

CHAPTER 1

Introduction

1.1 Introduction

Several metals are essential for proper functioning of living organisms while others are nonessential. Some nonessential metals are known to be toxic, even at very low levels (cadmium, lead, mercury, and arsenic), whereas metals which are biologically essential may also become hazardous at high levels (iron, zinc, and copper). Nonessential metals are suspected to enter metabolic pathways of chemically similar essential elements, thus having the potential to interfere with the normal functions of the latter. Reciprocally, essential metals can modify the adverse effects of toxic elements. Such metal-metal competitions are critical modifiers of metal toxicity and, from that respect, cadmium-zinc or cadmium-iron interactions are well documented examples (CNTC, 2005).

The two major symptoms of metal and chemical toxicity are the breakdown of the immune system, which encourages numerous diseases into the body, and damage to the organs and central nervous system. Toxic metals can be found in most soft tissues, especially the liver and kidneys, as well as in bones. Lipid-soluble metals may also cross the blood-brain barrier, a phenomenon that explains why methyl mercury, but not inorganic mercury, greatly affects the central nervous system. Placenta is another target tissue especially for lead, cadmium and mercury. Once absorbed, heavy metals are often poorly eliminated and the half-life of most of them in the human body is quite long (about 30 years for cadmium). As routes of excretion include milk secretion, metals can be transferred to newborns via lactation (CNTC,

2005). The primary treatment for most heavy metals is *chelation therapy*. Chelating agents are substances that go through the body to attract and bind heavy metals to be excreted.

Turmeric (*Curcuma longa* Linn., Zingiberaceae) is widely used in traditional oriental medicine, especially in India, China and also in Thailand. It has traditionally been used to treat many diseases including anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis and externally in the prevention and treatment of skin diseases (Jayaprakasha, *et al.*, 2005). According to scientific research, curcumin (Figure 1) the main yellow bioactive component of turmeric has been shown to have a wide spectrum of pharmacological effects including anti-carcinogenic, anti-inflammatory, antioxidant (Zsila, *et al.*, 2003a) and Alzheimer's prevention (Shen, *et al.*, 2005).

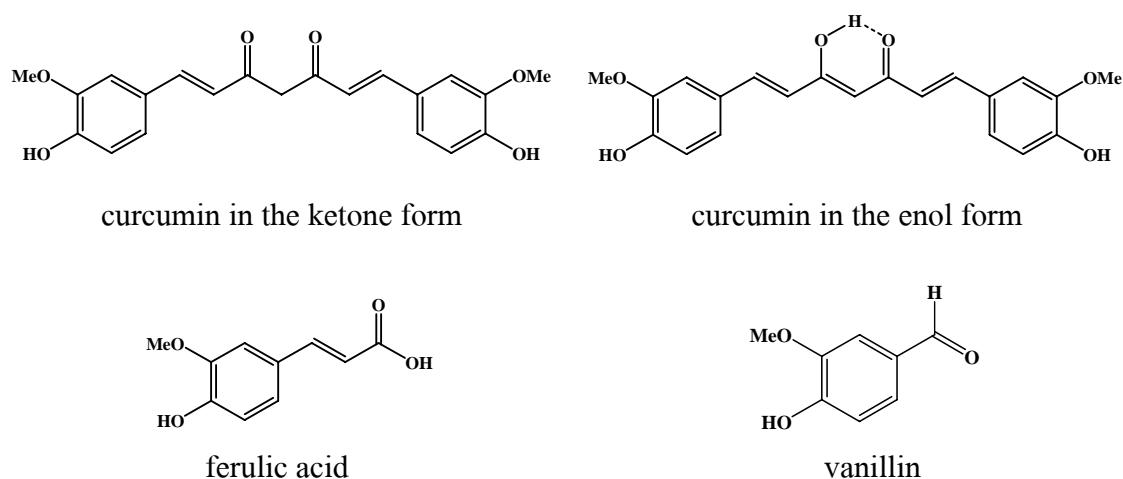


Figure 1 Structure of curcumin in the ketone and enol forms, ferulic acid, and vanillin

According to the chemical structure of curcumin {1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione}, the β -diketone moiety, curcumin can be a powerful natural chelating agent with its safety evaluation even administered at high

dose in human (Cotton and Wilkinson, 1980; Borsari, *et al.*, 2002; Chattopadhyay, 2004). The stability of curcumin chelation to the metal ion can be estimated by the complex formation constant (K). The higher the constant, the greater the affinity of the metal to the chelating agent and the more it is complexed. Complex formation constants are expressed as the logarithm or $\log K$ value. The higher the number of $\log K$ for a metal ion, the higher the affinity it is bound to the chelating agent. The $\log K$ values are useful for chelation therapy.

