



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

کلیه حقوق مادی مترتب بر نتایج مطالعات، ابتکارات و  
نوآوری های ناشی از تحقیق موضوع این پایان نامه  
متعلق به دانشگاه رازی است.



Razi University

**Faculty of Chemistry  
Department of Inorganic**

**M. Sc. Thesis**

**Title of the Thesis:**

**Interaction studies of the zinc and copper complexes of curcumin  
with calf thymus DNA using spectroscopic methods**

**Supervisors:  
Dr. N. Shahabadi**

**By:  
Fataneh khakrah**

**July 2012**

## *Acknowledgments*

Thanks God, the merciful and the passionate, for providing me the opportunity to step in the excellent world of science.

I would like to express my special thanks to Dr. Nahid Shahabadi the director of this thesis for her supervision, constructive guidance, excellent suggestion and great disposing efforts.

Special thanks go to my friends and all students in Inorganic research laboratory for providing a friendly environment during my stay in the research laboratory.

Special thanks to my dear father and to my compassionate mother for their encouragement that their prayers have been with me, and support thought the years.

Finally, I wish to express my gratitude to my partner and my dear daughter for moral support and patience during this work.

## Abstract

Curcumin is a polyphenolic diketone from turmeric. In this study two mononuclear complexes Zn(II)-Cur (1) and Cu(II)-Cur (2) (Cur = Curcumin, (1*E*,6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione) have been synthesized and characterized by elemental analysis (CHN), FT-IR, <sup>1</sup>H NMR and UV-Vis techniques. The interactions of the complexes with calf thymus DNA (CT- DNA) were investigated by absorption spectroscopy, fluorescence spectroscopy, circular dichroism and viscosity measurements. The binding constants of the complexes to CT-DNA calculated from UV-vis spectra were found to be  $1.26 \times 10^5 \text{ M}^{-1}$  ( Cu(II)- Cur) and  $6.61 \times 10^4 \text{ M}^{-1}$  ( Zn(II)- Cur) respectively. This indicating that DNA-complexes bonding have an affinity less than the classical intercalators, but it is comparable to complexes which bind to DNA mainly via groove binding mode. The binding affinity of Cu(II)-Cur complex to DNA was higher than that of Zn(II)-Cur. The results of CD spectra showed that binding of both complexes to DNA induced conformational changes in DNA (B DNA to A DNA). Moreover as the relative viscosity of CT-DNA showed an inappreciable increase in the presence of varying amounts of complexes. Fluorimetric studies showed that fluorescence enhancement was initiated by a static process in the ground state. As well as the thermodynamic data are lowering than those of previously reported for an intercalator and confirmed the non-intercalating interaction mode. Therefore experimental results suggested that both complexes interact with CT-DNA via groove-binding mode. Previous spectral data and molecular modeling calculations showed curcumin binds in the minor groove of the DNA double helix.

**Key words:** CT-DNA; Curcumin, Cu(II)-Cur complex, Zn(II)-Cur complex, Multispectroscopic techniques, Groove binding.

## Table of Contents

Contents	Page
<b>Chapter 1: Introduction</b>	
1. 1. Bioinorganic Chemistry.....	2
1.2. Medicinal Chemistry.....	3
1.3. Medicinal Inorganic Chemistry.....	3
1.4. Copper.....	4
1.4.1. Biological Application of Copper.....	4
1.4.2. Copper containing agents.....	5
1.5. Zinc.....	8
1.5.1. Biological applications of zinc.....	8
1.5.2. Zinc containing anticancer agent.....	9
1.6. Characteristics of DNA.....	10
1.6.1. DNA Forms.....	12
1.6.1.1. The B Form of DNA.....	13
1.6.1.2. The A Form of DNA.....	15
1.6.1.3. The Z Form of DNA.....	16
1.6.1.4. The H Form of DNA.....	16
1.6.1.5. Other Forms of DNA.....	17
1.7. Fundamental Interactions with Nucleic Acids.....	18
1.7.1. Covalent Interaction.....	17
1.7.1.1 Nucleophilic Sites Targeted by Soft Metal Ions on the bases..	18
1.7.2. Noncovalent Binding Modes.....	18
1.7.2.1. Eelectrostatic interactions.....	19
1.7.2.2. Groove binding.....	20
1.7.2.3. Classical intercalation.....	22
1.8. Curcumin.....	24
1.8.1. Chemical composition of turmeric.....	25
1.8.2. History and traditional uses of curcumin.....	26
1.8.3. Curcumin chemistry.....	27
1.8.4. Structure and pharmacology.....	27
1.8.5. Metal-binding chemistry.....	29

