SPECIALIST REFERRALS; AUSTRALIA

1989-1990.¹



(1) Muscular skeletal diseases rank forth and fifth as the main reasons for patient consultations with specialist and general practitioners , respectively. Appendix Figure 2 adapted from the Federal Department of Health and Family Services webpage: <http://www.aihw.gov.au/health/ahch5.html> 1998.

APPENDIX 3.

GRAPHITE FURNACE AAS PARAMETERS.

Furnace Time/Temperature Program					
Step	Temp (°C)	Time (sec)	Gas Flow (L/min)	Gas Type (Argon)	Read Command
1	85	15.0	3.0	Normal	No
2	95	14.0	3.0	Normal	No
3	98	10.0	3.0	Normal	No
4	120	30.0	3.0	Normal	No
5	800	5.0	3.0	Normal	No
6	800	12.0	3.0	Normal	No
7	800	2.0	0.0	Normal	No
8	2300	1.1	0.0	Normal	Yes
9	2300	2.0	0.0	Normal	Yes
10	2300	2.0	3.0	Normal	No

Replicates: 3

Calibration mode: Concentration

Sample introduction: Auto-mixing

Time constant: 0.05 s

Measurement time: 1.0 s

Tube: Varian™ coated graphite partition tube

Wavelength: 324.8 nm

Lamp current: 4 mA

Slit width: 0.5 nm

Measurement mode: Peak height

Background correction: On

Calibration				
Standards	Conc. of standard µg/L	Vol. of sample soln. (µL)	Vol. of diluent (µL)	Final volume (µL)
Reagent blank	-		50	50
Std. 1	10	10	40	50
Std. 2	20	20	30	50
Std. 3	30	20	20	50
Std. 4	40	40	10	50
Std. 5	50	50	Nil	50
Calib. Blank				50
Samples	-	50	Nil	50

Std. = Standard.

APPENDIX 4.

X-BAND EPR INSTRUMENT PARAMETERS.

Magnet and Receiver Parameters

Parameter	Cu(II) dimer	Cu(II) monomer (> 0.47 mM Cu(II))	Cu(II) monomer (< 0.47 mM Cu(II))
Spectrometer	Bruker EMX	Bruker EMX	Bruker ESP 300
Power	20 mW	20 mW	20 mW
Receiver gain	1×10^3	1.78×10^2	1.49×10^3
Signal channel conversion time	327 ms	41 ms	41 ms
Time constant	81 ms	20 ms	20 ms
Sweep time	335 ms	42 ms	42 ms
Modulation frequency	1×10^3 Hz	1×10^3 Hz	1×10^3 Hz
Amplitude	10 G	10 G	10 G
Sweep width	10,000 G	1000 G	1000 G
Centre field	5,000 G	3,000 G	3,000 G
No. of scans	1	1	10
Temperature	RT	4 K	4 K

APPENDIX 5.

XAFS ANALYSIS.

A5.1 Introduction

XAFS yields information on the number and type of atoms in the immediate environment of a specific absorbing metal atom and accurate absorber-neighbour distances;^{1,2} typically ± 0.02 Å for bond lengths of back-scattering atoms up to ~ 4.5 Å in distance from the absorbing metal centre.³ XAFS spectra are recorded following irradiation of the sample with high-energy monochromatic radiation from a synchrotron radiation source – with the emitted synchrotron radiation polarised and accelerated in a ring to near the speed of light by a series of magnets.⁴ The monochromatic X-ray beam is generated when the X-ray beam is passed through a monochromator.

At a metal-specific energy (e.g., ~ 9000 eV for Cu), sufficient energy is absorbed by the metal atom to promote a core electron in the metal centre to an unoccupied valence level.⁴ On promotion of the core electron, there is an abrupt increase in measured absorption, i.e., in the intensity of the incidence beam after the sample compared with intensity of the beam before the sample, for the transmission XAFS mode.² This increase in absorption is known as the XANES (X-ray absorption near-edge structure) and usually extends over 25 to 50 eV. The edge is where there is sufficient energy to eject the electron to the continuum and leads to XAFS oscillations, which are observed up to ~ 1000 eV thereafter.⁴

The long-range oscillations observed over this 1000–eV region are due to the interference between the photoelectron wave propagating from the x-ray absorbing metal atom and the wave back-scattered by the neighbouring atoms.^{2,4} If the back-scattered wave returns to the origin, in or out-of-phase with the outgoing photoelectron, there is a corresponding increase or decrease in the measured absorption.⁵ Typically, x-ray absorption spectra show decreasing absorption with increasing photon energy.⁴

Single-scattering (SS) and multiple-scattering (MS) analyses of the XAFS oscillations provide information about the environment of the absorbing atom.³ SS analysis

accounts for the single-scattering effects from identical atoms at a distance R_{as} surrounding the absorber,⁶ i.e., scattering from only one atom before returning to the absorber, with the number of legs travelled by the photoelectron in the scattering pathway (n) equal to 2. MS analysis includes effects from several sets of scatterers,⁴ i.e., $n > 2$. SS and MS refinement programs are used to minimise the difference between an observed XAFS spectrum and a theoretical XAFS spectrum based upon a model of the absorber site to determine the coordination and geometry around the absorber.⁶⁻⁹

A5.2 XAFS Theory

XAFS (χ) refers specifically to the relative oscillations of the x-ray absorption coefficient (μ) on the high-energy side of a specific metal X-ray edge compared to the smooth background absorption coefficient (μ_0); normalized by the absorption coefficient (μ_0) that would be absorbed for the free atom (Equation A 5.1):^{2,4}

$$\chi = \frac{\mu - \mu_0}{\mu_0} \quad \text{A5.1}$$

Since $\mu_r \approx \mu_0$, χ may alternatively be defined as:

$$\chi = \frac{\mu - \mu_0}{\mu_0} = \frac{\Delta\mu}{\mu_0} \quad \text{A5.2}$$

Specifically, the XAFS results from the interference between an outgoing photoelectron wave from the absorbing metal atom with a wavelength, λ_e , as defined by the de Broglie equation:

$$\lambda_e = h/p_e \quad \text{A5.3}$$

where h is Plank's constant and p_e is the momentum of the photoelectron and the back-scattered waves from surrounding atoms.^{4,5} The energy of the generated photoelectron ($E - E_0$) is the difference between the X-ray photon energy (E), where $E > E_0$, and the binding energy of the core electron (E_0).^{4,10} The amplitude of the XAFS oscillations are increased when the back-scattered waves are in-phase with the emitted waves and

decreased when the waves are out-of-phase. A general expression for the XAFS is given as:^{3,4}

$$\chi(k) = \sum_s \frac{N_s A_s(k) S_0^2}{k R_{as}^2} \exp(-2R_{as}/\lambda(k)) \exp(-2k^2 \sigma_{as}^2) \cdot \sin(2k R_{as} + \phi_{as}(k)) \quad \text{A5.4}$$

where $\chi(k)$ is the modulation in the absorption coefficient above the edge,

$k = \sqrt{2m(E - E_0)}/\hbar = 2\pi/\lambda_e$; ($\hbar = h/2\pi$) is the photoelectron wavevector, N_s is the number of scatterers at a distance R_{as} , A_s is the back-scattering amplitude, S_0^2 is an inelastic loss term, λ is the photoelectron mean-free path, σ^2 is the mean-square deviation in R_{as} , ϕ_{as}^2 is the phase shift that the photoelectron wave undergoes in passing through the potentials of the absorbing and scattering atoms ($\phi_{as} = 2\pi(2R_{as}/\lambda_e)$), and the sum is taken over all shells of the scatterers.³ In practice, the above equation is simplified as follows:³

$$\chi(k) = \sum_s \frac{N_s A'_s(k) S_0^2}{k R_{as}^2} \exp(-2k^2 \sigma_{as}^2) \sin(2k R_{as} + \Phi_{as}(k)) \quad \text{A5.5}$$

where A'_s is the effective amplitude,³ S is a scale factor³ and ϕ_{as}^2 is the Debye-Waller factor arising from thermal vibrations and static disorder of the back-scattering atoms.¹ The SS resolution (ΔR) of the XAFS data is given as:³

$$\Delta R = \pi/2\Delta k \quad \text{A5.6}$$

where Δk is magnitude of the photoelectron wave vector analysed. (For XAFS data where $\Delta k = 12 \text{ \AA}^{-1}$, resolution of the refined bond distances will be limited to $\sim 0.13 \text{ \AA}$ for SS analyses). A representation of the emitted and back-scattered photoelectron waves that give rise to the XAFS is shown in Figure A5.1.

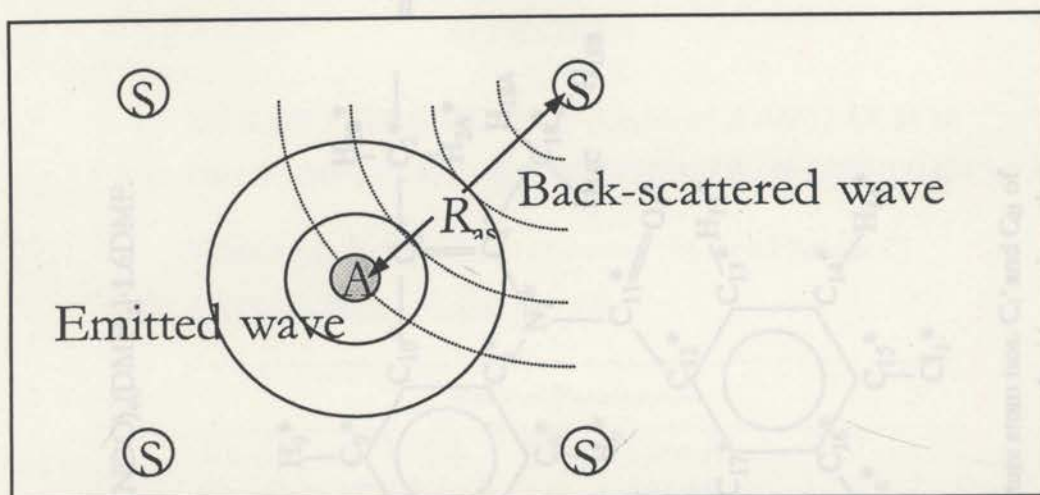


Figure A5.1. Emitted photoelectron waves (solid lines) propagate from absorber atom (A) to neighbouring scattering atom (S) separated by the distance R_{as} . The back-scattered waves (dotted line) modify the wave function at the absorber atom and give rise to XAFS.⁴

A5.3 References

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APPENDIX 6.

ATOMIC NUMBERING SYSTEM OF THE INDO MOIETIES OF $[\text{Cu}_2(\text{INDO})_4(\text{DMF})_2] \cdot 1.6\text{DMF}$.

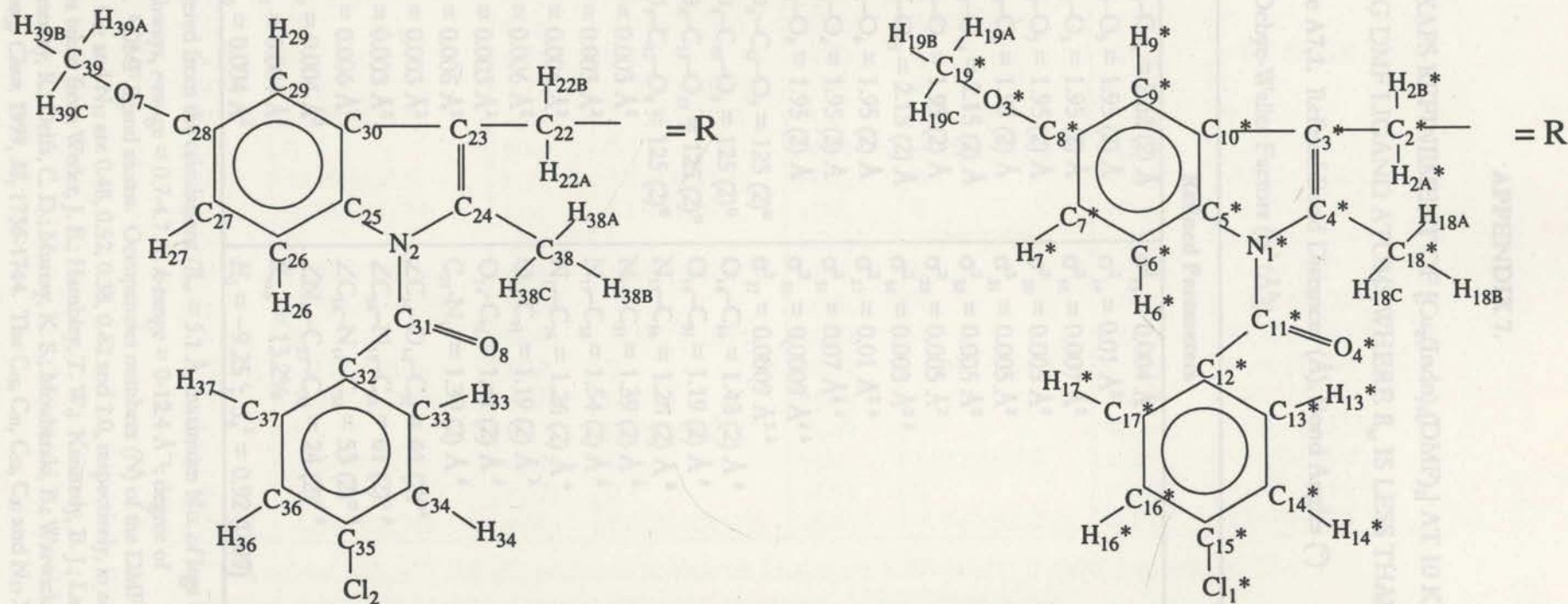


Figure A6.1. Figures of the atomic numbering system for the Indo moieties attached to crystal structure atom nos. C_1^* and C_{21} of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2] \cdot 1.6\text{DMF}$. Asterisks depict atom labeling assuming an inverted point of symmetry centered midway along the $\text{Cu} \cdots \text{Cu}$ vector (Figure 2.4). Taken from Weder, J. E., Hambley, T. W., Kennedy, B. J., Lay, P. A., MacLachlan, D., Bramley, R., Delfs, C. D., Murray, K. S., Moubaraki, B., Warwick, B., Biffin, J. R., Regtop, H. L. *Inorg. Chem.* 1999, 38, 1736-1744.

APPENDIX 7.

MS XAFS REFINEMENT OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ AT 10 K
INCLUDING DMF LIGAND ATOMS WHERE R_{as} IS LESS THAN 5.1 Å.

Table A7.1. Refined Bond Distances (Å), Bond Angles (°)
and Debye-Waller Factors (σ^2 (Å²)).^a

Refined Parameters	
$\text{Cu}_0\text{--Cu}_1 = 2.62$ (2) Å	$\sigma_{13}^2 = 0.004$ Å ²
$\text{Cu}_0\text{--O}_2 = 1.95$ (2) Å	$\sigma_{14}^2 = 0.01$ Å ²
$\text{Cu}_0\text{--O}_5 = 1.95$ (2) Å	$\sigma_{15}^2 = 0.007$ Å ²
$\text{Cu}_0\text{--O}_7 = 1.95$ (2) Å	$\sigma_{20}^2 = 0.005$ Å ²
$\text{Cu}_0\text{--O}_8 = 1.95$ (2) Å	$\sigma_{21}^2 = 0.005$ Å ²
$\text{Cu}_0\text{--O}_{14} = 2.15$ (2) Å	$\sigma_{22}^2 = 0.005$ Å ²
$\text{Cu}_1\text{--O}_3 = 1.95$ (2) Å	$\sigma_{23}^2 = 0.005$ Å ²
$\text{Cu}_1\text{--O}_{15} = 2.15$ (2) Å	$\sigma_{16}^2 = 0.003$ Å ^{2 b}
$\text{Cu}_1\text{--O}_6 = 1.95$ (2) Å	$\sigma_{17}^2 = 0.01$ Å ^{2 b}
$\text{Cu}_1\text{--O}_4 = 1.95$ (2) Å	$\sigma_{31}^2 = 0.07$ Å ^{2 b}
$\text{Cu}_1\text{--O}_9 = 1.95$ (2) Å	$\sigma_{32}^2 = 0.0009$ Å ^{2 b}
$\angle \text{O}_2\text{--C}_{11}\text{--O}_3 = 125$ (2)°	$\sigma_{27}^2 = 0.0009$ Å ^{2 b}
$\angle \text{O}_4\text{--C}_{10}\text{--O}_5 = 125$ (2)°	$\text{O}_{14}\text{--C}_{16} = 1.43$ (2) Å ^b
$\angle \text{O}_6\text{--C}_{13}\text{--O}_{13} = 125$ (2)°	$\text{O}_{14}\text{--C}_{31} = 1.19$ (2) Å ^b
$\angle \text{O}_9\text{--C}_{12}\text{--O}_8 = 125$ (2)°	$\text{N}_{17}\text{--C}_{16} = 1.26$ (2) Å ^b
$\sigma_1^2 = 0.003$ Å ²	$\text{N}_{17}\text{--C}_{31} = 1.39$ (2) Å ^b
$\sigma_2^2 = 0.003$ Å ²	$\text{N}_{17}\text{--C}_{32} = 1.54$ (2) Å ^b
$\sigma_3^2 = 0.006$ Å ²	$\text{N}_{17}\text{--C}_{16} = 1.26$ (2) Å ^b
$\sigma_4^2 = 0.006$ Å ²	$\text{O}_{14}\text{--C}_{31} = 1.19$ (2) Å ^b
$\sigma_5^2 = 0.003$ Å ²	$\text{O}_{14}\text{--C}_{16} = 1.41$ (2) Å ^b
$\sigma_6^2 = 0.006$ Å ²	$\text{C}_{31}\text{--N}_{17} = 1.39$ (2) Å ^b
$\sigma_7^2 = 0.003$ Å ²	$\angle \text{C}_{16}\text{--O}_{14}\text{--C}_{31} = 61$ (2)° ^{ob}
$\sigma_8^2 = 0.003$ Å ²	$\angle \text{C}_{16}\text{--N}_{17}\text{--C}_{31} = 61$ (2)° ^{ob}
$\sigma_9^2 = 0.006$ Å ²	$\angle \text{C}_{16}\text{--N}_{17}\text{--C}_{32} = 53$ (2)° ^{ob}
$\sigma_{10}^2 = 0.004$ Å ²	$\angle \text{N}_{17}\text{--C}_{27}\text{--C}_{32} = 24$ (2)° ^{ob}
$\sigma_{11}^2 = 0.004$ Å ²	$R_{\text{xafs}} = 13.2\%$
$\sigma_{12}^2 = 0.004$ Å ²	$E_0 = -9.25$ eV; $S_0^2 = 0.92$ (0.09)

^a No paths were filtered from the calculations ($R_{\text{as}} = 5.1$ Å, maximum No. of legs = 6; 262-unique-MS pathways, r -range = 0.7–4.7 Å; k -range = 0–12.4 Å⁻¹; degree of determinacy = 1.2). ^b DMF ligand atoms. Occupancies numbers (N) of the DMF XAFS model atoms C₁₆, C₃₁, C₃₂, C₂₇ and N₁₇ are 0.48, 0.52, 0.38, 0.62 and 1.0, respectively, in accordance with crystallographic data taken from Weder, J. E.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A.; MacLachlan, D.; Bramley, R.; Delfs, C. D.; Murray, K. S.; Moubaraki, B.; Warwick, B.; Biffin, J. R.; Regtop, H. L. *Inorg. Chem.* **1999**, *38*, 1736–1744. The C₁₆, C₃₁, C₃₂, C₂₇ and N₁₇ XAFS model atoms represent C₄₁, C_{41'}, C₄₂, C_{42'} and N₃, respectively, in the crystallographic data ^c E_0 is relative to the conventional energy edge of 8980.3 eV.