The present study focused on the complex formation constants of curcumin with as many metal ions as possible. A number of research groups have previously used UV-Vis spectroscopy to determine the complex formation (Bernabé-Pineda, *et al.*, 2004, Barik, *et al.*, 2005, Sundaryono, *et al.*, 2003). Their spectrophotometric data were fitted with commercial fitting program (such as SQUAD and LETAGROP-SPEFO) to generate the K values (Bernabé-Pineda, *et al.*, 2004, Sundaryono, *et al.*, 2003). Instead of using the expensive commercial program, this study attempted to correct the calibration curves to calculate the residual free ligand at the equilibrium, and the absorption of the residual free ligand was subtracted to generate the exact absorption of the complexes. Then the complex formation constants were calculated. All of these procedures were carried out with the basic Microsoft Window[®] program, the Microsoft[®] Office Excel.

1.2 Review of Literatures

1.2.1 Turmeric

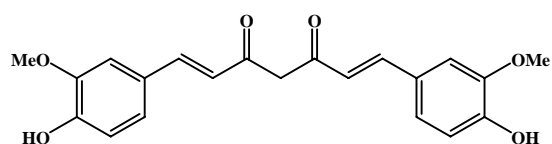
Turmeric (*Curcuma longa* L.) is a stemless rhizomatous herb; having rhizome fleshy, much branched, bright orange or yellow within and scented. Leaves emerge directly from the underground stem with overlapping petioles 8-15 cm long or more. They are light green, 30-40 by 8-110 cm, having thin ellipse-shaped or elongate lance-shaped blades. A cylindrical inflorescence about 10-15 by 5-7 cm, appears with the leaves and develops in their centre. It consists of large pale green, pouch like, curved bracts, each with two or more pale yellow flowers except in the upper part, where the bracts are white and green or pink and without flowers. The tube-shaped calyx is splitted in one side to unequally teeth. The corolla-tube is more or less funnel-shaped, not exerted beyond the bract, with 3-lobed limb and white. The lateral staminode petaloid is rather long and folded under the dorsal petal. There is a central yellow band at the labellum. A fertile stamen with short filament, broad and constricted at the apex is found in the floret. The anther is versatile and unusually spurred at the base; sometime with a small crest at the connective. The ovary consists of 3-locules, each locule contains 2 ovules. The capsules are ellipsoid. Seeds are rare (Bunyaphatsara, 1992).

Turmeric was described as *C. longa* by Linnaeus and its taxonomic position is as follows:

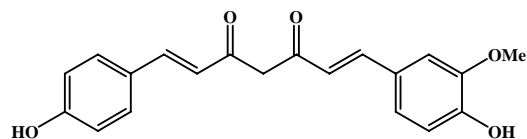
Class	Liliopsida
Subclass	Commelinids
Order	Zingiberales
Family	Zingiberaceae
Genus	<i>Curcuma</i>
Species	<i>Curcuma longa</i>

the wild turmeric is called *C. aromatica* and the domestic species is called *C. longa*. (Chattopadhyay, *et al.*, 2004)

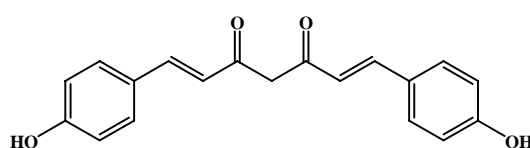
Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has α -phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%), and sesquiterpines (53%). Curcumin (difurulylmethane) (3-4%) is responsible for the yellow color, and comprises curcumin I (94%), curcumin II (6%), and curcumin III (0.3%) (Chattopadhyay, 2004).



Curcumin (curcumin I)



Demethoxycurcumin (curcumin II)



Bisdemethoxycurcumin (curcumin III)

Figure 2 Chemical structures of curcumin and curcumin derivatives

Several pharmacological activities and medicinal application of turmeric are known. Although curcumin has been isolated in the 19th century, extracts of the rhizomes of *C. longa* have been in used from the Vedic ages. Some of the medicinal applications of turmeric are mentioned in Table 1 (Chattopadhyay, 2004).

Table 1 Medicinal properties of turmeric (Chattopadhyay, 2004)

Turmeric finds medicinal applications in	Anemia, atherosclerosis, diabetes, edema, hemorrhoids, hepatitis, hysteria, indigestion, inflammation, skin disease, urinary disease, wound and bruise healing, psoriasis, anorexia, cough, liver disorders, rheumatism, sinusitis
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Turmeric powder, curcumin and its derivative, and many other extracts from the rhizomes were found to be bioactive. Some of biological activities of turmeric and its compounds were compiled by Chattopadhyay, are shown in Table 2 (Chattopadhyay, 2004).