<b>Contents</b>	<b>Page</b>
1.8.6. Anticancer effects.....	30
1.8.7. Anti Alzheimer.....	31
1.8.7.1. Mechanism of action of curcumin on Alzheimer's disease.....	32
1.8.8. Structure–activity relationship of curcumin.....	34
1.8.9. Curcumin interaction with copper and Zinc suggests one possible mechanism of action in Alzheimer’s disease.....	35
1.9. Analytical methods for investigating Complexes/ DNA interactions.....	35
1.9.1. Fluorescence spectroscopy.....	36
1.9.1.1. Applications of fluorescence spectroscopy.....	39
1.10. Simple theory of the absorption of light by molecules.....	40
1.10.1. Parameters measured in absorption spectroscopy.....	41
1.11. Circular dichroism spectroscopy.....	41
1.11.1. Simple theory of CD.....	41
1.11.2. Determination of the conformation of nucleic acids by electronic CD .....	44
1.12 Viscosimetry.....	45
 <b>Chapter 2: Experimental</b>	
2.1. Materials .....	49
2.2. Apparatus.....	51
2.3. Various section of this study.....	51
2.4. Synthesis of the complexes.....	52
2.4.1. Zinc(II) curcumin complex.....	52
2.4.2. Copper(II) curcumin complex.....	52
2.5. Preparation of buffers.....	52
2.6. Preparation of DNA, Zn(II) and Cu(II) complexes solutions interaction...	53
2.7. Method.....	53
2.7.1. UV-vis absorption measurements.....	53
2.7.2. Fluorescence measurements.....	54
2.7.3. Viscosity measurements.....	55
2.7.4. Circular dichroism measurements.....	55



<b>Contents</b>	<b>Page</b>
<b>Chapter 3: Results and Discussion</b>	
3.1. Scope of This Chapter.....	57
3.2. Synthesis and characterization of complexes .....	58
3.3. DNA interactions studies of Zn(II) and Cu(II) complexes.....	65
3.3.1. UV-Vis spectroscopy.....	65
3.3.1.1. Binding constant determination.....	67
3.3.2. Circular dichroism spectroscopy studies.....	70
3.3.3. Viscosity measurements.....	73
3.3.4. Fluorescence Studies.....	75
3.3.4.1. Equilibrium Binding Titration.....	79
3.3.4.2. Thermodynamic Studies.....	82
3.4. Conclusions.....	85
3.5. Suggestions.....	87
<b>References.....</b>	<b>88</b>