Table A7.2. Refined Positional Coordinates (x , y , z) of the 262-unique-MS-pathway Model incorporating the DMF ligand atoms where R_{as} is less than 5.1 Å.^a

x(1)	1.302 (0.021)	z(6)	-1.938 (0.018)	y(12)	0	x(22)	0
y(1)	0	x(7)	-1.118 (0.015)	z(12)	2.518 (0.027)	y(22)	0
z(1)	0	y(7)	0	x(13)	0	z(22)	4.017 (0.033)
x(2)	-1.118 (0.015)	z(7)	-1.938 (0.0179)	y(13)	0	x(23)	0
y(2)	1.938 (0.018)	x(8)	-1.118 (0.015)	z(13)	-2.518 (0.027)	y(23)	0
z(2)	0	y(8)	0	x(14)	-3.459 (0.146)	z(23)	-4.017 (0.033)
x(3)	1.118 (0.015)	z(8)	1.938 (0.0179)	y(14)	0	x(16) ^b	-3.912 (1.203)
y(3)	1.938 (0.0179)	x(9)	1.118 (0.015)	z(14)	0	y(16) ^b	1.115 (28.330)
z(3)	0	y(9)	0	x(15)	3.459 (0.146)	z(16) ^b	0.745 (42.367)
x(4)	1.118 (0.015)	z(9)	1.938 (0.018)	y(15)	0	x(17) ^b	-5.169 (3.255)
y(4)	-1.938 (0.0179)	x(10)	0	z(15)	0	y(17) ^b	1.288 (28.336)
z(4)	0	y(10)	-2.518 (0.027)	x(20)	0	z(17) ^b	0.752 (47.034)
x(5)	-1.118 (0.015)	z(10)	0	y(20)	-4.017 (0.033)	x(24) ^b	-4.63 (8.232)
y(5)	-1.938 (0.018)	x(11)	0	z(20)	0	y(24) ^b	0.237 (42.607)
z(5)	0	y(11)	2.518 (0.027)	x(21)	0	z(24) ^b	0.007 (64.185)
x(6)	1.118 (0.015)	z(11)	0	y(21)	4.017 (0.033)	x(25) ^b	-4.130 (5.611)
y(6)	0	x(12)	0	z(21)	0	y(25) ^b	2.027 (34.949)
x(26) ^b	-3.810 (32.945)	y(26) ^b	3.0135 (21.171)	z(26) ^b	-1.987 (58.675)	z(25) ^b	1.6169 (47.877)

^a The refined $x(0)$, $y(0)$ and $z(0)$ positional parameters for Cu₀ are 1.304 (0.021), 0 and 0, respectively. The number in brackets shows the error in the refined parameter. ^b DMF ligand atoms.

Table A7.2. Bond Distance (Å) and Angle (°) Restraints of the DMF Ligand Atoms less than 5.1 Å from Cu₀ incorporated into the 262-unique-MS pathway Model:^a Including the Occupancy Number (N) of the DMF Ligand Atoms.^a

Crystal Structure Atom	XAFS Model Atom Number (N)	Crystal Structure Parameter (Å)	Crystal Structure Parameter (°)	Distance from Cu ₀ (Å)
		$O_{14}-C_{16} = 1.40 (0.1) \text{ Å}$	$\angle C_{16}-N_{17}-C_{31} = 61 (5)^\circ$	
		$O_{14}-C_{31} = 1.19 (0.1) \text{ Å}$	$\angle N_{17}-C_{27}-C_{32} = 24 (5)^\circ$	
Cu ₀	1	$N_{17}-C_{16} = 1.262 (0.1) \text{ Å}$	$\angle C_{32}-C_{17}-C_{16} = 53 (5)^\circ$	
Cu ₁	1	$C_{31}-N_{17} = 1.39 (0.1) \text{ Å}$	$N(17) = 1.0 (0.1)^b$	10.59
Cu ₂	2	$N_{17}-C_{32} = 1.541 (0.1) \text{ Å}$	$N(16) = 0.48 (0.1)^b$	1.96
Cu ₃	11	$N_{17}-C_{16} = 1.262 (0.1) \text{ Å}$	$N(31) = 0.52 (0.1)^b$	3.11
Cu ₄	24	$C_{27}-C_{32} = 3.75 (0.1) \text{ Å}$	$N(32) = 0.38 (0.1)^b$	2.97
Cu ₅	31	$C_{27}-N_{17} = 3.51 (0.1) \text{ Å}$	$N(27) = 0.62 (0.1)^b$	4.25
Cu ₆	34	$\angle C_{31}-C_{16}-O_{14} = 62 (5)^\circ$		4.73
Cu ₇	35			4.73
Cu ₈	36			5.09
Cu ₉	37			6.11
Cu ₁₀	38			5.51
Cu ₁₁	39			6.82
Cu ₁₂	40			6.28
Cu ₁₃	41			6.13
Cu ₁₄	42			6.74
Cu ₁₅	43			3.81
Cu ₁₆	44			3.58
Cu ₁₇	45			7.21
Cu ₁₈	46			7.91
Cu ₁₉	47			7.79
Cu ₂₀	48			9.12
Cu ₂₁	49			9.83
Cu ₂₂	50			9.32
Cu ₂₃	51			8.02
Cu ₂₄	52			7.11
Cu ₂₅	53			5.87

^a Taken from Weder, J. E.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A.; MacLachlan, D.; Bramley, R.; Delfs, C. D.; Murray, K. S.; Moubaraki, B.; Warwick, B.; Biffin, J. R.; Regtop, H. L. *Inorg. Chem.* 1999, 38, 1736-1744. Number in brackets show the degree of freedom of the restraint. ^b Occupancy number of the DMF ligand atoms.

APPENDIX 8.

CRYSTAL STRUCTURE¹ AND MS XAFS NUMBERING OF [Cu₂(Indo)₄(DMF)₂].

Table A8.1. Crystal structure atom, corresponding XAFS model atom number (N), distance (Å) from XAFS Cu absorber (Cu₀) and positional parameters (x, y, z) of the crystal structure atoms ≤ 5.5 Å from Cu₀.

Crystal Structure Atom	XAFS Model Atom Number (N)	Crystal Structure Positional Parameters (x)	Crystal Structure Positional Parameter (y)	Crystal Structure Positional Parameter (z)	Distance from Cu ₀ (Å)
Cu ₀	0	-1.32	0	0	-
Cu ₁	1	1.32	0	0	-
Cl ₁	-	-	-	-	10.59
O ₁	2	-1.11	1.95	0	1.96
O ₂	3	1.12	1.95	0.03	3.11
C ₁	11	0.029	2.54	0.05	2.87
C ₂	20	-0.017	-4.03	-0.15	4.25
C ₃	24	-0.67	4.47	1.41	4.73
C ₄	-	-0.03	0.07	-4.04	5.44
N ₁	-	-	-	-	6.11
C ₅	-	-	-	-	5.93
C ₆	-	-	-	-	6.82
C ₇	-	-	-	-	6.88
C ₈	-	-	-	-	6.13
O ₃	-	-	-	-	6.74
C ₉	-	1.06378	0.90	-4.65	5.21
C ₁₀	-	2.13463	0.42	-5.32164	5.08
C ₁₁	-	-	-	-	7.21
O ₄	-	-	-	-	7.91
C ₁₂	-	-	-	-	7.73
C ₁₃	-	-	-	-	9.12
C ₁₄	-	-	-	-	9.83
C ₁₅	-	-	-	-	9.32
C ₁₆	-	-	-	-	8.02
C ₁₇	-	-	-	-	7.11
C ₁₈	-	-	-	-	5.87

Table A8.1. (cont.)

Crystal Structure Atom	XAFS Model Atom Number (<i>N</i>)	Crystal Structure Positional Parameter (<i>x</i>)	Crystal Structure Positional Parameter (<i>y</i>)	Crystal Structure Positional Parameter (<i>z</i>)	Distance from Cu ₀ (Å)
C ₁₉	-	-	-	-	6.48
C ₁₂	-	-	-	-	13.09
O ₅	6	1.11	0.016	-1.96	3.12
O ₆	7	-1.12	0.018	-1.95	1.96
C ₂₁	13	-0.007	0.04	-2.51	2.83
C ₂₂	23	-0.025	0.07	-4.04	4.24
C ₂₃	-	1.06	0.90	-4.65	5.296
C ₂₄	-	-	-	-	6.36
N ₂	-	-	-	-	7.29
C ₂₅	-	-	-	-	6.90
C ₂₆	-	-	-	-	7.74
C ₂₇	-	-	-	-	7.52
C ₂₈	-	-	-	-	6.43
O ₇	-	-	-	-	6.56
C ₂₉	-	0.32	3.29	-3.97	5.41
C ₃₀	-	-	-	-	5.68
C ₃₁	-	-	-	-	8.54
O ₈	-	-	-	-	9.095
C ₃₂	-	-	-	-	10.98
^b C _{33x}	30	-4.33	-2.06	2.46	4.41
C ₃₃	-	-	-	-	10.64
^b C _{34x}	25	-3.94	-2.89	1.46	4.17
C ₃₄	-	-	-	-	11.20
C ₃₅	-	-	-	-	12.08
C ₃₆	-	-	-	-	12.40
C ₃₇	-	-	-	-	11.88

Table A8.1. (cont.)

Crystal Structure Atom	XAFS Model Atom Number(N)	Crystal Structure Positional Parameter (x)	Crystal Structure Positional Parameter (y)	Crystal Structure Positional Parameter (z)	Distance from Cu ₀ (Å)
C ₃₈	-	-	-	-	6.77
C ₃₉	-	-	-	-	7.81
O ₉	14	-3.94	0.08	-0.05	2.14
^c C ₄₁	16	-3.92	1.13	0.76	2.94
^c C _{41'}	31	-4.63	0.25	0.02	3.33
N ₃	17	-5.17	1.295	0.76	4.14
^c C ₄₂	32	-4.14	2.05	1.62	3.85
^d C ₄₃	34	-6.55	1.42	0.95	5.51
^b C _{43x}	26	-3.34	2.13	-2.86	4.19
^c C _{42'}	27	-3.82	3.02	-1.99	4.398
^c C _{43'}	33	-5.78	2.50	1.46	5.33
O ₁₁	-	-	-	-	13.88
C ₄₄	-	-	-	-	13.66
N ₄	-	-	-	-	15.16
C _{45'}	-	-	-	-	15.14
C ₄₆	-	-	-	-	12.87
C ₄₇	-	-	-	-	14.12
Cu _{1a}	1	1.32	0	0	2.63
Cl _{1a}	-	-	-	-	12.07
O _{1a}	4	1.11	-1.95	0	3.11
O _{2a}	5	-1.12	-1.94	-0.029	1.95
C _{1a}	10	-0.03	-2.54	-0.05	2.84
C _{2a}	21	0.02	4.03	0.15	4.24
C _{3a}	-	0.67	-4.47	-1.41	5.09
C _{4a}	-	-	-	-	6.26
N _{1a}	-	-	-	-	6.98

Table A8.1. (cont.)

Crystal Structure Atom	XAFS Model Atom Number (<i>N</i>)	Crystal Structure Positional Parameter (<i>x</i>)	Crystal Structure Positional Parameter (<i>y</i>)	Crystal Structure Positional Parameter (<i>z</i>)	Distance from Cu ₀ (Å)
C _{5a}	-	-	-	-	6.42
C _{6a}	-	-	-	-	7.17
C _{7a}	-	-	-	-	6.81
C _{8a}	-	-	-	-	5.65
O _{3a}	-	-	-	-	5.85
^d C _{9a}	28	-0.92	-3.46	-3.19	4.72
C _{10a}	-	0.24	-4.14	-2.74	5.20
C _{11a}	-	-	-	-	8.30
O _{4a}	-	-	-	-	9.03
C _{12a}	-	-	-	-	8.97
C _{13a}	-	-	-	-	10.36
C _{14a}	-	-	-	-	11.17
C _{15a}	-	-	-	-	10.75
C _{16a}	-	-	-	-	9.484
C _{17a}	-	-	-	-	8.49
C _{18a}	-	-	-	-	6.99
C _{19a}	-	-3.08	-2.08	-4.28	5.08
Cl _{2a}	-	-	-	-	13.92
O _{5a}	8	-1.11	-0.02	1.96	1.97
O _{6a}	9	1.12	-0.02	1.95	3.12
C _{21a}	12	0.007	-0.02	2.51	2.84
C _{22a}	22	0.025	-0.07	4.04	4.26
^e C _{23a}	29	-1.06	-0.90	4.65	4.74
C _{24a}	-	-2.14	0.42	5.32	5.40
N _{2a}	-	-	-	-	6.14
C _{25a}	-	-	-	-	5.96

Table A8.1. (cont.)

Crystal Structure Atom	XAFS Model Atom Number (<i>N</i>)	Crystal Structure Positional Parameter (<i>x</i>)	Crystal Structure Positional Parameter (<i>y</i>)	Crystal Structure Positional Parameter (<i>z</i>)	Distance from Cu ₀ (Å)
C _{26a}	-	-	-	-	6.78
C _{27a}	-	-	-	-	6.85
C _{28a}	-	-	-	-	6.15
O _{7a}	-	-	-	-	6.63
C _{29a}	-	-0.32	-3.29	3.97	5.25
C _{30a}	-	-0.32	-2.34	4.56	5.13
C _{31a}	-	-	-	-	7.14
O _{8a}	-	-	-	-	7.54
C _{32a}	-	-	-	-	12.23
C _{33x}	-	-	-	-	6.498
C _{33a}	-	-	-	-	11.63
C _{34x}	-	-	-	-	6.18
C _{34a}	-	-	-	-	12.07
C _{35a}	-	-	-	-	13.07
C _{36a}	-	-	-	-	13.63
C _{37a}	-	-	-	-	13.24
C _{38a}	-	-	-	-	5.68
C _{39a}	-	-	-	-	7.75
O _{9a}	15	3.46	-0.08	0.05	4.77
C _{41a}	18	3.92	-1.13	-0.76	5.41
C _{41'}	-	-	-	-	5.95
N _{3a}	19	5.17	-1.295	-0.76	6.66
C _{42a}	-	-	-	-	6.05
C _{43a}	-	-	-	-	8.05
C _{43x}	-	-	-	-	6.01
C _{42'}	-	-	-	-	6.28

Table A8.1. (cont.)

Crystal Structure Atom	XAFS Model Atom Number(N)	Crystal Structure Positional Parameter (x)	Crystal Structure Positional Parameter (y)	Crystal Structure Positional Parameter (z)	Distance from Cu ₀ (Å)
C ₄₃ ^a	-	-	-	-	7.67
O _{11a}	-	-	-	-	15.53
C _{44a}	-	-	-	-	15.25
N _{4a}	-	-	-	-	16.695
C _{45a}	-	-	-	-	16.88
C _{46a}	O ₃ - O ₄	-	- 4	1.95 - 100.00%	14.44
C _{47a}	O ₁₁ - O ₆	-	- 1	2.16 - 13.60%	15.78

^a β -carbon atom of the indole ring of Indo, attached to XAFS model atom no. 11 (XAFS model atom no. 11 = crystal structure atom label C₁). ^b C atoms from neighbouring dimers. ^c Positional parameters for the disordered atoms of the axial DMF molecule closest to Cu₀. ^d C atom from a neighbouring dimer. ^e β -carbon atom of the indole ring of Indo, attached to XAFS model atom no. 12 (XAFS model atom no. 12 = crystal structure atom label C_{21a}). ^f Carbon atom of the aromatic ring of Indo, attached to XAFS model atom no. 10 (XAFS model atom no. 10 = crystal structure atom label C_{1a}).

The "a" annotation depicts atom labelling of the Indo group assuming an inverted point of symmetry centred midway along the Cu...Cu vector (Chapter 2; Figure 2.4). The positional parameters (x, y, z) for the XAFS model atom number are, therefore, equal and opposite in values according to Figure 2.4, e.g., the positional parameters (x, y, z) for the XAFS model atom number 2 (crystal structure atom O₁) are equal and opposite in values to those of XAFS model atom number 4 (crystal structure atom O_{1a}). The deviation of the atoms from the orthogonal plane of the Cu₂O₁₀ core was restrained in the MS XAFS models to 0.1 Å to reflect such deviations in the positional parameters of the crystal structure of [Cu₂(Indo)₄(DMF)₂],¹ e.g., the value of the crystal structure (z) positional parameter is 0.029 rather than 0 due to deviation from the orthogonal plane.

- (1) Weder, J. E.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A.; MacLachlan, D.; Bramley, R.; Delfs, C. D.; Murray, K. S.; Moubaraki, B.; Warwick, B.; Biffin, J. R.; Regtop, H. L. *Inorg. Chem.* **1999**, *38*, 1736-1744.

APPENDIX 9.

MULTIPLE SCATTERING PATHS.

Table A9.1. MS Paths and Importance Factors of the MS Model with a Maximum of 5 Legs per MS Pathway and $R_{\text{eff}} \leq 10.0 \text{ \AA}$ for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ excluding All Atoms from DMF Except the Oxygen Ligand.

ATOMS IN MS PATHWAY ^a	No. of legs ^b	Degeneracy	R_{eff} ^c ($\text{\AA}$)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	2	4	1.95	100.00%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	2	1	2.16	15.62%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	2	1	2.62	8.09%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$	2	4	2.84	38.90%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	8	3.03	45.79%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{Cu}_0$	2	4	3.11	21.30%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	4	3.21	15.44%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	8	3.32	7.54%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	8	3.58	4.60%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$	3	8	3.61	10.43%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	3	8	3.65	8.56%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	3.79	3.37%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	8	3.84	4.10%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	4	3.89	13.29%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	4	3.90	3.78%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	4	3.90	5.08%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	3	16	4.08	8.27%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0$	4	4	4.10	11.80%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{Cu}_0$	4	4	4.10	1.85%
$\text{Cu}_0 \rightarrow \text{C}_{10} \rightarrow \text{O}_4 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	4.14	3.25%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	8	4.15	1.67%

Table A9.1. (cont.)