Table 2 Biological activity of turmeric and its compounds (Chattopadhyay, 2004)

Compound/extract	Biological activity
Turmeric powder ethanol extract	Wound-healing
	Anti-inflammatory
	Hypolipemic
	Antitumour
	Antiprotozoan
Petroleum ether extract	Anti-inflammatory
	Antifertility
Alcoholic extract	Antibacterial
Chloroform extract	Antifungal
Aqueous extract	Antifertility
Volatile oil	Antiinflammatory
	Antibacterial
	Antifertility

Table 2 Biological activity of turmeric and its compounds (continued)

Compound/extract	Biological activity
Curcumin	Antibacterial
	Antiprotozoan
	Antiviral
	Hypolipemic
	Hypoglycemic
	Anticoagulant
	Antioxidant
	Antitumour
	Anticarcinogenic
Ar-turmerone	Antivenom
Methylcurcumin	Antiprotozoan
Demethoxycurcumin	Antioxidant
Bisdemethoxycurcumin	Antioxidant
Sodium curcuminates	Antiinflammatory
	Antibacterial

1.2.2 Curcumin

Curcumin, the major constituent of *Curcuma longa* L. (turmeric), has been first isolated as early as 1815 by Vogel and Pelletier, but it has attracted few modern studies. In 1870, it was crystallized by Daube and the structure was elucidated in 1910 by Lampe and co-workers, who later completed a synthesis. Renewed interest has been evoked by the recent discovery of relative sharing the 1,7-diaryl skeleton (Roughley and Whiting, 1973).

Curcumin has a molecular weight of 368.37 and a melting point of 183°C. Commercial grade curcumin contains the curcuminoids demethoxycurcumin (MW 338; typically 10–20%) and bisdemethoxycurcumin (MW 308; typically less than 5%) (Sharma, *et al.*, 2005). On UV-Vis spectrophotometric investigation, maximum light absorption of curcumin occurs around 400-430 nm depends on organic solvent (Jasim and Ali, 1989). Curcumin is relatively insoluble in water (Sharma, *et al.*, 2005), but best dissolves in methanol followed by acetone, normal amyl methyl ketone, ethanol, tetrahydrofuran, acetylacetone, chloroform, acetic acid, dimethyl sulfoxide, benzene, toluene, and CCl₄ (Jamis and Ali, 1989). The curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione) molecule and the related curcuminoid family resembles β -carotene in that they contain a conjugated section (in its enol form) with terminal rings, although curcumin contains phenyl rather than cyclohexenyl rings. Curcumin also contains carbonyl, methoxy and hydroxyl groups which may contribute to its activity, placing it in the category of phenolic antioxidant (Wright, 2002). Studies of Hang and Sreejayan in preclinical models of carcinogenesis have demonstrated that commercial grade curcumin has the same inhibitory effects as pure curcumin (Sharma, *et al.*, 2005). It is not known whether essential oils derived from *Curcuma* spp. have intrinsic activity akin to curcumin reported by Mau and co-worker (Sharma, *et al.*, 2005).

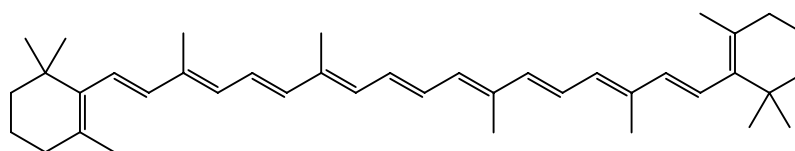


Figure 3 Chemical structure of β -carotene

1.2.2.1 Safety evaluation of turmeric and curcumin

The average intake of turmeric by Asians varies from 0.5 to 1.5 g/day/person, which produces no toxic symptoms. Male and female Wistar rats, guinea pigs and monkeys were fed with turmeric at much higher doses (2.5 g/kg body weight) than normally consumed by humans. No changes were observed in the appearance and weight of kidney, liver and heart. Also, no pathological or behavioral abnormalities were noticed and mortality were observed. No pathological, behavioral abnormalities or lethality was observed when curcumin was given to Wistar rats, guinea pigs and monkeys of both sexes at a dose of 300 mg/kg body weight. No adverse effects were observed on both growth and the level of erythrocytes, leukocytes, blood constituents such as haemoglobin, total serum protein, alkaline phosphatase, etc. Human clinical trials also indicate that curcumin has no toxicity when administered at dose of 1-10 g/day (Chattopadhyay, 2004).