## List of Figures

Contents	Page
Figure.1.1. Molecular structure of A) appc and B) atpc.....	5
Figure.1.2. Molecular structures of copper carboxamidrazones.....	6
Figure.1.3. Molecular structure of 5-amino-1-tolyimidazole-4-carboxylic acid.....	6
Figure.1.4. Molecular structure of Bis(5-amino-1-tolyimidazole-4-carboxylate) Cu(II).....	7
Figure.1.5. The normal right-handed "double helix" structure of DNA.....	10
Figure.1.6. Structures of the major pyrimidine and purine bases of DNA.....	11
Figure.1.7. watson-crick hydrogen bonds.....	12
Figure.1.8. Major secondary structures of DNA.....	13
Figure.1.9. Structure of DNA double helix showing major and minor grooves.....	15
Figure.1.10. Intra molecular triplex DNA (H DNA) form.....	17
Figure.1.11. This figure presentation of electrostatic interaction of a complex with DNA .....	20
Figure.1.12. Representative chemical structures of groove binding agents. The compounds as shown are protonated under physiological conditions of pH(up), crystal structure of netropsin binding to the minor groove of d(CGCAAATTTGCG) (left) and major groove(right)... ..	21
Figure.1.13. Groove binding of Hoescht 33258 to the minor groove of DNA (left) and the intercalation of ellipticine into DNA (right).....	22
Figure.1.14. Structures of DNA intercalating dyes.....	23
Figure.1.15. showing a molecule intercalated between two base pairs in a molecule of double-helical DNA.....	24
Figure.1.16. Medicinal properties of curcumin.....	25
Figure.1.17. Photo of rhizomes of <i>Curcuma longa</i> Linn plant and chemical structure of polyphenolic curcumin compound.....	26
Figure.1.18. Curcumin I,II, III (curcumin, demethoxycurcumin, bisdemethoxycurcumin), and keto- enoltautomers of curcumin.....	29
Figure.1.19. Nomenclature of regions of curcumin I. (A) $\beta$ -diketone or keto-enol; (B) phenolic; (C) alkene linker.....	30
Figure.1.20. Antitumor properties of curcumin.....	31

<b>Contents</b>	<b>Page</b>
Figure.1.21. Neuritic plaques are one of the characteristic structural abnormalities found in The brains of Alzheimer patients.....	32
Figure.1.22. Different mechanisms of action of curcumin in AD.....	33
Figure.1.23. Transitions giving rise to absorption and fluorescence emission pectra.....	37
Figure.1.24. Idealised absorption and emission spectra.....	38
Figure.1.25. Structures of common extrinsic fluors.....	40
Figure.1.26 Propagation of an electromagnetic wave through apace . The E and H vectors are mutually perpendicular at all times. ....	41
Figure.1.27. Production of plane-polarized light. A collection of waves falls on the polarizer, which passes only those components of the E vectors that are parallel to the axis of the polarizer.....	42
Figure.1.28. Generation of circularly polarized light. The E vectors of two electromagnetic waves are one-quarter wavelength out of phase and are perpendicular. The vector that is the sum of the E vectors of the two components rotates so that its tip follows a helical path (dotted line).....	43
Figure.1.29. Diagrams showing how right and left circularly polarized light combine: (A) if the two waves have the same amplitude, the result is plane-polarized light; and (B) if their amplitudes differ, the result is elliptically polarized light –that is, the head of the resultant vector will trace the ellipse shown as a dashed line. The lengths of the major and minor axes of the ellipse are a and b.....	44
Figure.1.30 .The length of DNA as a function of acridine orange concentration, as determined by electron microscopy.....	46
Figure.1.31. Ostwald Capillary vicometer.....	47
Figure.3.1. The chemical reactions and molecular structures of Zn(II) and Cu(II)-Cur complexes.....	59
Figure.3.2. UV-Vis spectra of curcumin, Zn(II)-Cur and Cu(II)-Cur complexes.....	60
Figure.3.3. IR spectrum of curcumin (A) Zn (II)-Cur (B) and Cu (II)-Cur (C) complexes...61	61
Figure.3.4. <sup>1</sup> HNMR spectra of curcumin.....	63