ATOMS IN MS PATHWAY ^a	No. of legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{O}_3 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	4	4.19	1.42%
$\text{Cu}_0 \rightarrow \text{C}_{21} \rightarrow \text{Cu}_0$	2	4	4.23	12.77%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	16	4.27	4.42%
$\text{Cu}_0 \rightarrow \text{C}_{20} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	8	4.27	23.90%
$\text{Cu}_0 \rightarrow \text{C}_{23} \rightarrow \text{C}_{13} \rightarrow \text{Cu}_0$	3	8	4.28	23.69%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	8	4.29	2.32%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	8	4.29	20.04%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	16	4.30	4.61%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{22} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	4	4.31	10.02%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{C}_{23} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	4.33	19.15%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.33	1.18%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{C}_{23} \rightarrow \text{C}_{13} \rightarrow \text{Cu}_0$	4	4	4.34	11.10%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{O}_3 \rightarrow \text{Cu}_0$	4	4	4.37	2.17%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	16	4.40	3.84%
$\text{Cu}_0 \rightarrow \text{C}_{21} \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	8	4.47	11.63%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{O}_3 \rightarrow \text{Cu}_0$	3	8	4.49	2.97%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{C}_{20} \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	5	8	4.51	14.08%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{C}_{21} \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.52	15.22%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	5	16	4.58	1.73%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.64	0.00%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	3	8	4.65	3.19%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	8	4.66	5.02%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0$	3	16	4.66	5.32%
$\text{Cu}_0 \rightarrow \text{C}_{10} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	8	4.69	9.62%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{O}_5 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	4.69	2.34%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{O}_2 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	4.69	5.58%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	4	8	4.73	8.21%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	8	4.77	4.81%

Table A9.1. (cont.)

ATOMS IN MS PATHWAY ^a	No. of legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	2	4.78	1.58%
$\text{Cu}_0 \rightarrow \text{O}_{15} \rightarrow \text{Cu}_0$	2	1	4.79	1.62%
$\text{Cu}_0 \rightarrow \text{O}_{15} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	2	4.79	1.55%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	8	4.79	4.43%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	8	4.83	2.95%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	8	4.84	4.72%
$\text{Cu}_0 \rightarrow \text{C}_{23} \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	3	8	4.85	2.86%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	5	8	4.87	4.04%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$	5	8	4.87	1.42%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	4.88	5.75%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{C}_{20} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	4.89	3.17%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{C}_{23} \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	4	8	4.91	4.81%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{11} \rightarrow \text{O}_3 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.91	2.13%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{O}_2 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.91	5.74%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	8	4.96	5.03%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.98	2.97%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.98	2.87%

^a Refer to Figure 2.4 for XAFS model numbering and coordination geometry. ^b The number of legs represents the path travelled by the photoelectron originating from and returning to the XAFS absorber atom Cu_0 . ^c The total distance travelled by the photoelectron R_{eff} is twice the value of R_{as} . ^d The importance factor represents the percent contribution of a path relative to the strongest MS path $\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$, e.g., the paths $\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$ and $\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$ are the next two strongest paths, representing 39% and 46% importance relative to path $\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$. It includes the contribution from the Debye-Waller factors.

Table A9.2. MS Paths and Importance Factors of the MS Model with a Maximum of 6 Legs per MS Pathway and $R_{\text{eff}} \leq 10.2 \text{ \AA}$ for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c ($\text{\AA}$)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	2	4	1.95	100.00%
$\text{Cu}_0 \rightarrow \text{C}_{14} \rightarrow \text{Cu}_0$	2	1	2.13	14.97%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	2	1	2.62	7.99%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0$	2	4	2.84	38.41%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	2	1	2.94	4.34%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	8	3.02	45.55%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{Cu}_0$	2	4	3.11	22.05%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	4	3.21	14.61%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	3	2	3.26	2.29%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	8	3.32	6.55%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	2	1	3.33	2.49%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	3	2	3.34	7.99%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	1	3.34	7.29%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	8	3.56	4.59%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	1	3.57	0.27%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0$	3	8	3.61	9.12%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	8	3.65	9.53%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	3.79	3.23%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	3	2	3.81	0.99%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	3.82	1.70%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	8	3.84	1.70%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	8	3.84	4.14%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$Cu_0 \rightarrow C_{25} \rightarrow Cu_0$	2	1	3.85	1.17%
$Cu_0 \rightarrow O_7 \rightarrow O_8 \rightarrow Cu_0$	3	4	3.89	14.06%
$Cu_0 \rightarrow O_2 \rightarrow Cu_0 \rightarrow O_5 \rightarrow Cu_0$	4	4	3.89	4.55%
$Cu_0 \rightarrow O_5 \rightarrow Cu_0 \rightarrow O_5 \rightarrow Cu_0$	4	4	3.89	4.19%
$Cu_0 \rightarrow O_5 \rightarrow Cu_0 \rightarrow O_8 \rightarrow Cu_0$	4	8	3.89	0.65%
$Cu_0 \rightarrow C_{16} \rightarrow O_2 \rightarrow Cu_0$	3	2	3.95	0.79%
$Cu_0 \rightarrow C_{25} \rightarrow C_{16} \rightarrow Cu_0$	3	2	4.03	1.26%
$Cu_0 \rightarrow C_{16} \rightarrow O_8 \rightarrow Cu_0$	3	2	4.07	0.57%
$Cu_0 \rightarrow C_{10} \rightarrow O_8 \rightarrow Cu_0$	3	16	4.08	7.04%
$Cu_0 \rightarrow O_{14} \rightarrow Cu_0 \rightarrow O_8 \rightarrow Cu_0$	4	8	4.09	0.88%
$Cu_0 \rightarrow C_{13} \rightarrow O_7 \rightarrow C_{13} \rightarrow Cu_0$	4	4	4.10	11.49%
$Cu_0 \rightarrow C_{10} \rightarrow O_4 \rightarrow C_{10} \rightarrow Cu_0$	4	4	4.10	1.31%
$Cu_0 \rightarrow C_{24} \rightarrow C_{16} \rightarrow O_{14} \rightarrow Cu_0$	4	2	4.13	0.68%
$Cu_0 \rightarrow O_{14} \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$	5	2	4.14	0.96%
$Cu_0 \rightarrow N_{17} \rightarrow Cu$	2	1	4.14	3.60%
$Cu_0 \rightarrow C_{13} \rightarrow O_6 \rightarrow O_7 \rightarrow Cu_0$	4	8	4.14	3.50%
$Cu_0 \rightarrow C_{10} \rightarrow Cu_1 \rightarrow Cu_0$	3	8	4.15	1.43%
$Cu_0 \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$	3	2	4.17	4.18%
$Cu_0 \rightarrow O_2 \rightarrow O_3 \rightarrow O_2 \rightarrow Cu_0$	4	4	4.19	1.22%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$	4	1	4.20	2.13%
$Cu_0 \rightarrow C_{16} \rightarrow C_{25} \rightarrow C_{16} \rightarrow Cu_0$	4	1	4.22	0.37%
$Cu_0 \rightarrow C_{22} \rightarrow Cu_0$	2	4	4.23	12.69%
$Cu_0 \rightarrow O_2 \rightarrow C_{11} \rightarrow O_7 \rightarrow Cu_0$	4	16	4.27	3.99%
$Cu_0 \rightarrow C_{21} \rightarrow O_2 \rightarrow Cu_0$	3	8	4.27	25.46%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$Cu_0 \rightarrow O_{14} \rightarrow C_{16} \rightarrow O_2 \rightarrow Cu_0$	4	2	4.27	0.15%
$Cu_0 \rightarrow O_{14} \rightarrow Cu_0 \rightarrow O_{14} \rightarrow Cu_0$	4	1	4.27	0.36%
$Cu_0 \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$	3	2	4.28	4.40%
$Cu_0 \rightarrow C_{22} \rightarrow C_{12} \rightarrow Cu_0$	3	8	4.28	26.73%
$Cu_0 \rightarrow C_{11} \rightarrow O_3 \rightarrow C_{11} \rightarrow O_2 \rightarrow Cu_0$	5	8	4.28	2.39%
$Cu_0 \rightarrow C_{13} \rightarrow O_7 \rightarrow C_{13} \rightarrow O_7 \rightarrow Cu_0$	5	8	4.28	18.92%
$Cu_0 \rightarrow C_{16} \rightarrow C_{24} \rightarrow C_{16} \rightarrow Cu_0$	4	1	4.29	0.14%
$Cu_0 \rightarrow O_3 \rightarrow O_8 \rightarrow Cu_0$	3	16	4.29	5.56%
$Cu_0 \rightarrow O_7 \rightarrow C_{23} \rightarrow O_7 \rightarrow Cu_0$	4	4	4.31	11.67%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$	4	2	4.31	2.27%
$Cu_0 \rightarrow C_{11} \rightarrow C_{21} \rightarrow O_2 \rightarrow Cu_0$	4	8	4.32	19.54%
$Cu_0 \rightarrow O_2 \rightarrow O_3 \rightarrow C_{11} \rightarrow O_2 \rightarrow Cu_0$	5	8	4.33	1.30%
$Cu_0 \rightarrow Cu_1 \rightarrow C_{10} \rightarrow O_5 \rightarrow Cu_0$	4	8	4.33	0.53%
$Cu_0 \rightarrow C_{11} \rightarrow O_3 \rightarrow Cu_1 \rightarrow Cu_0$	4	8	4.33	0.45%
$Cu_0 \rightarrow C_{11} \rightarrow C_{21} \rightarrow C_{11} \rightarrow Cu_0$	4	4	4.34	11.48%
$Cu_0 \rightarrow C_{25} \rightarrow O_{14} \rightarrow Cu_0$	3	2	4.34	0.52%
$Cu_0 \rightarrow C_{25} \rightarrow C_{16} \rightarrow O_{14} \rightarrow Cu_0$	4	2	4.35	0.62%
$Cu_0 \rightarrow O_4 \rightarrow C_{10} \rightarrow O_4 \rightarrow Cu_0$	4	4	4.37	2.62%
$Cu_0 \rightarrow Cu_1 \rightarrow O_3 \rightarrow O_2 \rightarrow Cu_0$	4	8	4.37	0.33%
$Cu_0 \rightarrow C_{16} \rightarrow O_{14} \rightarrow C_{16} \rightarrow Cu_0$	4	1	4.38	0.55%
$Cu_0 \rightarrow O_{14} \rightarrow C_{16} \rightarrow O_8 \rightarrow Cu_0$	4	2	4.38	0.13%
$Cu_0 \rightarrow C_{11} \rightarrow O_2 \rightarrow O_7 \rightarrow Cu_0$	4	16	4.39	3.11%
$Cu_0 \rightarrow O_{14} \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$	4	1	4.42	1.67%
$Cu_0 \rightarrow N_{17} \rightarrow C_{24} \rightarrow Cu_0$	3	2	4.423	1.15%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{ss} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.44	1.81%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	4	2	4.46	0.81%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	4	2	4.46	1.02%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.47	1.55%
$\text{Cu}_0 \rightarrow \text{C}_{21} \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	8	4.47	12.71%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	6	4	4.47	8.10%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	6	4	4.47	4.25%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	3	2	4.47	0.40%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.47	1.31%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{O}_3 \rightarrow \text{Cu}_0$	3	8	4.48	3.15%
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.49	1.30%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{C}_{20} \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	5	8	4.51	10.18%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	5	8	4.52	0.22%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.52	1.04%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{C}_{21} \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	8	4.52	10.63%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.53	0.39%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	1	4.54	0.94%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.54	0.62%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.54	3.62%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.55	3.26%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	2	4.56	0.50%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{O}_4 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	4	4	4.57	0.76%
$\text{Cu}_0 \rightarrow \text{C}_{10} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	3	8	4.57	0.14%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.57	0.91%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.58	1.72%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	16	4.58	1.35%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	2	4.59	0.31%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.59	0.22%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.61	0.26%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	3	2	4.62	0.37%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{C}_{10} \rightarrow \text{Cu}_0$	3	8	4.62	2.45%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	3	8	4.63	3.52%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.63	0.83%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	8	4.64	4.01%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	2	4.64	0.27%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	2	4.65	0.32%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	3	2	4.65	0.26%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0$	3	16	4.66	5.31%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	2	4.66	0.33%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.68	0.15%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	2	4.68	0.10%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	2	4.68	0.36%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	2	4.68	0.19%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	1	4.68	0.21%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{O}_7 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	4.69	1.96%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{O}_8 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	4.69	5.05%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.69	0.68%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	8	4.69	11.26%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{ss} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.69	0.41%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.69	0.51%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	2	4.71	0.22%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{C}_{23} \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	6	4	4.71	4.05%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	2	4.72	0.42%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	1	4.72	0.21%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{O}_9 \rightarrow \text{Cu}_0$	4	8	4.72	7.73%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.73	0.88%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	3	2	4.73	0.28%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.74	0.90%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	2	4.75	1.27%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	4	2	4.75	0.39%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.76	0.17%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	2	4.76	0.18%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	2	4.76	0.18%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	2	4.76	0.24%
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{25} \rightarrow \text{Cu}_0$	3	2	4.76	0.37%
$\text{Cu}_0 \rightarrow \text{O}_{15} \rightarrow \text{Cu}_0$	2	1	4.77	2.10%
$\text{Cu}_0 \rightarrow \text{O}_{15} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	3	2	4.77	2.54%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{O}_{15} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	4	1	4.77	1.02%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	5	2	4.77	0.52%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	2	4.77	0.31%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	5	2	4.77	0.27%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	2	4.77	0.12%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	3	8	4.77	4.74%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.78	0.37%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.78	0.41%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	2	4.78	0.65%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	2	4.79	0.14%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	2	4.79	0.54%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_1 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	4.79	1.61%
$\text{Cu}_0 \rightarrow \text{N}_{13} \rightarrow \text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	4.79	3.48%
$\text{Cu}_0 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	16	4.79	0.18%
$\text{Cu}_0 \rightarrow \text{C}_{10} \rightarrow \text{O}_4 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	16	4.79	1.25%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{N}_{17} \rightarrow \text{C}_{25} \rightarrow \text{Cu}_0$	4	2	4.79	0.57%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.80	0.18%
$\text{Cu}_0 \rightarrow \text{C}_{10} \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	16	4.80	1.41%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{C}_{10} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	8	4.81	2.21%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	3	2	4.82	0.26%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	8	4.82	3.68%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.82	0.11%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.83	0.27%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	5	2	4.83	0.08%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{O}_3 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	16	4.84	1.12%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{25} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	1	4.84	0.05%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.84	0.15%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	16	4.84	1.48%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{C}_{10} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	16	4.84	1.77%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.85	0.21%
$\text{Cu}_0 \rightarrow \text{C}_{22} \rightarrow \text{O}_9 \rightarrow \text{Cu}_0$	3	8	4.85	2.40%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.86	0.24%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	5	8	4.87	4.07%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{C}_{10} \rightarrow \text{O}_4 \rightarrow \text{C}_{10} \rightarrow \text{Cu}_0$	5	8	4.87	1.37%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	2	4.88	0.17%
$\text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	8	4.88	6.96%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	2	4.89	0.09%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	2	4.89	0.08%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	2	4.89	0.04%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	4.89	0.10%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{C}_{21} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	8	4.89	2.53%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{N}_{17} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	2	4.90	0.22%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	2	4.90	0.72%
$\text{Cu}_0 \rightarrow \text{C}_{13} \rightarrow \text{C}_{23} \rightarrow \text{O}_6 \rightarrow \text{Cu}_0$	4	8	4.91	3.57%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	8	4.91	2.04%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{O}_7 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	5	8	4.91	5.46%
$\text{Cu}_0 \rightarrow \text{C}_{11} \rightarrow \text{O}_2 \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	4	8	4.91	0.30%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{Cu}_1 \rightarrow \text{Cu}_0$	4	8	4.91	1.03%
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	4	2	4.91	0.35%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.91	0.73%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	2	4.92	0.14%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{O}_7 \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	4	16	4.93	0.55%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	4.93	0.09%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{ss} ^c (Å)	Importance Factor ^d
$Cu_0 \rightarrow C_{24} \rightarrow C_{16} \rightarrow O_8 \rightarrow Cu_0$	4	2	4.94	0.08%
$Cu_0 \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_{14} \rightarrow C_{16} \rightarrow Cu_0$	5	2	4.94	0.21%
$Cu_0 \rightarrow C_{16} \rightarrow C_{24} \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$	5	2	4.94	0.25%
$Cu_0 \rightarrow O_{14} \rightarrow C_{24} \rightarrow C_{16} \rightarrow O_8 \rightarrow Cu_0$	5	2	4.95	0.09%
$Cu_0 \rightarrow C_{16} \rightarrow C_{25} \rightarrow N_{17} \rightarrow Cu_0$	4	2	4.95	0.47%
$Cu_0 \rightarrow O_2 \rightarrow C_{16} \rightarrow O_2 \rightarrow Cu_0$	4	1	4.96	0.09%
$Cu_0 \rightarrow C_{10} \rightarrow O_5 \rightarrow O_2 \rightarrow Cu_0$	4	8	4.96	5.57%
$Cu_0 \rightarrow C_{10} \rightarrow O_5 \rightarrow Cu_0 \rightarrow O_2 \rightarrow Cu_0$	5	8	4.97	1.36%
$Cu_0 \rightarrow O_5 \rightarrow C_{10} \rightarrow Cu_0 \rightarrow O_2 \rightarrow Cu_0$	5	8	4.97	0.99%
$Cu_0 \rightarrow O_2 \rightarrow C_{11} \rightarrow Cu_0 \rightarrow O_2 \rightarrow Cu_0$	5	8	4.97	2.27%
$Cu_0 \rightarrow C_{12} \rightarrow O_8 \rightarrow Cu_0 \rightarrow O_5 \rightarrow Cu_0$	5	16	4.97	0.07%
$Cu_0 \rightarrow C_{13} \rightarrow O_7 \rightarrow Cu_0 \rightarrow O_7 \rightarrow Cu_0$	5	8	4.97	2.13%
$Cu_0 \rightarrow O_2 \rightarrow C_{11} \rightarrow Cu_0 \rightarrow O_7 \rightarrow Cu_0$	5	16	4.97	0.12%
$Cu_0 \rightarrow C_{13} \rightarrow Cu_0 \rightarrow O_{14} \rightarrow Cu_0$	4	8	4.98	0.56%
$Cu_0 \rightarrow O_6 \rightarrow O_3 \rightarrow C_{11} \rightarrow Cu_0$	4	16	4.98	1.15%
$Cu_0 \rightarrow O_2 \rightarrow C_{11} \rightarrow O_3 \rightarrow O_8 \rightarrow Cu_0$	5	16	4.98	0.62%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow C_{25} \rightarrow C_{16} \rightarrow Cu_0$	5	2	4.98	0.95%
$Cu_0 \rightarrow O_7 \rightarrow O_{14} \rightarrow O_8 \rightarrow Cu_0$	4	4	4.98	1.04%
$Cu_0 \rightarrow O_7 \rightarrow O_{14} \rightarrow O_7 \rightarrow Cu_0$	4	4	4.98	1.43%
$Cu_0 \rightarrow O_7 \rightarrow O_{14} \rightarrow O_5 \rightarrow Cu_0$	4	8	4.98	0.16%
$Cu_0 \rightarrow O_5 \rightarrow C_{10} \rightarrow C_{13} \rightarrow O_7 \rightarrow Cu_0$	5	8	4.99	0.53%
$Cu_0 \rightarrow O_{14} \rightarrow C_{16} \rightarrow O_{14} \rightarrow O_5 \rightarrow Cu_0$	5	2	4.99	0.15%
$Cu_0 \rightarrow O_{14} \rightarrow C_{16} \rightarrow O_{14} \rightarrow O_7 \rightarrow Cu_0$	5	2	4.99	0.09%
$Cu_0 \rightarrow O_2 \rightarrow O_{14} \rightarrow C_{16} \rightarrow O_{14} \rightarrow Cu_0$	5	2	4.99	0.02%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{ss} ^c (Å)	Importance Factor ^d
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	4.99	0.02%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$	3	2	5.00	0.12%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	2	5.00	0.23%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{25} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	5.01	0.18%
$\text{Cu}_0 \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{C}_{16} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	1	5.02	0.09%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{O}_7 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	16	5.02	0.88%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{O}_4 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	16	5.02	0.58%
$\text{Cu}_0 \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{C}_{16} \rightarrow \text{Cu}_0$	5	2	5.02	0.52%
$\text{Cu}_0 \rightarrow \text{Cu}_1 \rightarrow \text{O}_6 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	16	5.03	0.39%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{O}_2 \rightarrow \text{C}_{11} \rightarrow \text{Cu}_0$	4	2	5.03	0.13%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	6	2	5.03	1.01%
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{Cu}_0$	4	2	5.04	0.31%
$\text{Cu}_0 \rightarrow \text{C}_{25} \rightarrow \text{C}_{16} \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	2	5.05	0.14%
$\text{Cu}_0 \rightarrow \text{C}_{20} \rightarrow \text{C}_{10} \rightarrow \text{O}_4 \rightarrow \text{Cu}_0$	4	8	5.05	4.45%
$\text{Cu}_0 \rightarrow \text{N}_{17} \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	5.05	0.48%
$\text{Cu}_0 \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_6 \rightarrow \text{C}_{13} \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	6	8	5.05	2.32%
$\text{Cu}_0 \rightarrow \text{O}_9 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{C}_{12} \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	6	8	5.05	2.36%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{O}_3 \rightarrow \text{Cu}_0$	3	4	5.05	0.70%
$\text{Cu}_0 \rightarrow \text{O}_5 \rightarrow \text{Cu}_1 \rightarrow \text{O}_2 \rightarrow \text{Cu}_0$	4	4	5.05	0.34%
$\text{Cu}_0 \rightarrow \text{O}_8 \rightarrow \text{Cu}_1 \rightarrow \text{O}_8 \rightarrow \text{Cu}_0$	4	4	5.05	0.63%
$\text{Cu}_0 \rightarrow \text{O}_2 \rightarrow \text{Cu}_1 \rightarrow \text{O}_7 \rightarrow \text{Cu}_0$	4	8	5.05	0.05%
$\text{Cu}_0 \rightarrow \text{C}_{16} \rightarrow \text{C}_{24} \rightarrow \text{N}_{17} \rightarrow \text{O}_{14} \rightarrow \text{Cu}_0$	5	2	5.05	0.24%
$\text{Cu}_0 \rightarrow \text{O}_4 \rightarrow \text{Cu}_1 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	5.05	0.45%
$\text{Cu}_0 \rightarrow \text{O}_3 \rightarrow \text{Cu}_1 \rightarrow \text{O}_5 \rightarrow \text{Cu}_0$	4	8	5.05	0.54%