1.2.2.2 Influence of pH

Curcumin is a bis- α,β -unsaturated β -diketone. As such, curcumin exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane (Wang, *et al.*, 1997). At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor (Jovanovic, *et al.*, 1999). This is because, in the keto form, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C–H carbon bonds on this carbon are very weak due to delocalization of the unpaired electron on the adjacent oxygens (Sharma, *et al.*, 2005). In contrast, above pH 8, the enolate form of the heptadienone chain predominates, and curcumin acts mainly as an electron donor. A mechanism more typical for the scavenging activity of phenolic antioxidants suggested by Jovanovic and co-worker (Sharma, *et al.*, 2005).

Dyrssen and co-worker studied on the acid dissociation constant of curcumin in aqueous ethanolic media. The UV-Vis spectra at different pH values of 1×10^{-5} mol/L of curcumin solution in 1:1 ethanol-water solution are shown in Figure 4. The shifts indicates that several acid-base equilibria must be involved. Starting with the neutral form (H_3Cur), the absorbance had a maximum at 430 nm for a pH of 3-7. When more acid was added, a maximum absorption band shifted to 555 nm with an isosbestic point at 470 nm. If, instead, alkali was added to the neutral solution, another maximum absorption band appeared at about 520 nm. Owing to decomposition, the alkali shifted the maximum to shorter wavelength, *ca.* 480 nm, the curcumin being quite unstable. Addition to a solution until pH 12 did not reverse the last reaction. The behavior of curcumin at different pH stages can be summarized in Figure 5 (Dyrssen, *et al.*, 1972).

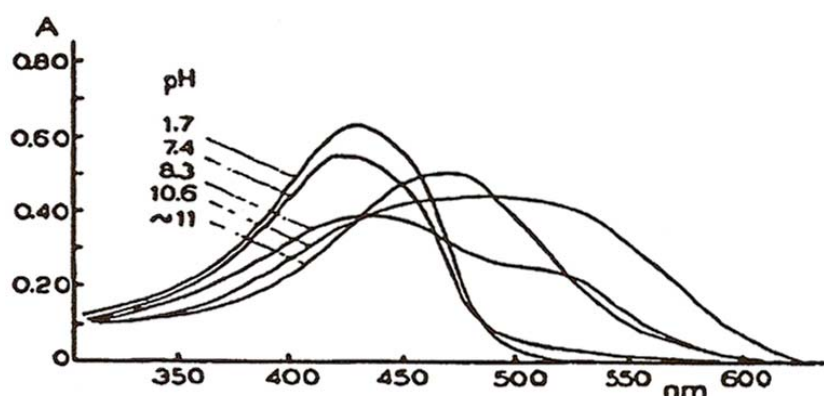


Figure 4 The absorbance of curcumin in a 1:1 water-ethanol solution measured at different pH values (Dyrssen, *et al.*, 1972)

Curcumin in enol form has three ionizable protons, corresponding to the enol group and two phenolic groups. The theoretical study by Shen and Ji showed the calculated absorption maximum at 419 nm for the enol form. This absorption is red shifted in comparison with that of the diketone form. The calculated absorption maximum of enolic proton dissociation is about 429 nm. On the other hand, the

dissociation of phenolic proton gives a large bathochromic shift in the absorption maximum to 531 nm (Shen and Ji, 2007). In accordance with the study of Zsila and co-worker, acid dissociation of the phenolic proton of curcumin causing a large red shift of the main absorption band, and the deprotonation of second phenolic and enolic play only secondary role in spectral changes (16 and 12 nm red shift) (Zsila, *et al.*, 2003a; Zsila, *et al.*, 2003b).