<b>Contents</b>	<b>Page</b>
Figure.3.4.1. <sup>1</sup> H NMR spectra of Zn(II)-Cur (A) and Cu(II)-Cur (B) complexes.....	64
Figure.3.5. <sup>1</sup> H NMR labeling of curcumin.....	64
Figure.3.6. Absorption spectrum of Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes (5×10 <sup>-5</sup> M <sup>-1</sup> ), in the absence and presence of increasing amounts of CT-DNA.....	67
Figure.3.7. The plot of [DNA]/(ε <sub>a</sub> -ε <sub>f</sub> ) versus [DNA] for Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes.....	69
Figure.3.8. Circular dichroism spectra of CT-DNA (5× 10 <sup>-5</sup> M <sup>-1</sup> ) in Tris-HCl (50 mM) in the presence of increasing amounts of Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes .....	73
Figure.3.9. Effect of increasing amounts of Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes on the Viscosity of CT-DNA (5 × 10 <sup>-5</sup> M) in 50 mM Tris buffer.....	75
Figure.3.10. Fluorescence spectra of the Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes in the absence and presence of the increasing amounts of DNA (solid lines) in aqueous solution at 25 °C.....	78
Figure.3.11. Stern–Volmer plot for the observed fluorescence enhancement of Cu(II)-Cur (A) and Zn(II)-Cur (B) complexes upon addition of CT-DNA at different temperatures.....	79
Figure.3.12. Van't Hoff plot for the interaction of DNA with Cu(II)-Cur complex (A) and Zn(II)-Cur complex (B) at pH 7.4.....	84
Figure.3.13. Relative spatial positions of two curcumin molecules bound adjacent to each other in the minor groove of the right - handed helix (sugar - phosphate backbones:yellow; base pairs: dark blue).....	87

## List of Tables

<b>Contents</b>	<b>Page</b>
Table.3.1. Elemental analysis data for Zn(II)-Cur and Cu(II)-Cur complexes.....	62
Table.3.2. The <sup>1</sup> H NMR data of curcumin and Cu(II) and Zn(II)-Cur complexes in CH <sub>3</sub> CH <sub>2</sub> OH.....	62
Table.3.3. The comparison of K <sub>b</sub> values of Cu(II), Zn(II)-Cur complexes, curcumin, and analogs of curcumin with CT-DNA.....	70
Table.3.4. Dynamic enhancement and bimolecular enhancement constants of Cu-Cur-DNA and Zn-Cur-DNA complexes at different temperatures .....	81
Table.3.5. Binding constants (K <sub>f</sub> ) and number of binding sites (n) of Cu-Cur-DNA and Zn- Cur-DNA complexes at different temperatures.....	81
Table.3.6. Termodinamics parameters of binding Cu(II)-Cur and Zn(II)-Cur complexes to CT- DNA.....	85

## Abbreviations and Symbols

LUMO	lowest unoccupied molecular orbital
HUMO	highest unoccupied molecular orbital
CT – DNA	calf thymu DNA
CD	circular dichroism
$K_D$	dynamic enhancement constant
$K_B$	bimolecular enhancement constant
$K_F$	binding constant
$\tau_0$	lifetime
MLCT	Metal-to-ligand charge transfer
Cur	Curcumin
AD	Alzheimer's disease
acac–	Acetylacetonate
appc	2-acetylpyridine-pyridine-2-carboxamidrazone
atpc	2-acetylthiophene-pyridine-2-carboxamidrazone

# **Chapter One**

## **Introduction**

## 1.1. Bioinorganic chemistry

Bioinorganic chemistry is best considered as understanding all aspects of the role of metal ions in biology and has been traditionally heavily involved in understanding their processing, incorporation into protein and the nature and function of metalloproteins. Advances in our understanding of how cells process metals and the genetic basis of disease is naturally expanding the traditional directions of bioinorganic chemistry toward an appreciation of its medical importance especially with respect to the role of metalloproteins in human health and disease [1].

Inorganic or metal-containing medicinal compounds may contain either (a) chemical elements essential to life forms-iron salts used in the treatment of anemia- or (b) nonessential/toxic elements that carry out specific medicinal purposes- platinum containing compounds as antitumor agents or technetium and gadolinium complexes as medicinal diagnostic tools. Introducing metal ions into a biological system may be carried out for therapeutic or diagnostic purposes, although these purposes overlap in many cases.