Table A9.2. (cont.)

Atoms in MS Pathway ^a	No. of Legs ^b	Degeneracy	R_{as} ^c (Å)	Importance Factor ^d
$Cu_0 \rightarrow O_4 \rightarrow Cu_1 \rightarrow O_7 \rightarrow Cu_0$	4	16	5.05	0.39%
$Cu_0 \rightarrow C_{25} \rightarrow N_{17} \rightarrow C_{24} \rightarrow Cu_0$	4	2	5.05	0.14%
$Cu_0 \rightarrow O_4 \rightarrow Cu_1 \rightarrow O_4 \rightarrow Cu_0$	4	4	5.06	0.31%
$Cu_0 \rightarrow O_6 \rightarrow Cu_1 \rightarrow O_9 \rightarrow Cu_0$	4	4	5.06	0.34%
$Cu_0 \rightarrow O_4 \rightarrow Cu_1 \rightarrow O_6 \rightarrow Cu_0$	4	8	5.06	0.48%
$Cu_0 \rightarrow O_4 \rightarrow Cu_0 \rightarrow O_2 \rightarrow Cu_0$	4	8	5.06	0.57%
$Cu_0 \rightarrow O_9 \rightarrow Cu_0 \rightarrow O_5 \rightarrow Cu_0$	4	16	5.06	0.14%
$Cu_0 \rightarrow O_4 \rightarrow Cu_0 \rightarrow O_5 \rightarrow Cu_0$	4	8	5.06	1.60%
$Cu_0 \rightarrow C_{25} \rightarrow O_7 \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$	5	2	5.06	0.15%
$Cu_0 \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_2 \rightarrow Cu_0$	4	2	5.07	0.10%
$Cu_0 \rightarrow C_{24} \rightarrow C_{16} \rightarrow N_{17} \rightarrow C_{16} \rightarrow Cu_0$	5	2	5.07	0.42%
$Cu_0 \rightarrow C_{16} \rightarrow Cu_0 \rightarrow O_{14} \rightarrow Cu_0$	4	2	5.08	0.22%
$Cu_0 \rightarrow O_2 \rightarrow C_{16} \rightarrow O_8 \rightarrow Cu_0$	4	2	5.08	0.06%
$Cu_0 \rightarrow C_{16} \rightarrow N_{17} \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$	6	2	5.08	0.50%
$Cu_0 \rightarrow C_{16} \rightarrow C_{25} \rightarrow N_{17} \rightarrow O_{14} \rightarrow Cu_0$	5	2	5.09	0.16%
$Cu_0 \rightarrow C_{16} \rightarrow C_{25} \rightarrow C_{16} \rightarrow C_{24} \rightarrow Cu_0$	5	2	5.09	0.63%
$Cu_0 \rightarrow O_9 \rightarrow C_{22} \rightarrow C_{12} \rightarrow O_8 \rightarrow Cu_0$	5	8	5.09	2.34%
$Cu_0 \rightarrow O_6 \rightarrow C_{13} \rightarrow C_{23} \rightarrow O_7 \rightarrow Cu_0$	5	8	5.09	4.64%
$Cu_0 \rightarrow Cu_1 \rightarrow O_8 \rightarrow C_{12} \rightarrow O_8 \rightarrow Cu_0$	5	8	5.09	0.24%
$Cu_0 \rightarrow O_9 \rightarrow C_{12} \rightarrow O_9 \rightarrow Cu_1 \rightarrow Cu_0$	5	8	5.09	0.94%
$Cu_0 \rightarrow C_{16} \rightarrow C_{25} \rightarrow C_{16} \rightarrow C_{24} \rightarrow O_{14} \rightarrow Cu_0$	6	2	5.09	0.58%

^a Refer to Figure 2.4 for XAFS model numbering and coordination geometry. ^b The number of legs represents the path travelled by the photoelectron originating from and returning to the XAFS absorber atom Cu_0 . ^c The total distance travelled by the photoelectron R_{eff} is twice the value of R_{as} . ^d The importance factor represents the percent contribution of a path relative to the strongest MS path $Cu_0 \rightarrow O_2 \rightarrow Cu_0$, e.g., the paths $Cu_0 \rightarrow C_{12} \rightarrow Cu_0$ and $Cu_0 \rightarrow C_{11} \rightarrow O_2 \rightarrow Cu_0$ are the next two strongest paths, representing 38.4% and 45.6% importance relative to path $Cu_0 \rightarrow O_2 \rightarrow Cu_0$. It includes the contribution from the Debye-Waller factors.

APPENDIX 10.

THE ANTI-FERROMAGNETIC INTERACTION OF Cu(II) DIMERS.

The electron-spin-electron-spin interaction for two coupled electrons between two Cu(II) sites, assuming an anti-ferromagnetic interaction sufficiently strong so that the singlet-triplet separation is significantly greater than D (the symmetric axial crystal-field splitting parameter) can be characterised by the zero-field spin Hamiltonian:^{1,2}

$$H_z = D(S_z^2 - S(S+1)/3) + E(S_x^2 - S_y^2) + A_1 S_z I_z \quad \text{A10.1}$$

where the axial direction is along the Cu-Cu vector. As perpendicular hyperfine constituents are unresolved, they are not included in the equation. The zero-field splitting parameters, D , E and A_1 can be obtained from ZF-EPR spectra using Equation A10.1. Furthermore, values of g were derived from X-band powder EPR spectra, as described by the spin Hamiltonian:

$$H = \beta B g S + D(S_z^2 - 2\beta) + E(S_x^2 - S_y^2) \quad \text{A10.2}$$

where, B , the external magnetic field, g , the anisotropic Lande splitting tensor, S , the total spin vector, and D , and E , the zero-field splitting parameters derived directly from the observed zero-field EPR spectrum. It has been proposed that the magnetic interaction in Cu(II) dimers involves a super-exchange mechanism.³ Specifically, interaction via one continuous (short) σ -bonding pathway, by an overlap of the orbital containing the unpaired electron on each of the Cu(II) d orbitals with the filled s , p , d or hybrid orbitals of the bridging atom, is proposed.¹⁵ The delocalised "magnetic orbitals" of the two Cu(II) centres overlap either orthogonally or non-orthogonally to produce *potential* (parallel spin coupling; ferromagnetism) or *kinetic* (anti-parallel spin coupling; antiferromagnetism) exchange, respectively, of which kinetic exchange predominates.³ The theory, as described by Anderson,⁴ predicts that the singlet state is stabilised in the kinetic exchange by a pairing of the two electrons (and so is intrinsically anti-ferromagnetic), whilst in potential exchange the

triplet state is stabilised over the singlet state according to Hund's rule.⁵ Furthermore, the energy difference between the singlet-triplet states (J) can be written as:

$$J = b_{12}^2 / U - J_{12} \quad \text{A10.3}$$

where, b_{12}^2 is a transfer integral, i.e., a kinetic energy term due to the stabilized delocalised singlet, U is the energy difference between the perturbed, i.e., an electron removed from one Cu(II) and transferred to the other Cu(II), and unperturbed state and J_{12} is the transfer integral for the triplet state.⁶ When J is negative, the diamagnetic state will be the ground state, and the triplet state will lie at a distance $-2J$ above the diamagnetic state.

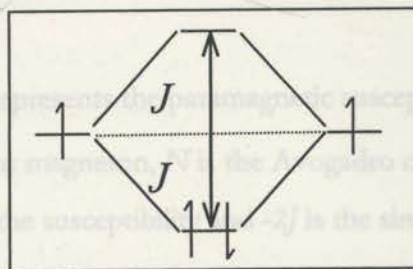


Figure A10.1. Antiferromagnetic spin singlet interaction of separate Cu(II) ions.

This corresponds to anti-ferromagnetism rather than ferromagnetism⁷ (Figure A10.1), with the absolute value of the magnitude of J decreasing with increasing inter-electron distance.⁵ By convention the singlet-triplet separation is $2J^6$ or $-2J$, where a positive $-2J$ value means anti-ferromagnetic interactions. For the purpose of this thesis, the singlet-triplet anti-ferromagnetic exchange interaction will be defined as J .

Using variable temperature EPR spectroscopy, the triplet state can be more readily distinguished from the singlet state as the temperature approaches 0 K.⁵ Typically for Cu(II) dimers, as the temperature decreases, there is a decreasing population of the thermally populated triplet state relative to the singlet state. There is, consequently, a decrease in the observed EPR intensity of the triplet state at low temperature, possibly leading to eventual disappearance of the signal, and corresponding dominance of the EPR spectra by any monomeric Cu(II) impurity ($S = 1/2$).⁸ There is, however, an increase in the EPR amplitude of the triplet state on cooling due to a narrowing of the line width.⁸

The experimental determination of the value of J for dinuclear Cu(II) complexes, with exchange interactions between the Cu(II) centres, defined as:

$$H_{\text{ex}} = -2J\hat{S}_1\hat{S}_2 \quad \text{A10.4}$$

is obtained by fitting the variable temperature magnetic susceptibility data of powder samples to the Bleaney-Bowers equation:⁷

$$\chi_A = \frac{N \beta^2 \bar{g}^2}{3kT} (1-x) \left[\frac{3\exp(-2J/kT)}{3\exp(-2J/kT) + 1} \right] + N\alpha + \frac{4x}{T} \quad \text{A10.5}$$

where $\bar{g}$ is the average g value for the dimer, χ_A represents the paramagnetic susceptibility per Cu, x is the fraction of monomer, β is the Bohr magneton, N is the Avogadro constant, $N\alpha$ is a temperature independent contribution to the susceptibility and $-2J$ is the singlet-triplet separation.

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APPENDIX 11.

ROOM TEMPERATURE MAGNETIC DATA.

Table A11.1. Calculated Diamagnetic Susceptibility of the Ligands.¹⁻³

Ligand	χ_D (cgs)
DMF	-3.83×10^{-5}
DMA	-5.02×10^{-5}
NMP	-6.56×10^{-5}
OH ₂	-1.30×10^{-5}
IndoH	-1.85×10^{-4}
$[(CH_3COO)_2]$	-6.0×10^{-5}

Table A11.2. Room Temperature Gram (χ_g), Molar (χ_M) and Paramagnetic (χ_P) Susceptibilities per Cu of $[Cu_2(Indo)_4L_2]$ (L = DMF, DMA, NMP, OH₂) and $[Cu_2(OAc)_4(OH_2)_2]$.

Complex	χ_g (cgs)	χ_M (cgs mole ⁻¹)	χ_P (cgs)
$[Cu_2(Indo)_4(DMF)_2] \cdot DMF \cdot 2H_2O$	5.76×10^{-7}	5.21×10^{-4}	9.96×10^{-4}
$[Cu_2(Indo)_4(DMA)_2] \cdot 3H_2O$	5.26×10^{-7}	4.68×10^{-4}	9.87×10^{-3}
$[Cu_2(Indo)_4(NMP)_2] \cdot 2H_2O$	3.78×10^{-7}	3.28×10^{-4}	8.11×10^{-4}
$[Cu_2(Indo)_4(OH_2)_2] \cdot 1.5H_2O$	5.10×10^{-7}	4.12×10^{-4}	9.20×10^{-4}
$[Cu_2(Indo)_4(OH_2)_2]$	7.18×10^{-7}	5.71×10^{-4}	1.05×10^{-3}
$[Cu_2(OAc)_4(OH_2)_2]$	4.19×10^{-6}	8.36×10^{-4}	8.96×10^{-4}
IndoH ^a	-6.51×10^{-7}	-2.33×10^{-4}	—

^a The measured diamagnetic susceptibility of IndoH (χ_{Dmeas}) = -2.33×10^{-4} cgs.

References

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APPENDIX 12.

X-BAND EPR Cu(II) MONOMER DETERMINATIONS.

The Cu(II) monomer content was determined at 4 K by comparing the value of the double integral (DI) of the Cu(II) monomer X-band EPR spectrum in the range 2000-4000 G (Appendix Four) with calibration solution samples of CuCl₂. A typical X-band EPR Cu(II) monomer calibration curve derived from solution samples of CuCl₂ in the range 0.03-0.30 mg Cu(II) mL⁻¹ is shown in Figure A12.1, along with the line-of-best fit and 1% error bars. Glycerol (20% v/v) or dimethylsulfoxide (DMSO) (20% v/v) was added to the calibration samples to ensure a vitrified sample suitable for low temperature EPR spectroscopy.

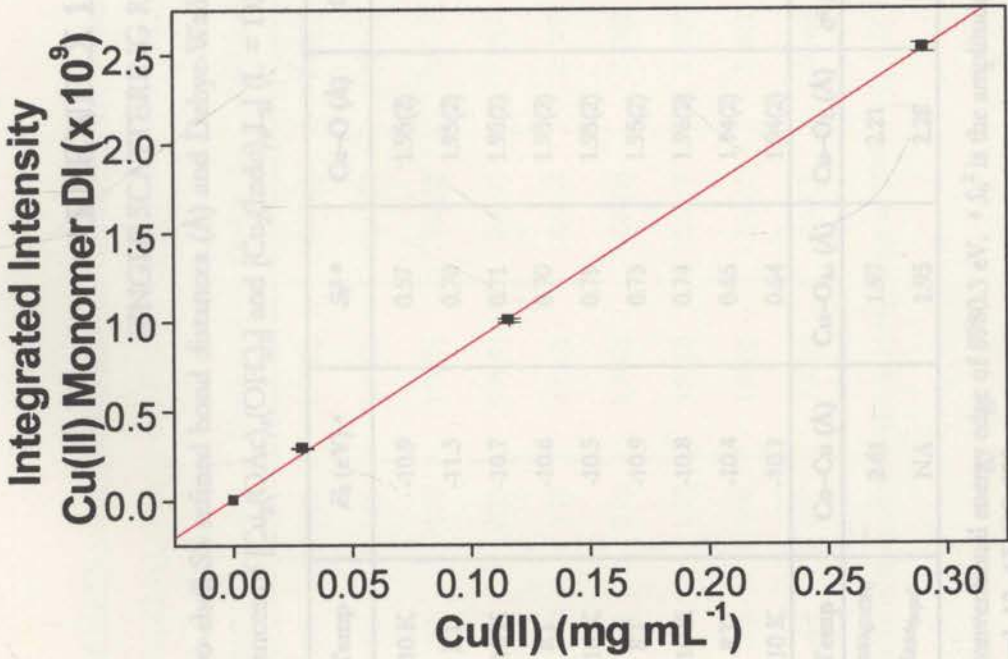


Figure A12.1. A typical X-band EPR Cu(II) monomer calibration curve of integrated signal intensity (arbitrary units and the [Cu(II)] mg mL⁻¹) showing the line-of-best fit and 1% error bars.

APPENDIX 13.

SINGLE SCATTERING RESULTS.

Table A13.1. The two-shell SS refined bond distances (Å) and Debye-Waller factors (σ^2 (Å²)) for the Cu...Cu vector length, mean Cu-O_{Ac} and Cu-O_L bond distances of [Cu₂(OAc)₄(OH₂)₂] and [Cu₂(Indo)₄L₂] (L = DMF, DMA, NMP, OH₂).