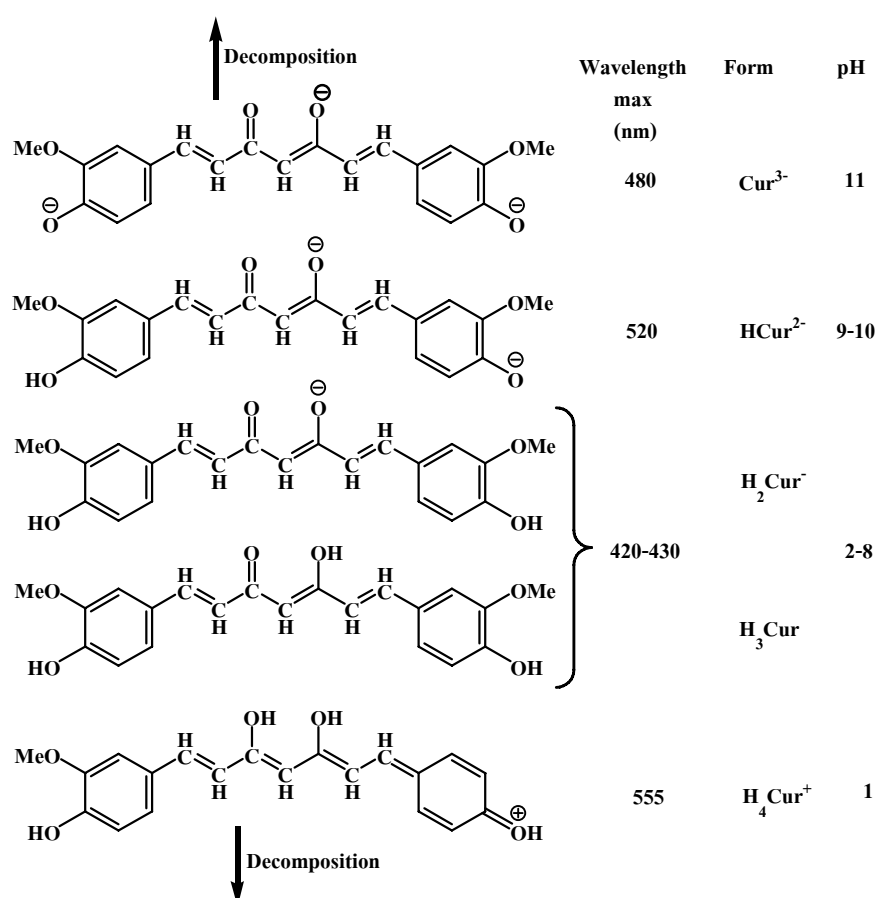


Figure 5 The behavior of curcumin at different pH stages (Dyrssen, *et al.*, 1972)

Curcumin behavior in acid and basic aqueous media was studied by Bernabé-Pineda and co-worker. Curcumin in basic media had a red color with the maximum absorption bands were observed at 463 and 261 nm along with the presence of a third shoulder-like signal at 360 nm. While in acid media, it had a yellow color

with the maximum absorption bands showed a hypsochromic shift in the main signal from 463 nm in basic media to 422 nm in acid media (Bernabé-Pineda, *et al.*, 2004).

Jasim and Ali studied the UV-Vis spectra of curcumin in 12 polar and nonpolar organic solvents, such as methanol, acetone, normal amyl methyl ketone (NAMK), ethanol, tetrahydrofuran (THF), acetylacetone, chloroform, acetic acid, dimethyl sulfoxide (DMS), benzene, toluene, and CCl₄. The pH versus λ_{\max} plot indicated all systems were stable at pH 5.5-7.5 (Jasim and Ali, 1989).

1.2.2.3 Stability of curcumin solution

Tønnesen and Wang reported that curcumin was unstable at basic pH and underwent alkaline hydrolysis in alkali (higher pH) solution. Hydrolytic decomposition was reported even in *in vitro* physiological condition (isotonic phosphate buffer, pH 7.2). It underwent photodegradation when being exposed to light in solution as well as in solid form (Ansari, *et al.*, 2005). Their report went on that in basic pH solution, curcumin was unstable and was degraded within 30 min to *trans*-6(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin. Under acidic conditions, the degradation of curcumin was much slower, with less than 20% of total curcumin decomposed at 1 hour (Wang, *et al.*, 1997).

Curcumin has a poor light stability, about 5% decrease in absorbance due to curcumin has been measured during the time for typical sample preparation when clear rather than amber glassware is used. Curcumin decomposed when being exposed to sunlight, both in ethanolic and methanolic extracts and as a solid. Vanillin, vanillic acid, ferulic aldehyde and ferulic acid were identified as the degradation products (Wang, *et al.*, 1997).

To determine the effect of the UV radiation (using a xenon arc, 150 Watt lamp as a source) on the stability of various curcumin systems, the latter were exposed for 60 min, and no shift in λ_{\max} was detected. And the color of the systems

were stable for a week when exposed to diffused light and for not less than 1 month when stored at 5-10°C (Jasim and Ali, 1989).

1.2.3 Curcumin-Metal complexes

Curcumin, (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione) can be considered as a two feruloyl parts linked by a methylene bridge. In the middle of curcumin molecule, there is a β -diketone moiety which is resemble to acetylacetone, the most common β -diketoenolato ligand that forms stable metal chelate ring. Binding of acetylacetone to metal can take place in several ways, such as acetylacetonato ion complexes (Figure 7a), neutral acetylacetone complexes (Figure 7b), olefin complexes (Figure 7c), or carbon- bounded complexes (Figure 7e) (Cotton and Wilkison, 1980; Nakamoto, 1986).