In 1991, Peter Sadler noted that most elements of the periodic table, up to and including bismuth with an atomic number of 83, have potential uses as drugs or diagnostic agents. Inorganic compounds have found usage in chemotherapeutic agents such as [2]:

1. Anticancer agents like cis-[Pt (NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>]
2. The gold-containing antiarthritic drug Auranofin.
3. Metal-mediated antibiotics like bleomycin, which requires iron or other metals for activity
4. Technetium-99m and other short-lived isotopes used as radiopharmaceuticals in disease diagnosis and treatment.



5. Magnetic resonance imaging (MRI)-enhancing gadolinium compounds.

6. Antibacterials, antivirals, antiparasitics, and radiosensitizing agents.

## **1.2. Medicinal chemistry**

Medicinal chemistry involves the study of the interaction of drugs with biological systems at the molecular level, and the design and synthesis of such drugs. Medicinal chemistry requires intimate knowledge of the metabolism and stability, as well as target interactions of the drug [1].

Medicinal applications of metals can be traced to almost 5000 years back but the lack of experience of traditional medicinal chemists and pharmacologists in dealing with biologically active metal complexes, poses a substantial activation energy barrier to their identifying active metal complexes and shepherding them to the clinic. This factor retards the development of metallo-pharmaceuticals. However, it provides enterprising transition metal chemists with opportunities to pioneer the development of exciting new drugs [3].

## **1.3. Medicinal inorganic chemistry**

Inorganic chemistry is playing a role in the biotechnology revolution currently on going worldwide. AnorMed and Kinetek pharmaceuticals, in Canada, currently have metal complexes in clinical trials. The field of inorganic chemistry in medicine may usefully be divided into two main categories - drugs which target metal ions in some form, whether free or protein-bound, and secondly, metal-based drugs where the central metal ion is usually the key feature of the mechanism of action. Metal-based drugs are a commercially important sector of the pharmaceutical business. Applications continue to grow and approaches to further clinically useful agents are ever more sophisticated [1].

## 1.4. Copper

It is the 29th element on the Periodic Table, located between nickel and zinc in the first row of transition elements. Copper has eleven known isotopes, of which only two,  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$ , are present in significant amounts, with natural abundances of 30.91 and 69.09% respectively, resulting in an atomic weight of 63.546. The ground state electronic configuration of elemental copper is  $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^1$  or  $[\text{Ar}] 3d^{10} 4s^1$  [4]. Common oxidation states of copper include the less stable copper (I) state,  $\text{Cu}^+$ ; and the more stable copper (II) state,  $\text{Cu}^{2+}$ , which forms blue or blue-green salts and solutions. Under unusual conditions, a +3 state and even an extremely rare +4 state can be obtained [4].

### 1.4.1. Biological application of copper

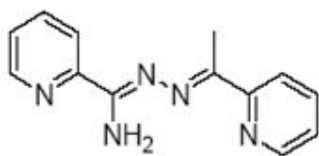
Though the use of copper dates back to antiquity, the recognition of its biological importance is of a more recent origin. Copper has been found to be required essential trace element for the normal functioning of plants, animals and most microorganisms. As a trace metal, it is present in tissues and fluids at parts per million (ppm) or parts per billion (ppb) concentrations [5]. Issues pertaining to the absorption, transport, and function of Cu in the body may be relevant to an investigation of the pharmacology and biodistribution of Cu-NSAIDs. The following section briefly discusses the various biological role of Cu, its biodistribution and its function in inflammation. Copper was first shown to be an essential biological element in the 1920s when anemia was found to result from Cu-deficient diets in animals [6] and addition of Cu salts corrected this affliction [6,7]. It is now recognized as an essential trace element for many biological functions [8]. It serves as a catalytic component in many enzymes, e.g. it is an important constituent of metalloproteins (exhibiting oxidative reductase activity, e.g. oxidases or hydroxylases) [9], and in such enzymes as lysyloxidase (required for connective tissue) and cytochrome oxidase (electron

transport protein) [10]. Copper also influences specific gene expression in mammalian cells [11,12], nerve myelation and endorphin action [13], with Cu deficiency impairing immunity [14-16]. The role of trace metallic elements, such as Cu in inflammation, is of great interest given their function as co-factors in metabolic processes involving articular/connective tissue and the immune system [17] and their effect on PG synthesis [18,19-22].