Complex	Temp	E_0 (eV) ^a	S_0^2 ^b	Cu-O (Å)	Cu-Cu (Å)	σ^2 (Cu-O) (Å ²)	σ^2 (Cu-Cu) (Å ²)	R_{ref} (%)
[Cu ₂ (OAc) ₄ (OH ₂) ₂] ^c	10 K	-10.9	0.57	1.95(2)	2.60(2)	0.003	0.002	13.8
[Cu ₂ (Indo) ₄ (DMF) ₂]	RT	-11.3	0.70	1.95(2)	2.63 (2)	0.004	0.007	10.2
[Cu ₂ (Indo) ₄ (DMF) ₂]	10 K	-10.7	0.71	1.95(2)	2.62 (2)	0.003	0.004	18.9
[Cu ₂ (Indo) ₄ (DMA) ₂]	RT	-10.6	0.70	1.95(2)	2.66(2)	0.004	0.007	12.8
[Cu ₂ (Indo) ₄ (DMA) ₂]	10 K	-10.5	0.79	1.95(2)	2.65(2)	0.004	0.004	14.4
[Cu ₂ (Indo) ₄ (NMP) ₂]	RT	-10.9	0.73	1.95(2)	2.65(2)	0.005	0.007	11.6
[Cu ₂ (Indo) ₄ (NMP) ₂]	10 K	-10.8	0.74	1.96(2)	2.64(2)	0.004	0.003	13.2
[Cu ₂ (Indo) ₄ (OH ₂) ₂]	RT	-10.4	0.65	1.94(2)	2.63(2)	0.003	0.006	14.2
[Cu ₂ (Indo) ₄ (OH ₂) ₂]	10 K	-10.1	0.64	1.94(2)	2.62(2)	0.002	0.002	20.5
Sample	Temp	Cu-Cu (Å)	Cu-O _{Ac} (Å)	Cu-O _L (Å)	σ^2 (Cu-Cu) (Å ²)	σ^2 (Cu-O _{Ac}) (Å ²)	σ^2 (Cu-O _L) (Å ²)	R_{ref} (%)
[Cu ₂ (OAc) ₄ (OH ₂) ₂] ^c	Glass(EtOH)	2.61	1.97	2.21	0.006	0.002	0.005	NA
[Cu ₂ (OAc) ₄ (OH ₂) ₂] ^c	Glass(aqua)	NA	1.95	2.28	NA	0.006	0.007	NA

^a E_0 is relative to the conventional energy edge of 8980.3 eV. ^b S_0^2 is the amplitude reduction factor. ^c Taken from Nomura, M.; Yamaguchi, T., *J. Physiq.* **1986**, C8, Suppl. 12, 47, 619-622.

Table A13.2. The three-shell SS XAFS refined bond distances (Å) and Debye-Waller factors (σ^2 (Å²)) for the Cu...Cu vector length, mean Cu–O_(Ac) and Cu–O_L bond distances of [Cu₂(OAc)₄(OH₂)₂] and [Cu₂(Indo)₄L₂] (L = DMF, DMA, NMP, OH₂).

Complex	Temp	E_0 (eV) ^a	S_0^2 ^b	Cu–O _(Ac) (Å)	Cu–Cu (Å)	Cu–O _L (Å)	σ^2 (Cu–O _{Ac}) (Å ²)	σ^2 (Cu–Cu) (Å ²)	σ^2 (Cu–O _L) (Å ²)	R_{XAF} (%)
[Cu ₂ (OAc) ₄ (OH ₂) ₂]	10 K	-8.9	0.85	1.96(2)	2.61(2)	2.24(2)	0.004	0.005	0.004	11.0
[Cu ₂ (Indo) ₄ (DMF) ₂]	RT	-9.6	1.01	1.96(2)	2.64(2)	2.25(2)	0.005	0.01	0.008	7.0
[Cu ₂ (Indo) ₄ (DMF) ₂]	10 K	-8.9	1.12	1.96(2)	2.63(2)	2.25(2)	0.005	0.007	0.002	12.1
[Cu ₂ (Indo) ₄ (DMA) ₂]	RT	-9.2	0.95	1.96(2)	2.66(2)	2.25(2)	0.005	0.009	0.01	10.9
[Cu ₂ (Indo) ₄ (DMA) ₂]	10 K	-9.62	1.13	1.96(2)	2.66(2)	2.25(2)	0.005	0.009	0.01	11.9
[Cu ₂ (Indo) ₄ (NMP) ₂]	RT	-9.3	0.99	1.96(2)	2.66(2)	2.24(2)	0.005	0.009	0.01	9.6
[Cu ₂ (Indo) ₄ (NMP) ₂]	10 K	-9.5	1.05	1.96(2)	2.65(2)	2.26(2)	0.004	0.005	0.01	10.5
[Cu ₂ (Indo) ₄ (OH ₂) ₂]	RT	-9.3	0.99	1.95(2)	2.64(2)	2.26(2)	0.005	0.01	0.004	7.6
[Cu ₂ (Indo) ₄ (OH ₂) ₂]	10 K	-8.9	0.97	1.95(2)	2.64(2)	2.24(2)	0.003	0.005	0.003	16.9

^a E_0 is relative to the conventional energy edge of 8980.3 eV. ^b S_0^2 is the amplitude reduction factor.

Table A13.3. The Seven-shell SS Model, Refined Bond Distances (Å) and Debye-Waller factors (σ^2 (Å²)) for the Cu...Cu, Mean Cu–O_(Ac) and Cu–O_L Vector Distances from the XAFS Analysis of Solid [Cu₂(Indo)₄(DMF)₂] at 10 K.

XAFS Parameter	Shell No.	N_s	Distance from Cu ₀ (Å) ^a	Refined XAFS Parameter (Restrained SS Model)	Refined XAFS Parameter (Unrestrained SS Model)
R_{xaf}	—	—	—	39.5%	30.9%
E_0 ^b	—	—	—	-7.4 eV	-8.3 eV
S_0^2 ^c	—	—	—	0.78	0.79
Cu–O _L ^d (Å)	1	1	2.143(5)	2.15(2) Å ^k	2.22(2)
Cu–O _{Ac} ^e (Å)	2	4	1.96	1.96(2) Å	1.96(2)
Cu–Cu ^f (Å)	3	1	2.630(1)	2.63(2) Å	2.64(2)
Cu–C _{Ac} ^g (Å)	4	4	2.84	2.82(2) Å	2.87(2)
Cu–O _{Ac} ^h (Å)	5	4	3.11	3.16(2) Å	3.14(2)
Cu–C _{Indo} ⁱ (Å)	6	4	4.24	4.24(2) Å ^j	5.01(2)
Cu–O _L ^j (Å)	7	1	4.773(6)	4.71(2) Å ^m	5.65(2) ^g

^a Taken from Weder, J. E.; Hambley, T. W.; Kennedy, B. J.; Lay, P. A.; MacLachlan, D.; Bramley, R.; Delfs, C. D.; Murray, K. S.; Moubaraki, B.; Warwick, B.; Biffin, J. R.; Regtop, H. L. *Inorg. Chem.* **1999**, *38*, 1736-1744. ^b E_0 is relative to the conventional energy edge of 8980.3 eV. ^c S_0^2 is the amplitude reduction factor. ^d XAFS model atom No. = 14. ^e XAFS model atom Nos. = 2, 5, 7, 8. ^f XAFS model atom No. = 1. ^g XAFS model atom Nos. = 10, 11, 12, 13. ^h XAFS model atom Nos = 3, 4, 6, 9. ⁱ XAFS model atom Nos = 20, 21, 22, 23. ^j XAFS model atom No. = 15. ^k Vector distance symmetry restraints of 2.14(2) Å. ^l Vector distance symmetry restraint of 4.24(2) Å. ^m Vector distance symmetry restraint of 4.77 Å. ⁿ $(R_{\text{Cu}(1)\text{-O}(15)} = (R_{\text{Cu-O15}} - R_{\text{Cu-Cu}})$, i.e., = 3.01(2) Å).

APPENDIX 14.

ADDITIONAL MS REFINEMENT MODELS of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, DMA, NMP, OH_2) at 10 K and RT.

Table A14.1. MS XAFS Refined Bond Distances (Å), Debye-Waller Factors (σ^2 (Å²)) and Bridging O–C–O Bond Angle (°) of $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ (L = DMF, DMA, NMP) Using 65 and 130 Unique Multiple Scattering Paths.

Complex	No. of Paths	Tem P	Bond distance (Å)			Bond angle (°)	Debye-Waller factor (σ^2 (Å ²))			XAFS parameter		
			Cu–Cu	Cu–O _{Ac}	Cu–O _L		Cu–Cu	Cu–O _{Ac}	Cu–O _L	E_0 (eV) ^c	S_0^2 ^d	R_{xaf} (%) ^e
$[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ ^a	65	RT	2.63(2)	1.95(2)	2.15(2)	125(1)	0.006	0.008	0.02	-9.97	0.91	12.6
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ ^a	65	10 K	2.62(2)	1.95(2)	2.11(2)	125(1)	0.003	0.005	0.01	-9.34	0.91	14.0
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ ^a	65	RT	2.63(2)	1.95(2)	2.11(2)	125(1)	0.007	0.009	0.02	-9.92	0.91	12.9
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ ^a	65	10 K	2.63(2)	1.96(2)	2.14(2)	125(1)	0.003	0.005	0.009	-9.37	0.92	12.45
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ ^a	65	RT	2.63(2)	1.95(2)	2.11(2)	125(1)	0.007	0.008	0.01	-9.74	0.91	13.9
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ ^b	130	10 K	2.63(2)	1.95(2)	2.11(2)	125(1)	0.003	0.005	0.01	-9.52	0.91	13.3
$[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ ^b	130	RT	2.63(2)	1.95(2)	2.09(2)	125(1)	0.007	0.009	0.02	-10.16	0.90	12.3
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ ^b	130	10 K	2.63(2)	1.96(2)	2.11(2)	125(1)	0.003	0.005	0.01	-9.69	0.90	15.1
$[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ ^b	130	RT	2.63(2)	1.95(2)	2.10(2)	125(1)	0.006	0.008	0.01	-10.01	0.91	13.2

^a Cu–O_L starting distance 2.14 Å. ^b Cu–O_L starting distance 2.14 Å. ^c E_0 is relative to the conventional energy edge of 8980.3 eV. ^d S_0^2 is the amplitude reduction factor. ^e Determinacy (N_i/N_p) of the models ~2.0.

Table A14.2. MS XAFS Refined Bond Distances (Å), Debye-Waller Factors (σ^2 (Å²)) and Bridging O–C–O Bond Angle (°) of [Cu₂(Indo)₄L₂] (L = OH₂) at Room Temperature.

Complex	No. of Paths	Temp	Bond distance (Å)			Bond angle (°)	Debye-Waller factor (σ^2 (Å ²))			XAFS parameter		
			Cu–Cu	Cu–O _{Ac}	Cu–O _L		Cu–Cu	Cu–O _{Ac}	Cu–O _L	E_0 (eV) ^a	S_0^2 ^b	R_{xaf} (%)
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	130	RT	2.63(2)	1.95(2)	2.19(2)	125(1)	0.006	0.01	0.04	-9.19	0.91	14.5
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^d	130	RT	2.63(2)	1.95(2)	2.20(2)	125(1)	0.006	0.01	0.04	-9.18	0.91	10.5
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	65	RT	2.63(2)	1.95(2)	2.22(2)	125(1)	0.006	0.01	0.04	-9.05	0.90	11.3
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^d	65	RT	2.62(2)	1.95(2)	2.21(2)	125(1)	0.006	0.01	0.02	-9.16	0.90	11.0
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	65	RT	2.63(2)	1.95(2)	2.14(2)	125(1)	0.006	0.01	0.02	-9.37	0.89	11.8

^a E_0 is relative to the conventional energy edge of 8980.3 eV. ^b S_0^2 is the amplitude reduction factor.. ^c Cu–O_L starting distance 2.14 Å, ^d Cu–O_L starting distance 2.00 Å. ^e Cu–O_L distance restrained to 2.14(2) Å.

Table A14.3. MS XAFS Refined Bond Distances (Å), Debye-Waller Factors (σ^2 (Å²)) and Bridging O–C–O Bond Angle (°) of [Cu₂(Indo)₄(OH₂)₂] at 10 K

Complex	No. of Paths	Temp	Bond distance (Å)			Bond angle (°)	Debye-Waller factor (σ^2 (Å ²))			XAFS parameter		
			Cu–Cu	Cu–O _{Ac}	Cu–O _L		Cu–Cu	Cu–O _{Ac}	Cu–O _L	E_0 (eV) ^a	S_0^2 ^b	R_{xaf} (%)
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	65	10 K	2.62(2)	1.95(2)	2.21(2)	125(1)	0.003	0.008	0.008	-8.41	0.91	15.3
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^d	65	10 K	2.62(2)	1.95(2)	2.22(2)	125(1)	0.003	0.005	0/007	-8.56	0.92	14.9
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	130	10 K	2.62(2)	1.95(2)	2.22(2)	125(1)	0.003	0.04	0.04	-8.81	0.92	14.1
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^d	130	10 K	2.62(2)	1.95(2)	2.15(2)	125(1)	0.002	0.05	0.05	-9.50	0.91	17.2
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^c	65	10 K	2.62(2)	1.95(2)	2.14(2)	125(1)	0.002	0.01	0.01	-8.94	0.89	16.8
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^f	130	10 K	2.62(2)	1.94(2)	–	125(1)	0.002	–	–	-10.11	0.89	16.9
[Cu ₂ (Indo) ₄ (OH ₂) ₂] ^{d,g}	65	10 K	2.61(2)	1.95(2)	2.25(1)	125(1)	0.004	0.006	0.04	-9.59	1.11	11.9

^a E_0 is relative to the conventional energy edge of 8980.3 eV. ^b S_0^2 is the amplitude reduction factor. ^c Cu–O_L starting distance 2.14 Å. ^d Cu–O_L starting distance 2.00 Å. ^e Cu–O_L distance restrained to 2.14(2) Å. ^f Cu–O_L distance is not refined. ^g XAFS weighting factor (W) = 8.

APPENDIX 15.

THE Cu K-EDGE MS XAFS AND FOURIER TRANSFORMS (OBSERVED, SIMULATED & RESIDUAL) OF $[\text{Cu}_2(\text{Indo})_4\text{L}_2]$ ($\text{L} = \text{DMF}, \text{DMA}, \text{NMP}, \text{OH}_2$) At 10 K and RT.

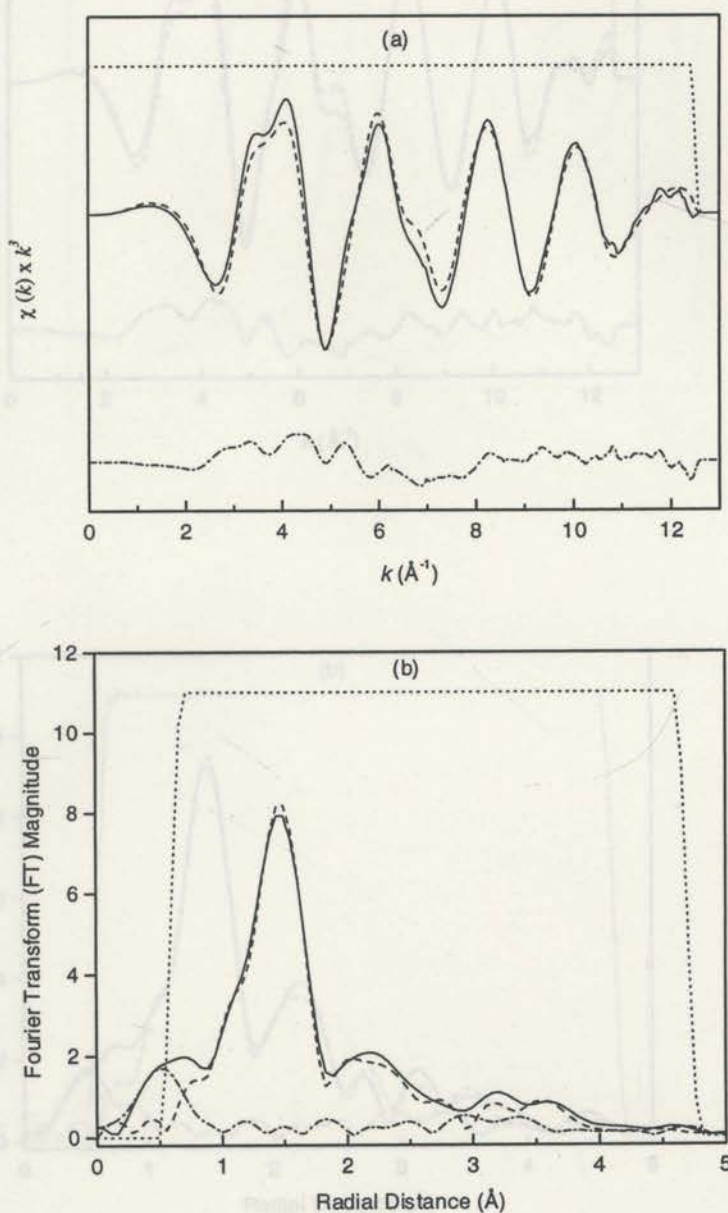


Figure A15.1. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) Fourier transforms of the XAFS data for solid $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ at RT using 65 MS paths and Cu-O_L starting distance of 2.14 $\text{\AA}$ ($R_{\text{xaf}} = 12.9\%$). The region shown by (...) is the r - and k -range used in the Fourier filter.

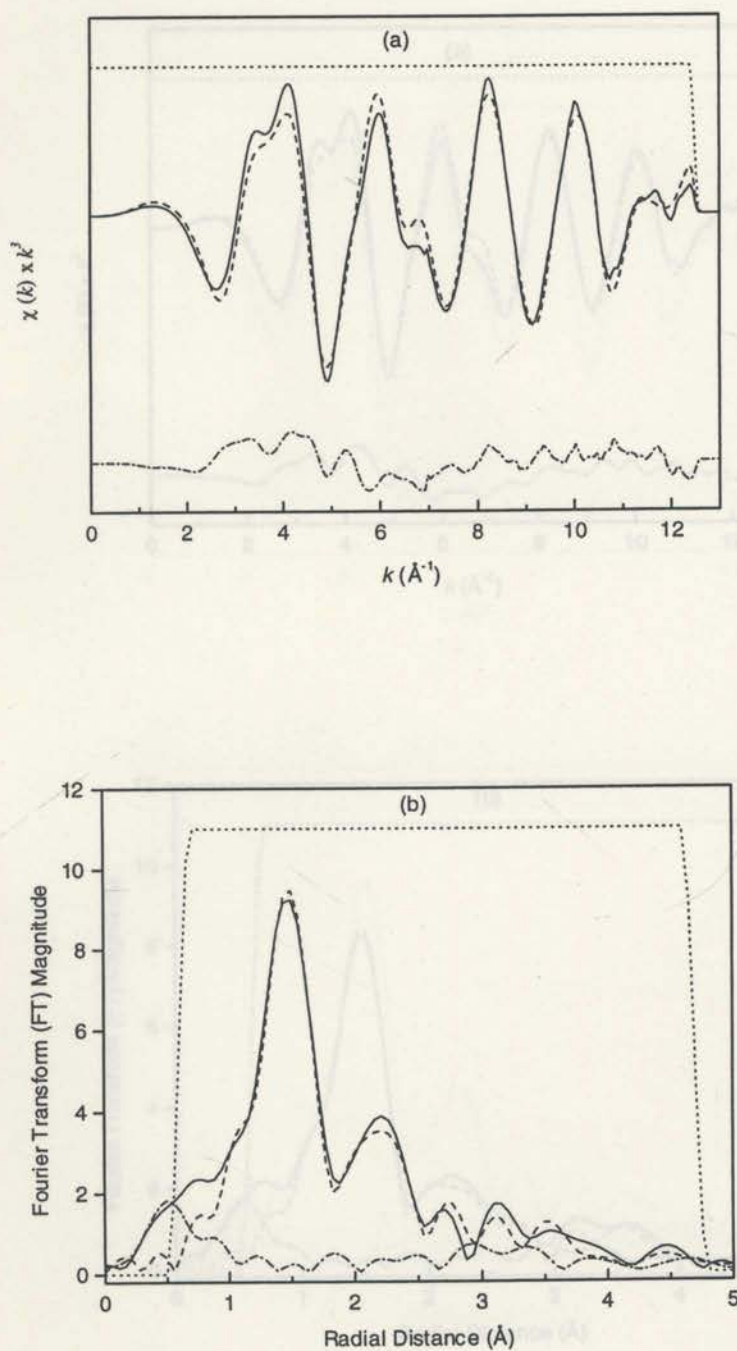


Figure A15.2. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) MS Fourier transforms of the XAFS data for $[\text{Cu}_2(\text{Indo})_4(\text{DMA})_2]$ at 10 K using 65 MS paths and Cu-O_L starting distance of 2.14 Å ($R_{\text{xaf}} = 14.0\%$). The region shown by (...) is the r - and k -range used in the Fourier filter.