This β -diketone has the property of forming stable anion as a result of enolization followed by ionization:

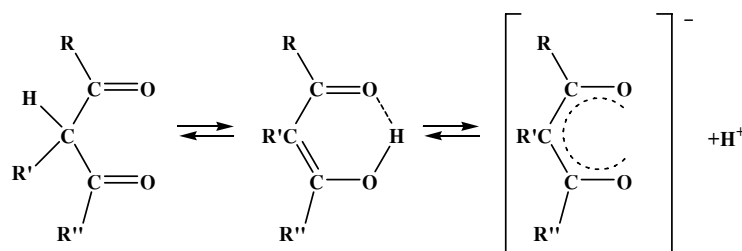


Figure 6 Enolization and ionization pattern of β -ketoenolato ligand
(Cotton and Wilkison, 1980)

These β -diketoenolate ions form very stable chelate complexes with a great range of metal ions. Among the commonest types of diketo complexes are those with the stoichiometries $\text{M}(\text{dike})_3$ and $\text{M}(\text{dike})_2$. Tetradiketo complexes $\text{M}(\beta\text{-dike})_4$ are usually nonrigid. Substance of composition $\text{M}(\text{dike})_2$ are almost invariably oligomeric, unless the replacement groups are very bulky ones such as $(\text{CH}_3)_3\text{C}-$.

Moreover, The methylene moiety in the middle of acetylacetonone molecule can also bond with the metal anion, notably for rhodium, iridium, palladium, and platinum (Cotton and Wilkison, 1980; Nakamoto, 1986).

β -diketone acetylacetonone is an example, can occasionally act as neutral ligands, being bound either through oxygen (Figure 7b) or for certain metals that can form olefin complexes (Figure 7c). When bonding as a unidentate ligand acetylacetonone used one of its oxygen atoms or its γ -carbon atom as a binding site with metal ion as shown in Figures 7d and 7e, respectively (Cotton and Wilkison, 1980; Nakamoto, 1986).

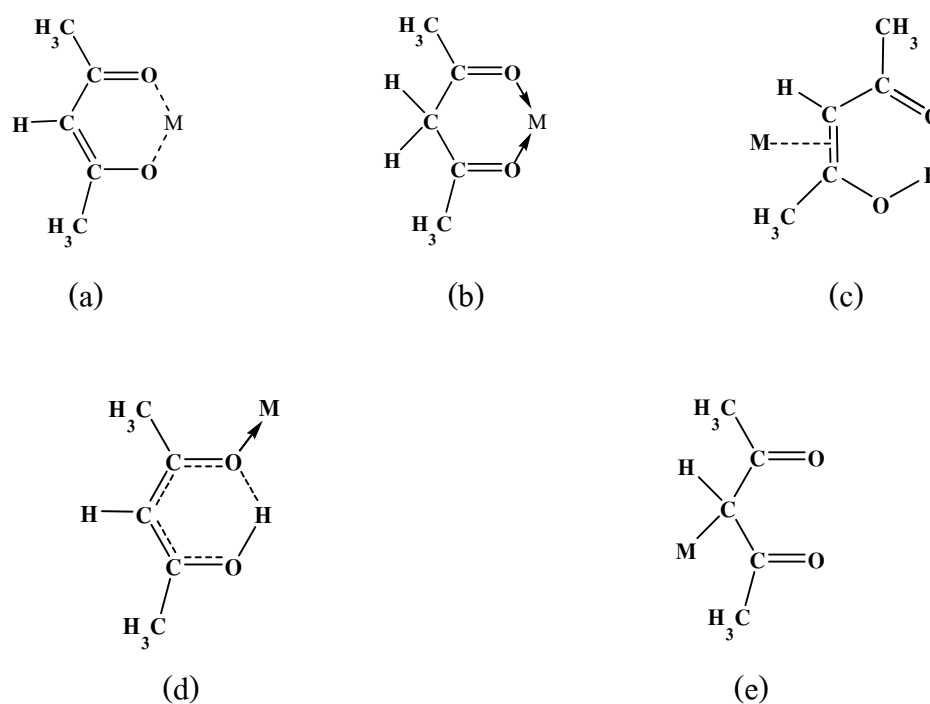


Figure 7 Possible binding sites of acetylacetonone ligand to metal ion

On the other hand, besides the β -diketone moiety, curcumin also has, on each side, a set of conjugated bonds ending with a phenyl ring with 3-methyl-4-hydroxy substituents. It is recognized that the polyphenols can act as metal chelating

agents (Andjelković, *et al.*, 2006), so more interactions between curcumin and metal ions can be through its terminal molecule of curcumin.

A half of curcumin molecule relevant to the dehydrozingerone (DHZ) (Jovanovic, *et al.*, 1999; Wright, 2002), and ferulic acid (Zsila, *et al.*, 2003b). The polyphenols, dehydrozingerone, ferulic acid and vanillin are cyclic derivative of benzene with 3-methyl-4-hydroxy substituents that compared with half curcumin. Which these molecules have been used to study to understanding the reaction of terminal partial part of curcumin molecule.