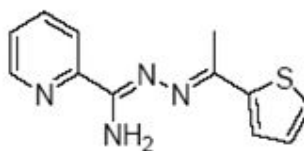
### 1.4.2. Copper containing anticancer agents

The use of copper for the treatment of cancer dates back to early 1980 with the report by Petering [23] on the activity of copper thiosemicarbazones. More recent work has involved the use of copper complexes of carboxamidrazones [24] and carboxylates [25]. The carboxamidrazones are interesting because they have similar structures to the thiosemicarbazones which were the first copper complexes to be reported for anticancer activity. Both classes of copper complexes have been explored by Padhye et al [24,25]. The carboxamidrazone chemistry involves the use of the ligands 2-acetylpyridine-pyridine-2-carboxamidrazone (appc) and 2-acetylthiophene-pyridine-2-carboxamidrazone (atpc). These ligands are readily synthesized by refluxing pyridine-2-carboxamidrazone with excess 2-acetyl pyridine or 2-acetyl thiophene, respectively, in EtOH for 2 hours. The molecular structures of appc and atpc are shown in (Fig1.1).

The copper complexes of these ligands were also readily synthesized by adding equimolar amounts of appc or atpc and CuCl<sub>2</sub> dihydrate to methanol and refluxing for 1 hour. The products precipitated out of solution as dark green crystalline material. The molecular structures of [Cu (appc) Cl<sub>2</sub>], A, and [Cu (atpc) Cl<sub>2</sub>], B, are shown in (Fig1.2).

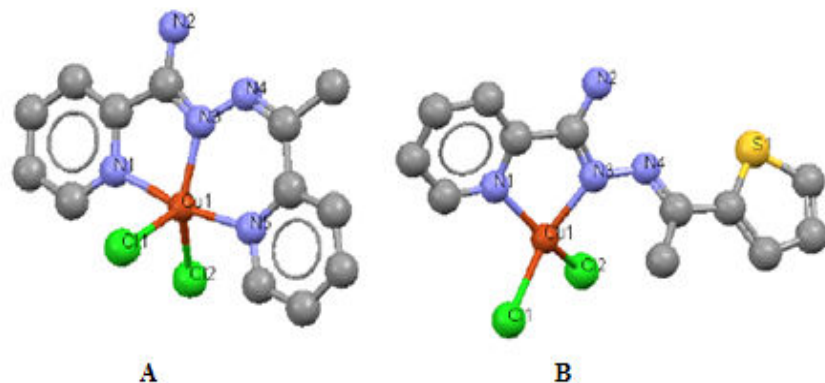


A



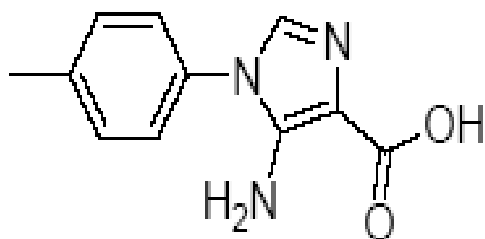
B

**Fig. 1.1. Molecular Structure of A) appc and B) atpc.**



**Fig. 1.2. Molecular Structures of Copper Carboxamidrazones.**

The copper carboxylate chemistry involves the use of the ligand 5-amino-1-tolylimidazole-4-carboxylic acid, shown in (Fig 1.3), which can readily be obtained by the alkaline hydrolysis of ethyl-5-amino-1-tolylimidazole-4-carboxylate. The copper complex was then synthesized by reacting two equivalents of 5-amino-1-tolylimidazole-4-carboxylic acid with one equivalent of copper nitrate in methanol at pH 7. The product precipitates as the pure green solid shown in (Fig1.4).



**Fig. 1.3. Molecular Structure of 5-amino-1-tolylimidazole-4-carboxylic acid.**