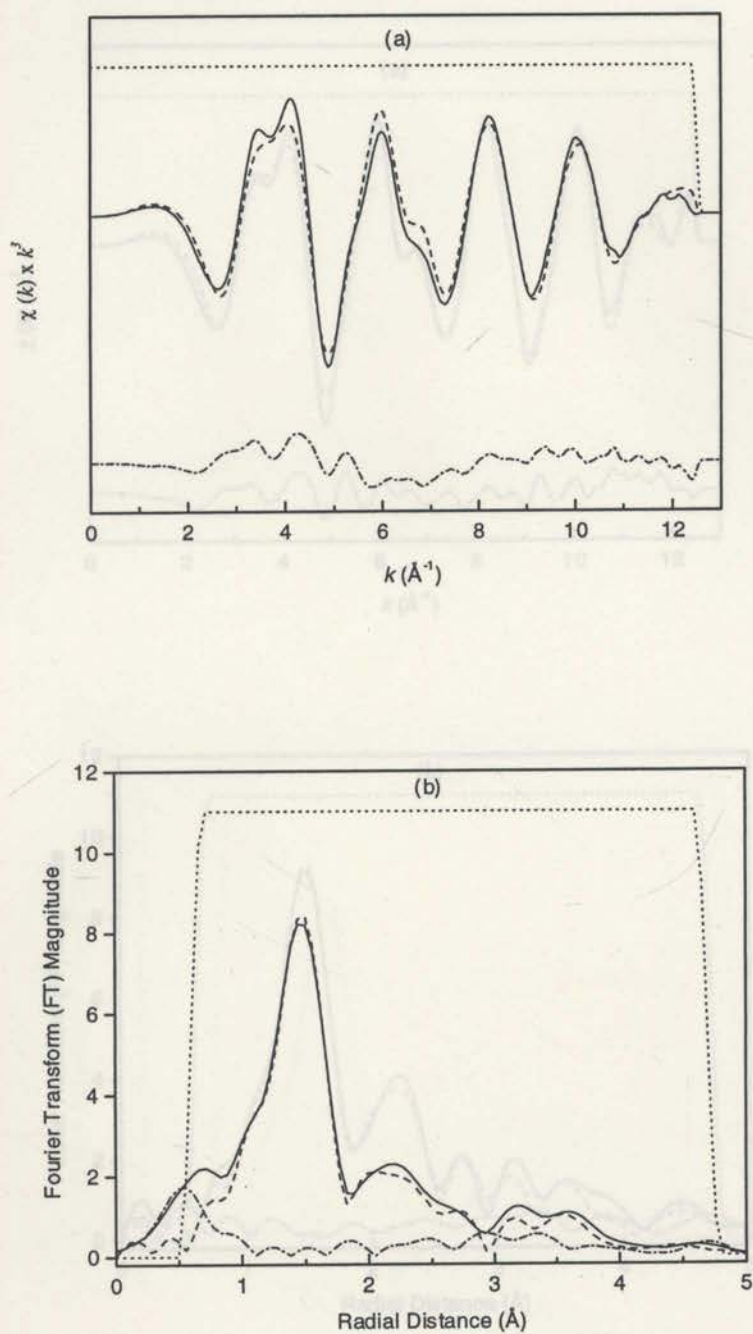


Figure A15.3. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) MS Fourier transforms for solid $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ at RT using 65 MS paths and Cu-O_L starting distance of 2.14 Å ($R_{\text{xaf}} = 13.9\%$). The region shown by (···) is the r - and k -range used in the Fourier filter.

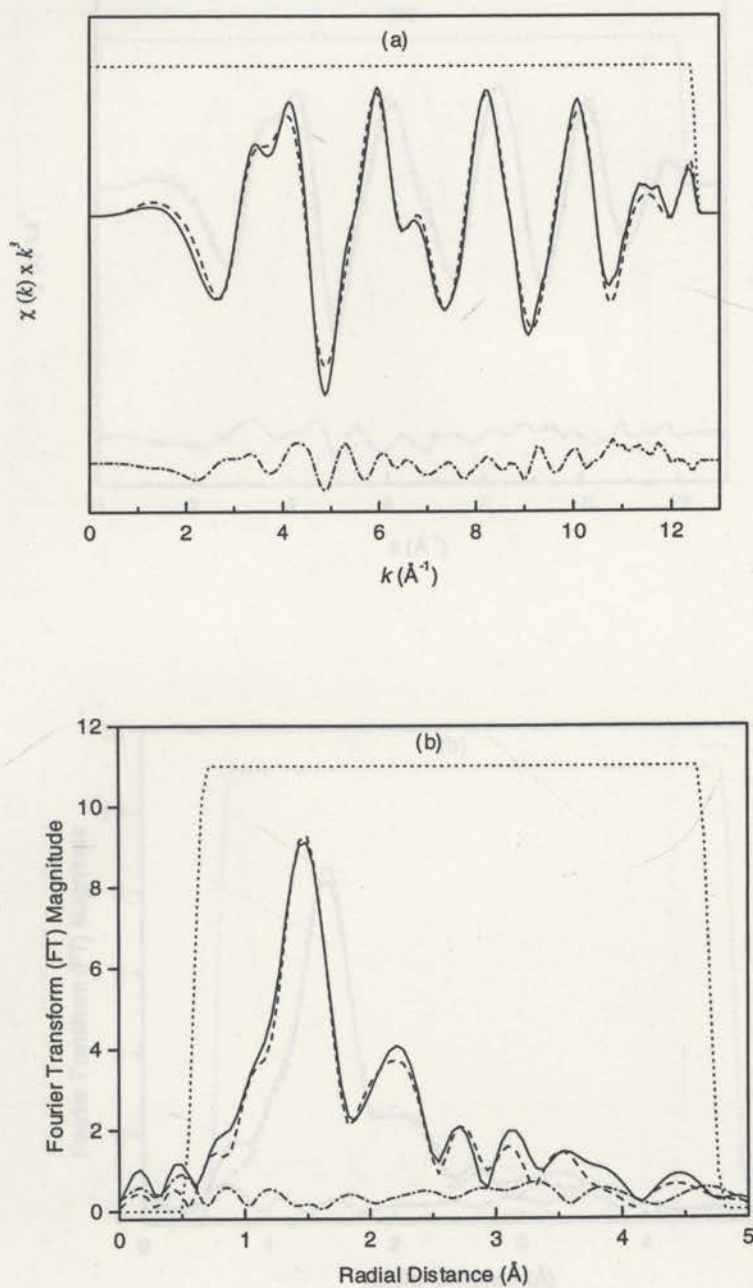


Figure A15.4. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) MS Fourier transforms for solid $[\text{Cu}_2(\text{Indo})_4(\text{NMP})_2]$ at 10 K using 65 MS paths and Cu-O_L starting distance of 2.14 $\text{\AA}$ ($R_{\text{xaf}} = 12.5\%$). The region shown by (...) is the r - and k -range used in the Fourier filter.

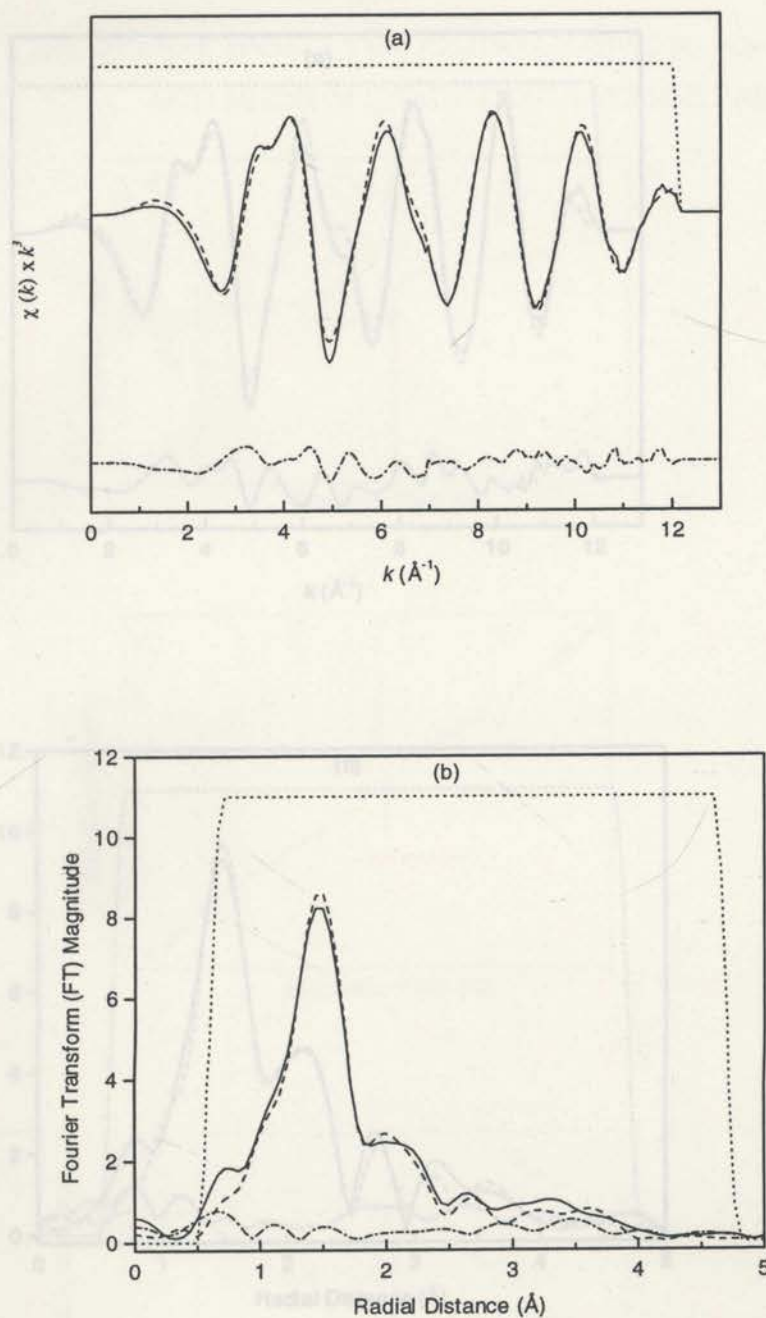


Figure A15.5. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) Fourier transforms for $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]$ at RT with the bond distance restraints on Cu–O_L of 2.14(2) $\text{\AA}$ using 65 MS paths and Cu–O_L starting distance of 2.14 $\text{\AA}$ ($R_{\text{xaf}} = 11.8\%$). The region shown by (···) is the r - and k -range used in the Fourier filter.

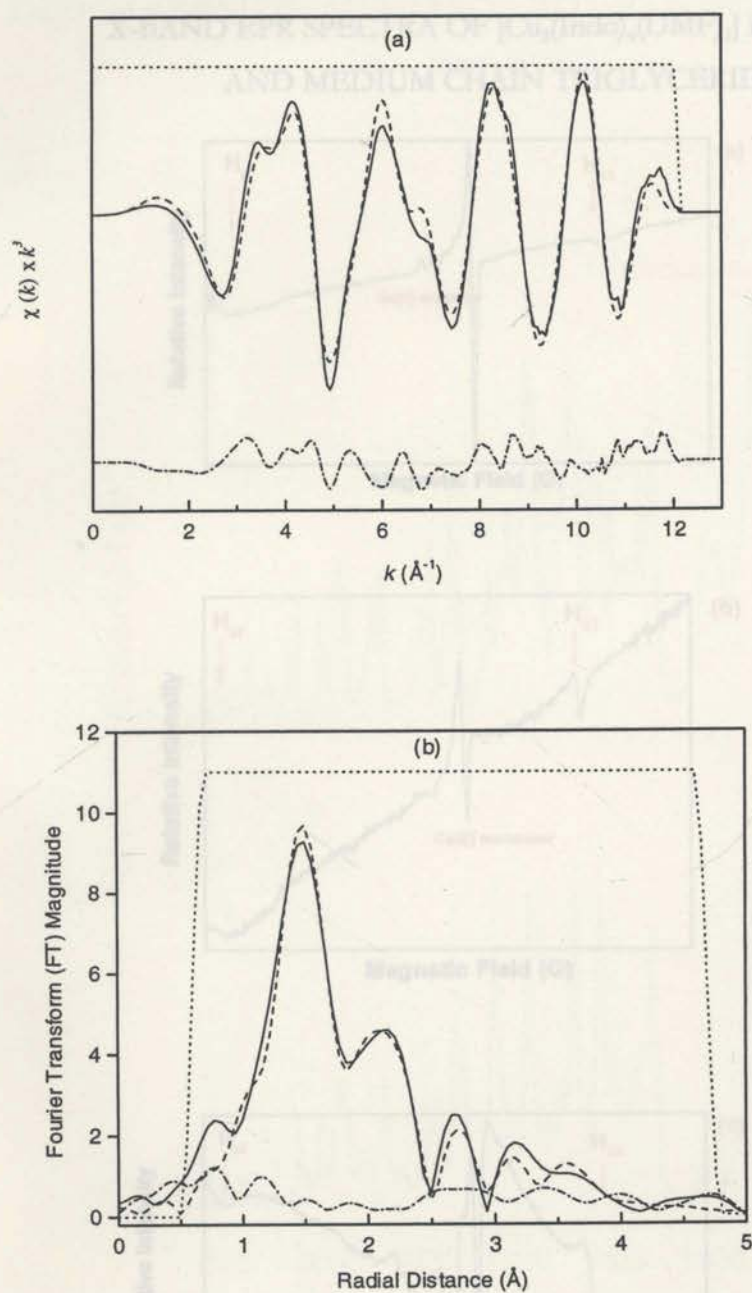


Figure A15.6. The observed (solid line), simulated (dashed line) and residual (hashed line) of the (a) Cu-K edge MS XAFS and (b) MS Fourier transforms for solid $[\text{Cu}_2(\text{Indo})_4(\text{OH})_2]$ at 10 K with the bond distance restraints on Cu–O_L of 2.14(2) Å using 65 MS paths ($R_{\text{xaf}} = 15.3\%$). The region shown by (···) is the r - and k -range used in the Fourier filter.

Figure A16.1 X-Band EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMP})_2]$ at ~ 100 K in (a) *Acetone*, (b) *neat DMP*, and (c) *medium chain oligoetheric paste* showing the resonances at ~ 500 G (H_{iso}) and ~ 4720 G (H_{ax}) due to the spin-triplet state of the dimer complex.

APPENDIX 16.

X-BAND EPR SPECTRA OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in *Avigesic Forte*, DMF AND MEDIUM CHAIN TRIGLYCERIDE PASTE.

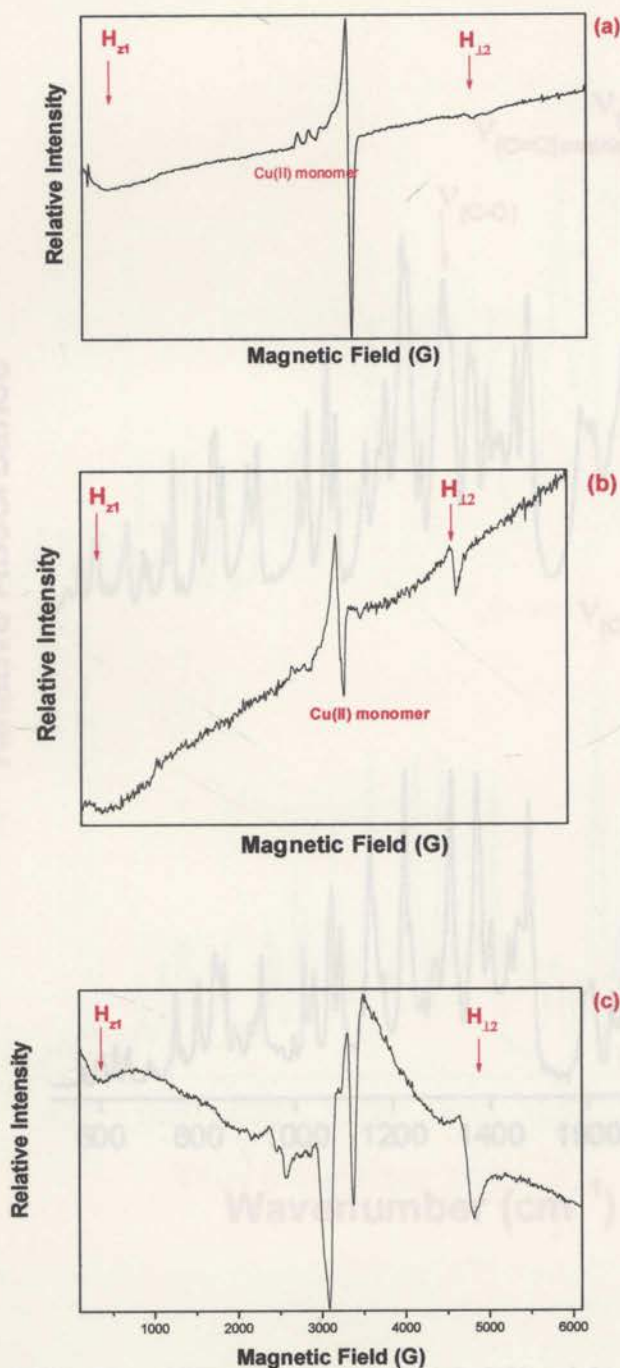


Figure A16.1 X-Band EPR spectra of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ at ~100 K in (a) *Avigesic Forte*, (b) neat DMF; and (c) medium chain triglyceride paste showing the resonances at ~500 G (H_{z1}) and ~4720 G (H_{L2}) due to the spin-triplet state of the dimer complex.

APPENDIX 17.

A COMPARISON OF THE IR SPECTRA OF THE SOLID RESIDUE OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ OBTAINED FROM HCl (pH 1.6) WITH INDOH AND $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$.