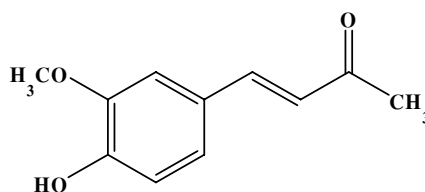


Figure 8 Structure of dehydrozingerone;
4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one

1.2.3.1 Curcumin-boron complex

In 1960-1972, much interest was focused on curcumin as a new photometric reagent for determining boron, based on the intensive color occurring in chelates due to the stabilization of particular ligand structure where the complexed element has no influence upon the position and intensity of the spectrum (Uppström, 1968; Dyrssen, *et al.* 1972). Curcumin in the enol form which changes into a mesomeric form (Figure 9) in a strongly acid solution. This form shows a continuous conjugation with charge resonance and has long wavelength bands at 550 nm (18020 cm^{-1}) with extinction coefficient $\epsilon_{\text{max}} = 73.6 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ (Perkampus, 1992).

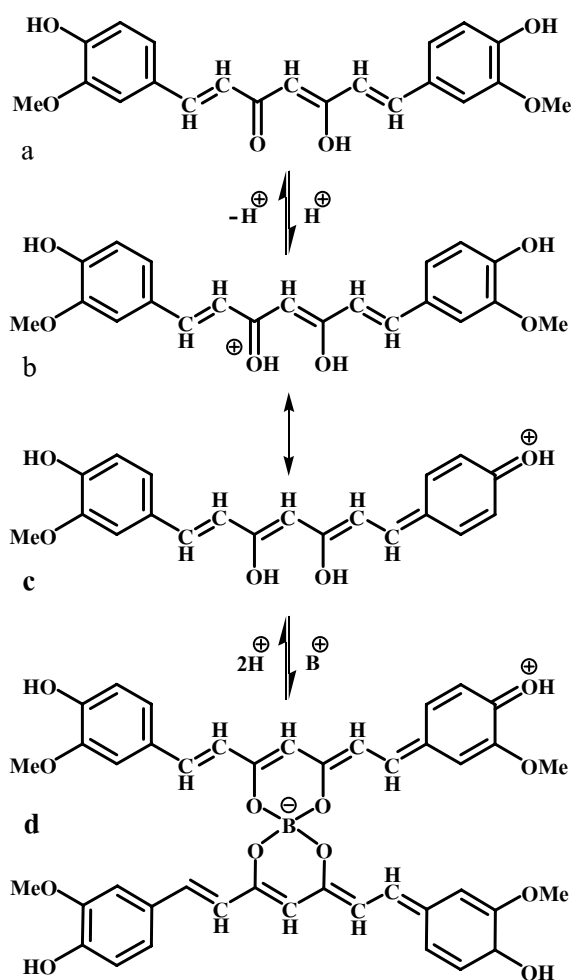


Figure 9 Complexing mechanism for boron with curcumin (Perkampus, 1992)

The mesomeric form (Figure 9c) has a structure with two enolic OH-groups which is selective for boron. This structure fixed by ester chelate formation if boric acids are present. The position of the maximum absorption and extinction coefficient of 1:1 complex is similar to that of the protonated molecule. However, since boron is capable of binding two curcumin ligands the extinction coefficient of this 1:2 complex is then twice as large. Instead of $\epsilon=73.6 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ for the chelate with one curcumin ligand in ethanol as solvent this value can increase to $\epsilon=146 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ for the chelate with two curcumin ligands (Perkampus, 1992).

In recent years, curcumin was used to determine trace amount of boron by means of spectrophotometry, e.g. boron in rice, bean, tap water (Benfu and

Hongying, 1996), steels (Kunihiro, *et al.*, 2003), glass and nitride based ceramic materials (Udo and Lothar, 2004). Furthermore, curcumin was used for treatment of boron-containing water (Haruki, *et al.*, 1998).

Some methods to prepare curcumin have been reported, and the best one involves a boron complex of 2,4-pentanedione to protect C-3 from Knoevenagel condensation (Pedersen, *et al.*, 1985). In order to avoid Knoevenagel condensation at C-3 of 2,4-pentanedione, it is necessary to protect C-3 by a boron complex, which is suggested to have the structure in Figure 10. Then the condensation will occur only on the terminal methyl groups. In the case of 3-substituted 2,4-pentanediones it should not be necessary to use a boron complex but experiments without the complex did not succeed (Pedersen, *et al.*, 1985).