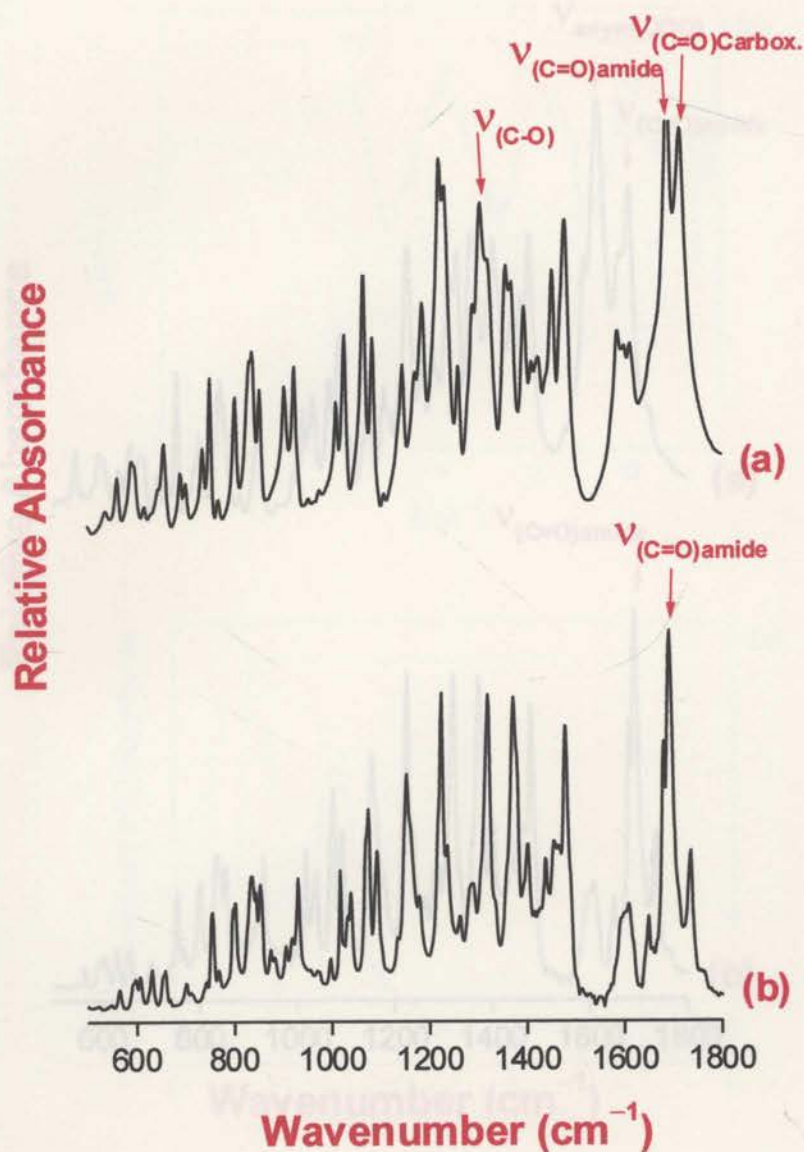


Figure A17.1. The solid-state IR spectra of (a) IndoH and (b) residue from HCl (pH 1.6) following the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. A comparison of the solid residues obtained from artificial “gastric juice” of IndoH (0.25 mg mL^{-1}) and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.08 mg mL^{-1}) are shown in Figure 4.3.

APPENDIX 18.

THE XAPS AND CORRESPONDING FOURIER TRANSFORM
FOR $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ AT 10 K IN PHARMACEUTICAL
FORMULATIONS.

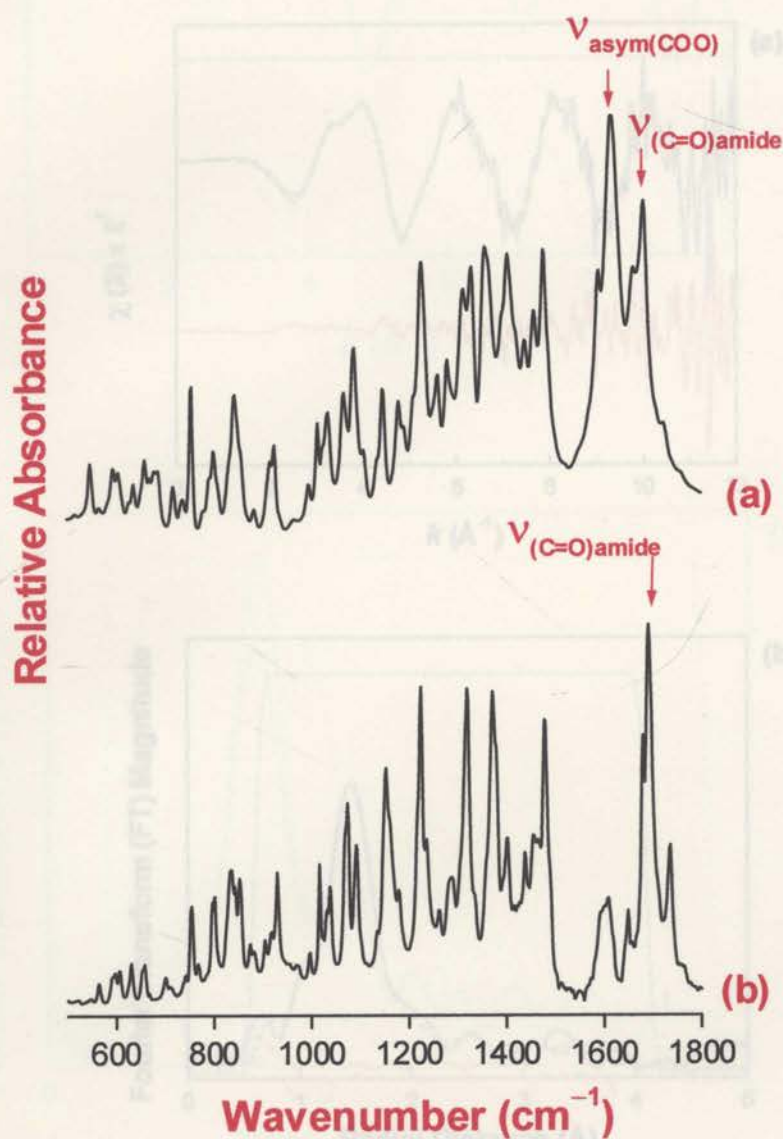


Figure A17.2. The solid-state IR spectra of (a) $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ and (b) residue from HCl (pH 1.6) following the addition of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$. A comparison of the solid residues obtained from artificial “gastric juice” of IndoH (0.25 mg mL^{-1}) and $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (0.08 mg mL^{-1}) are shown in Figure 4.3.

APPENDIX 18.

THE XAFS AND CORRESPONDING FOURIER TRANSFORM
FOR $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ AT 10 K IN PHARMACEUTICAL
FORMULATIONS.

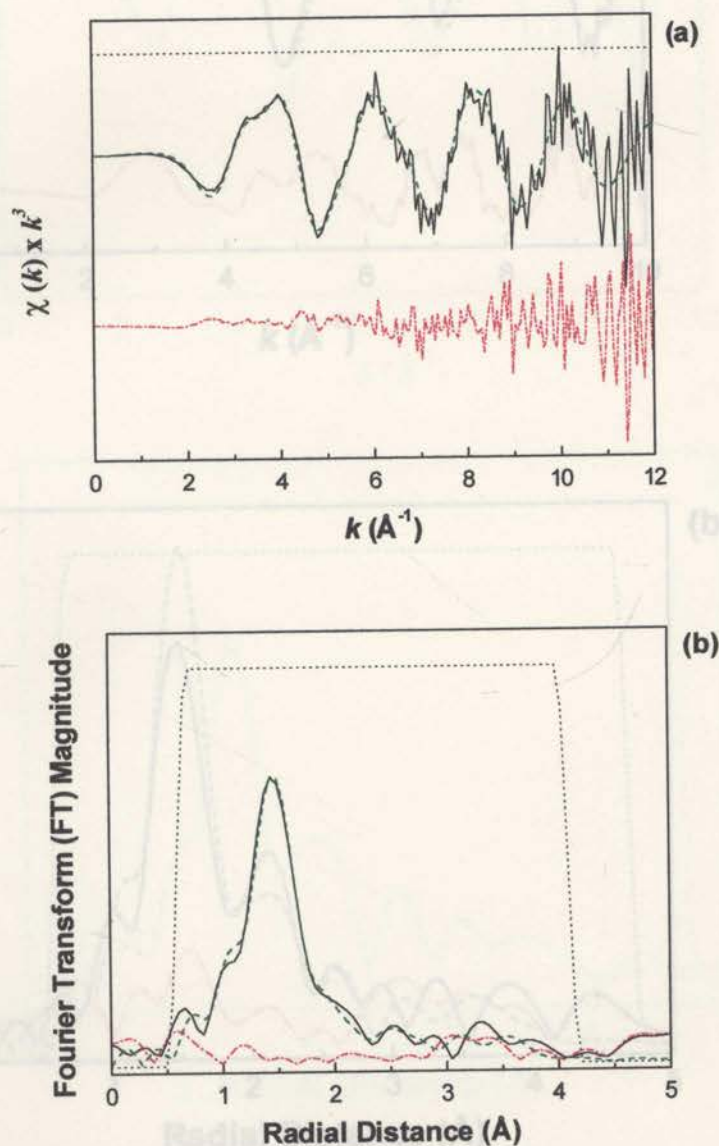


Figure A18.1. The MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding Fourier transform observed (—), simulated (···) and residual (·-·-) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in MCT paste, $R_{\text{xf}} = 14.4\%$. The region shown by (···) is the k -window used in the Fourier filter.

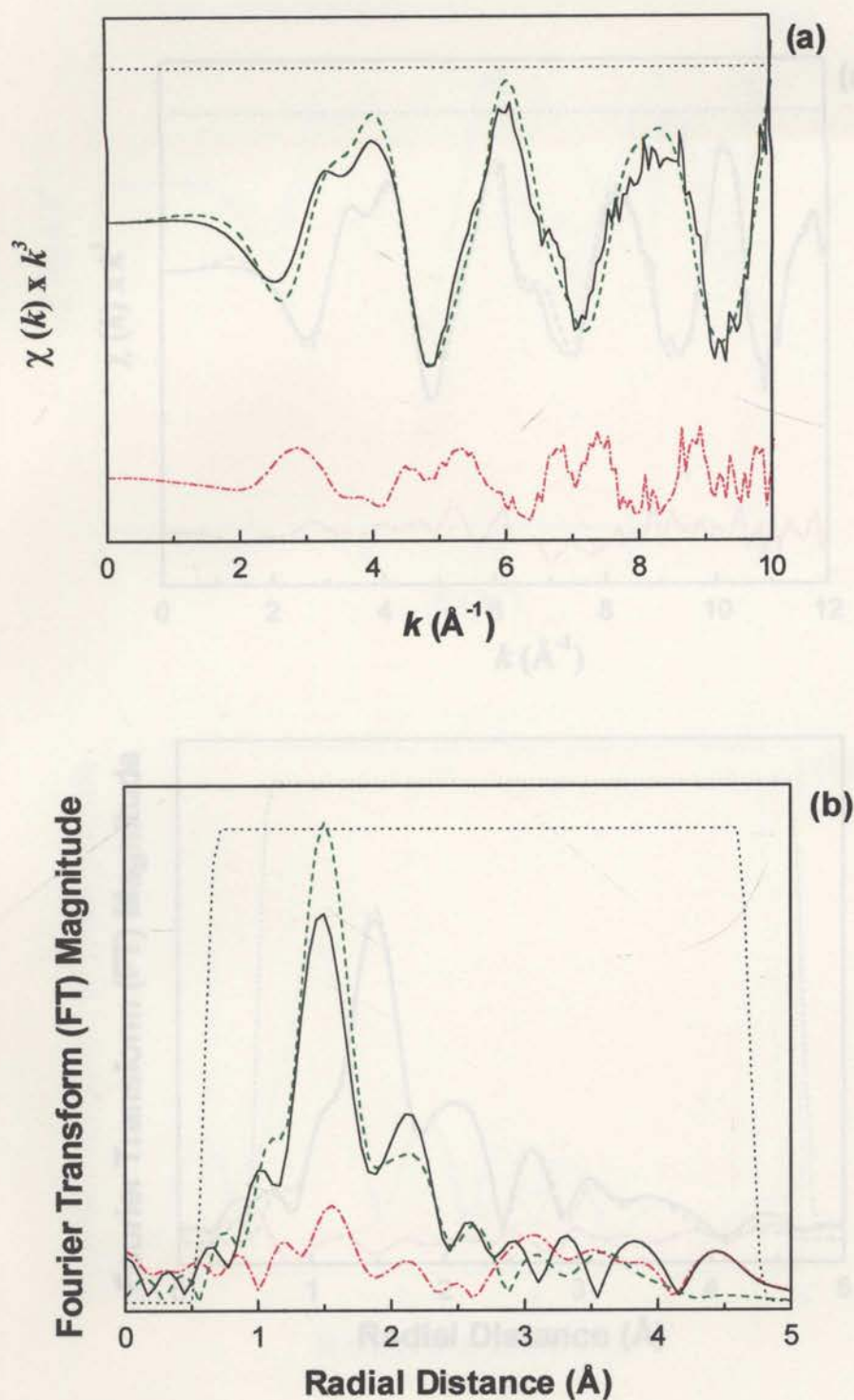


Figure A18.2. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding Fourier transform observed (—), simulated (---) and residual (— · —) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in *CN-Algesic* (carbopol) paste, $R_{\text{xaf}} = 41\%$. The region shown by (---) is the k -window used in the Fourier filter. Excessive noise in the XAFS data in the region greater than $10 \text{ \AA}^{-1}$ prevented the use of a larger k -range.

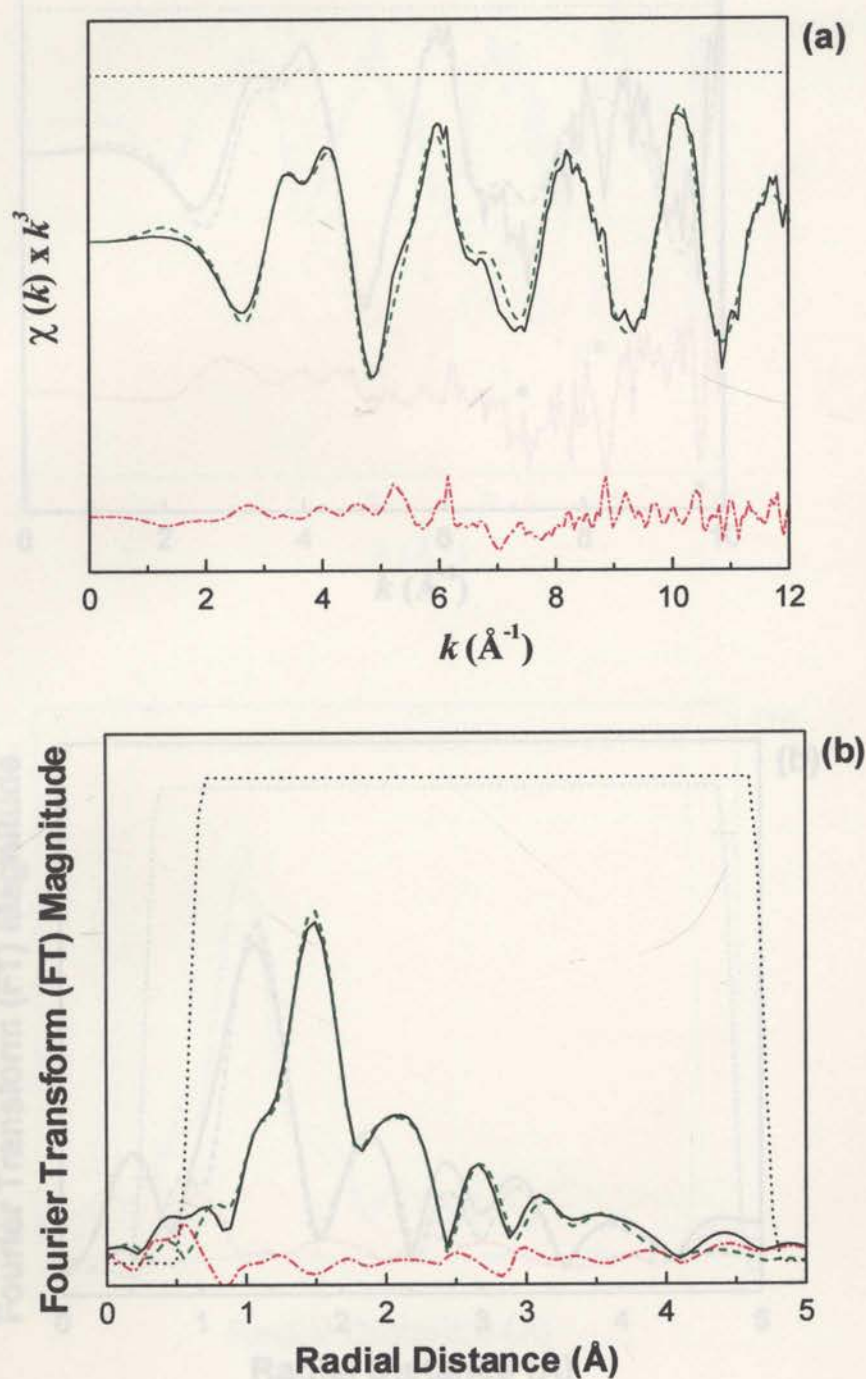


Figure A18.3. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding Fourier transform observed (—), simulated (---) and residual (· · ·) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in paraffin paste, $R_{\text{xtf}} = 13.5\%$. The region shown by (· · ·) is the k -window used in the Fourier filter.

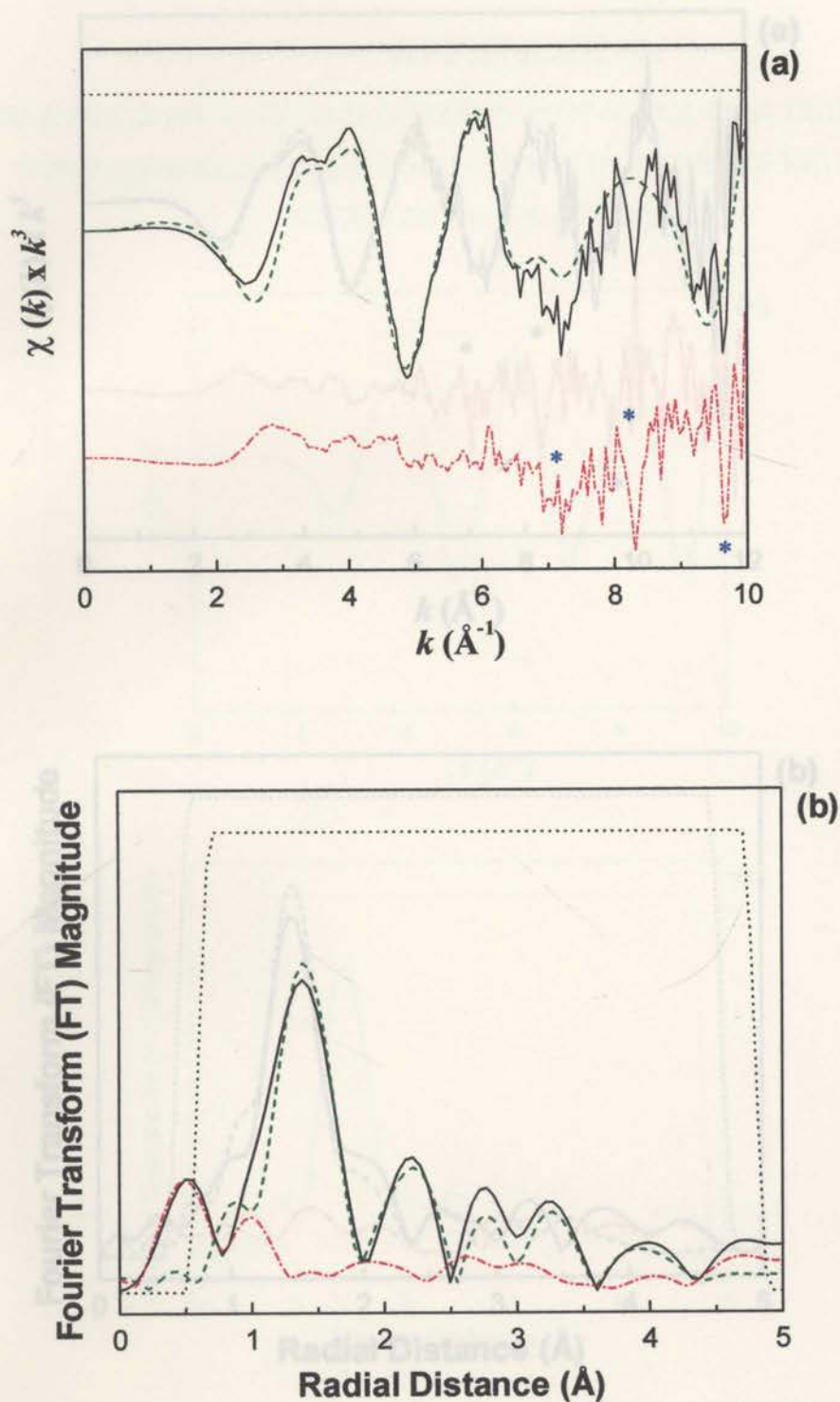


Figure A18.4. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding Fourier transform observed (—), simulated (---) and residual (·-·-) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ in *Avigesic Forte*, $R_{\text{saf}} = 26\%$. The region shown by (---) is the k -window used in the Fourier filter. The asterisks (*) represent glitches in the XAFS data, which could not be satisfactorily edited.

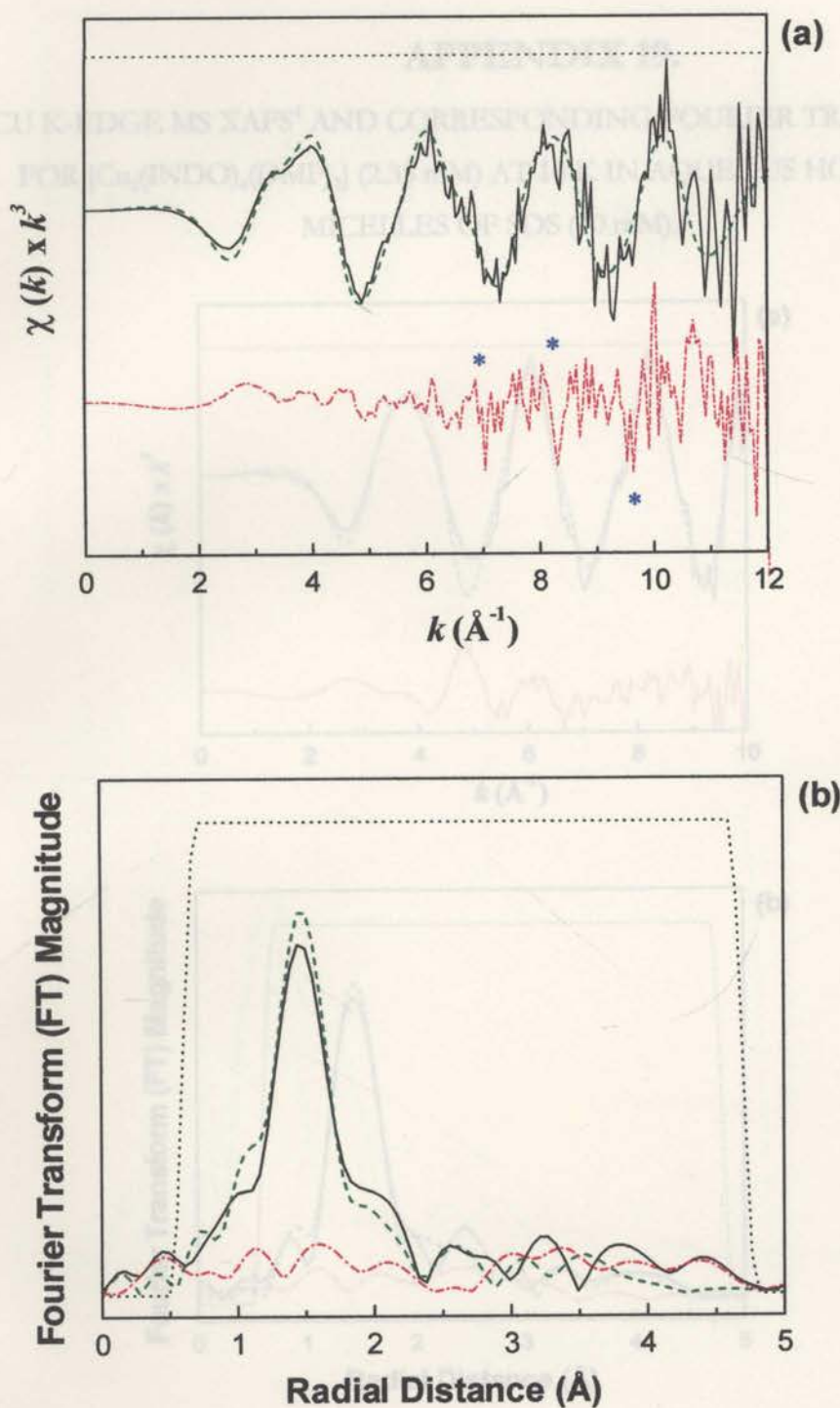


Figure A18.5. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding FT observed (—), simulated (---) and residual (---) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (2.35 mM) in MilliQ water and SDS (40 mM) at 10 K using a 130-unique MS-XAFS model (Chapter 3), starting Cu-O_L bond distance of 2.00 Å and Monte Carlo calculation of error. The region shown by (---) is the k -window used in the Fourier filter. The asterisks (*) represent glitches in the XAFS data, which could not be satisfactorily edited.

X-ray absorption spectra of the freshly prepared micelle solutions were recorded at the Stanford Synchrotron Radiation Laboratory (SSRL) on the focused beamline 9-3 (3 GeV, 95–55 mA) using a Si(220) double-crystal monochromator detuned 50% to reduce the flux (in the optics reject the harmonics). Data were collected as fluorescence spectra using a 30-element Ge array detector.

APPENDIX 19.

Cu K-EDGE MS XAFS¹ AND CORRESPONDING FOURIER TRANSFORM
 FOR $[\text{Cu}_2(\text{INDO})_4(\text{DMF})_2]$ (2.35 mM) AT 10 K IN AQUEOUS HCl (pH 1.6)
 MICELLES OF SDS (40 mM).

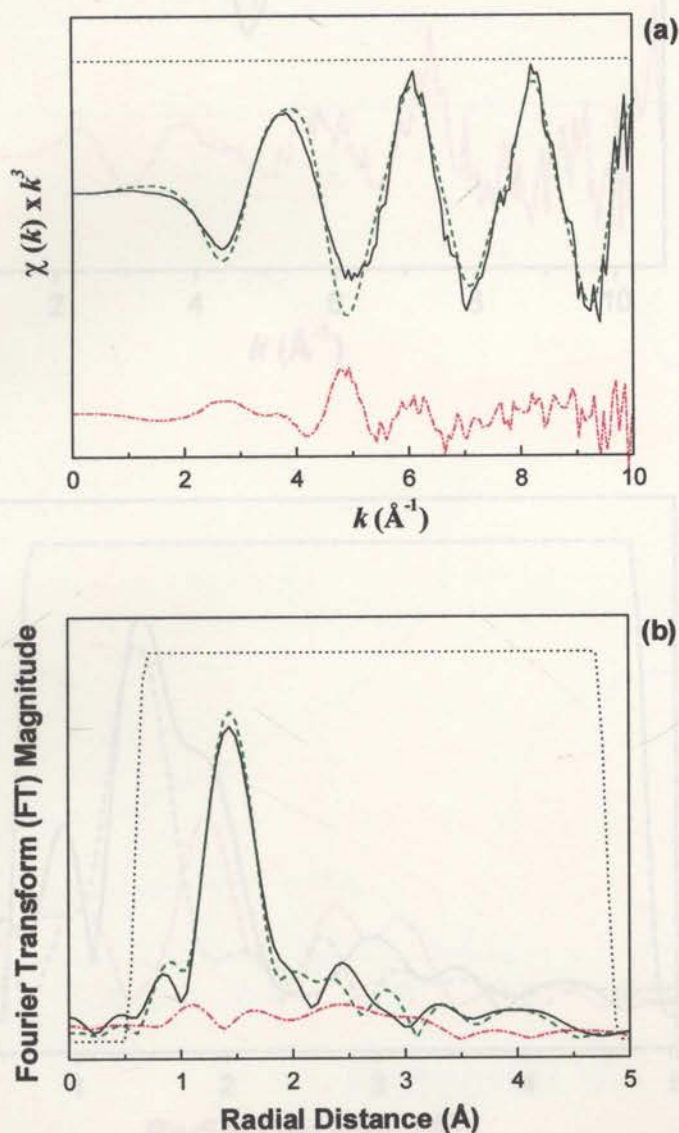


Figure A19.1. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding FT observed (—), simulated (···) and residual (---) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (2.35 mM) in MilliQ water and SDS (40 mM) at 10 K using a 130-unique-MS-XAFS model (Chapter 3), starting Cu-O_L bond distance of 2.00 Å and Monte Carlo calculation of errors due to noise in the collected data ($R_{\text{xaf}} = 20.5\%$, determinacy of the model (N_i/N_p) = 1.6). The refined bond distances and XAFS parameters: Cu···Cu 2.62(2) Å; Cu-O_{Ac} 1.95(2) Å; Cu-O_L 2.10(2) Å; $E_0 = 11.1$, $S_0^2 = 0.89$. Excessive noise in the XAFS data in the region greater than 10 Å⁻¹ prevented the use of a larger k -range.

¹ X-ray absorption spectra of the freshly prepared micelle solutions were recorded at the Stanford Synchrotron Radiation Laboratory (SSRL) on the focused beamline 9-3 (3 GeV, 95-55 mA) using a Si[220] double-crystal monochromator detuned 50% to reduce the flux (as the optics reject the harmonics). Data were collected as fluorescence spectra using a 30-element Ge array detector.

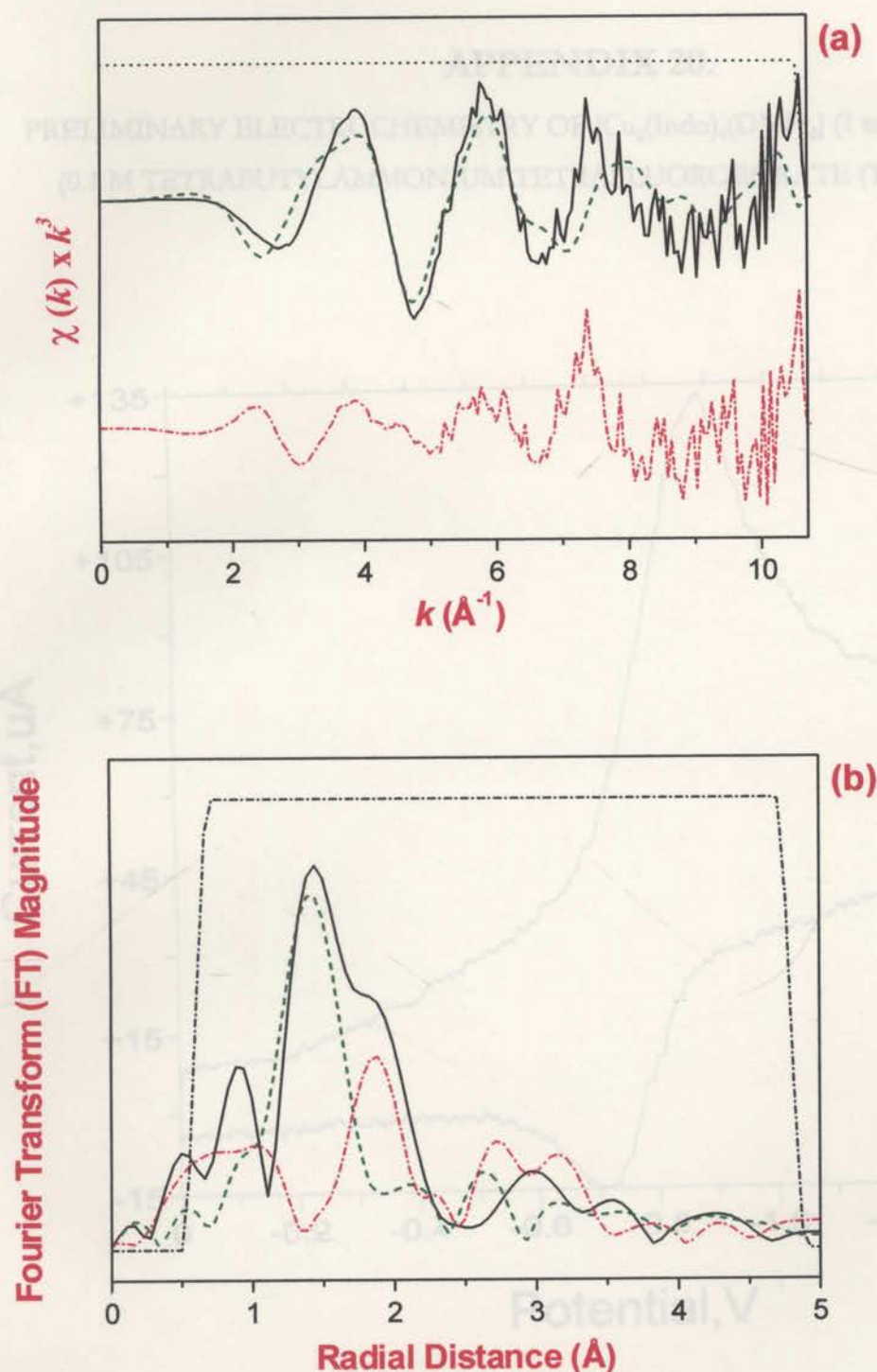


Figure A19.2. MS analysis of the Cu K-edge (a) XAFS data and (b) corresponding FT observed (—), simulated (···) and residual (---) for $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (2.35 mM) in HCl (pH 1.6) and SDS (40 mM) at 10 K using a 130-unique-MS-XAFS Cu dimer model (Chapter 3) and starting Cu- O_L bond distance of 2.00 Å ($R_{\text{xaf}} = 54\%$). The determinacy of the model = 1.9.

APPENDIX 20.

PRELIMINARY ELECTROCHEMISTRY OF $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mM) IN DMF
(0.1 M TETRABUTYLAMMONIUM TETRAFLUOROBORATE (TBATFB)).

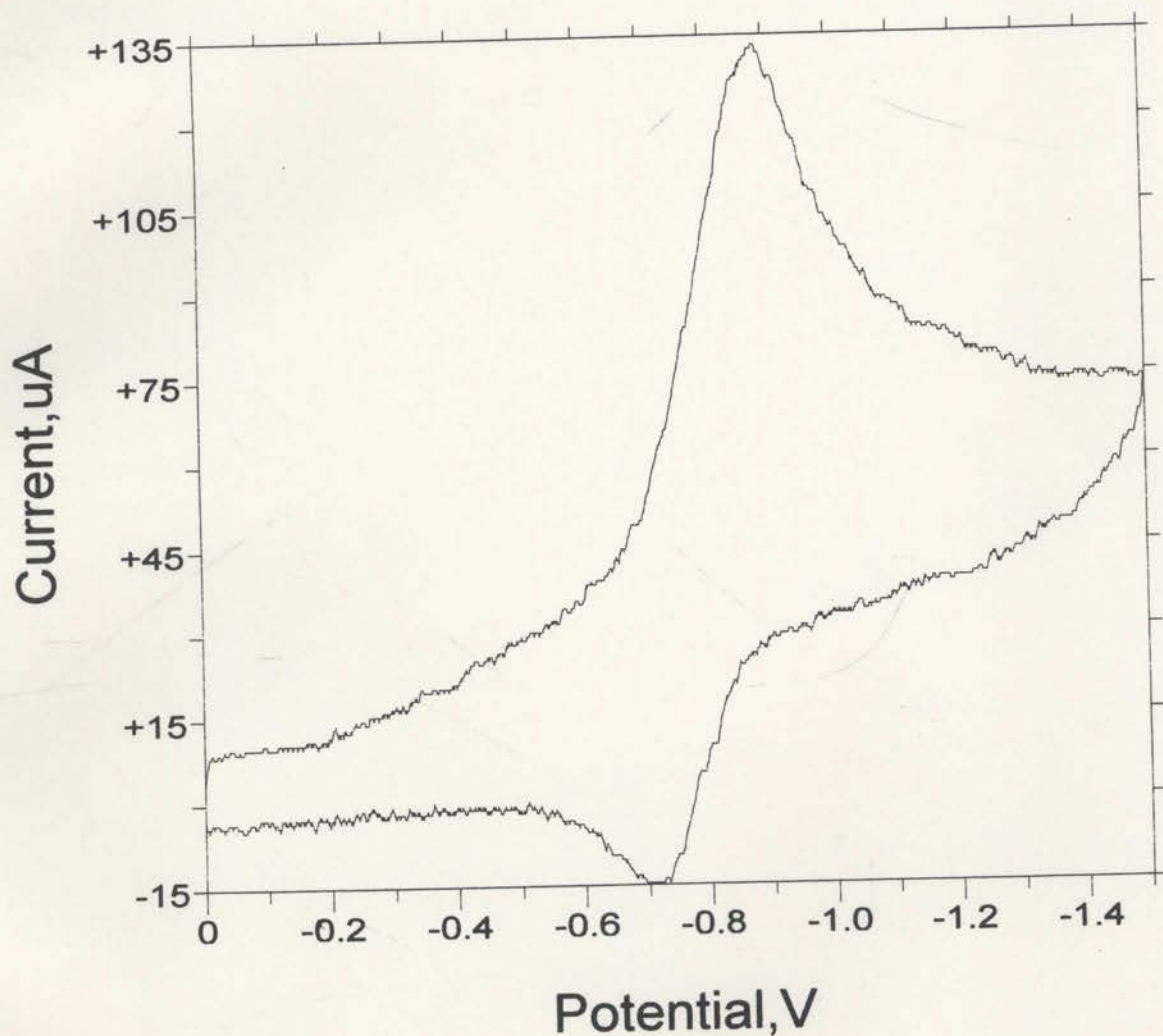


Figure A19.1. The cyclic voltammogram of $[\text{Cu}_2(\text{Indo})_4(\text{DMF})_2]$ (1 mM) measured versus Ag/AgCl/(6 M NaCl) in DMF (0.1 M TBATFB) using a Pt working electrode and a scan rate of 1000 mV s^{-1} . No iR compensation was applied. A quasi-reversible redox reaction with a reduction and re-oxidation potential (E_p) of -855 mV and -733 mV , respectively, is observed. The cyclic voltammogram of the ferrocenium/ferrocene couple, as a redox standard under the same conditions, has not as yet been measured.