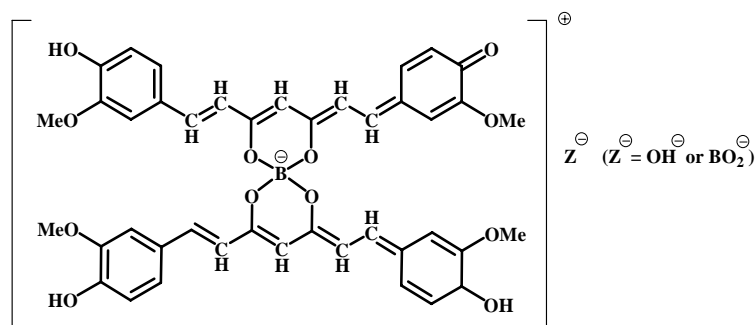


Figure 10 The structure of boron complex (Pedersen, *et al.*, 1985)

Rao and co-worker deduced the structure of complex formed by the reaction of curcumin with BF_3 etherate. The NMR studies (^1H , ^{13}C , ^{11}B and ^{19}F) indicated that the two keto-oxygens in curcumin were bonded to boron. This involved deprotonation of the enolic OH with concomitant delocalization of the double bonds leading to quinone form of one half of the curcumin molecule. The ^{11}B and ^{19}F NMR spectra of the complex indicated that the negative charge was present on the boron atom and two fluorine atoms were attached to boron atom in the complex, the third

fluorine atom was probably removed as HF. Based on NMR studies structure as Figure 11 was proposed for the complex. By means spectrophotometry, the stoichiometry of the 1:1 complex was determined by Job's method, the decrease in absorbance of λ_{\max} 420 nm and the increase in the absorbance at 500 nm (due to the formation of red colored complex) were monitored as the concentration of added BF_3 etherate was increased. Scatchard analysis of the spectra data gave a binding constant value for 1:1 complex as $6.69 \times 10^4 \text{ M}^{-1}$ (Rao, *et al.*, 1988).

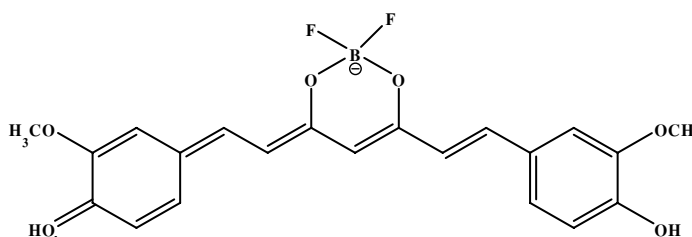


Figure 11 Structure of curcumin-boron complex (Rao, *et al.*, 1988)

1.2.3.2 Curcumin-iron complex

Iron, the most abundant transition metal in man, is essential for oxygen transport and many redox reactions. Only slight disturbances to the delicate balance between iron intake and iron loss can push the body into condition of iron overload or iron deficiency (Hynes and O'Coinceanainn, 2004). Several studies have been carried out with curcumin-Fe(III) and curcumin-Fe(II) in non-aqueous and aqueous media that refer to the formation of complexes, although the experimental condition for each of the studies were different.

Tønnesen and Greenhill discussed the function of curcumin in the reduction of Fe(III) to Fe(II) and in oxygen radical reactions. The interaction between curcumin and Fe(II) was indicated by a new absorbance at 500 nm and a decrease in the absorbance of curcumin (428 nm). By the spectrophotometric method, a 1:1 complex between curcumin and Fe(II) was formed. Interactions between curcumin

(1×10^{-5} M) and Fe(III) were demonstrated by an increase in the absorbance at 500 nm in the concentration range 1×10^{-6} - 1×10^{-5} M Fe(III). There was no corresponding decreases in the absorbance at 428 nm. In the concentration over 1×10^{-5} - 1×10^{-4} M Fe(III), an increase in the absorbance at 520 nm was detected. A further increase in the iron concentration led to a bathochromic shift in the curcumin spectrum. And the reduction of Fe(III) to Fe(II) can be determined by the increase in absorbance at 510 nm (phenanthroline-complex method) (Tønnesen and Greenhill, 1992).

Borsari and co-workers determined the pK_a values of curcumin and diacetylcurcumin by means of spectroscopic and potentiometric measurements. The interaction of Fe(III) with curcumin and diacetylcurcumin, in water/methanol 1:1 solution, led to the formation of complex species $[\text{FeH}_2\text{Cur}(\text{OH})_2]$ and $[\text{FeDCur}(\text{OH})_2]$ (H_2Cur and DCur = curcumin or diacetylcurcumin monoanion, respectively) which prevented metal hydroxide prevails near pH 7. At more basic condition the prevailing species are $[\text{FeH}_2\text{Cur}(\text{OH})_3]^-$ and $[\text{FeDCur}(\text{OH})_3]^-$, which prevented metal hydroxide precipitation. The stability constants, evaluated from potentiometric data, the stability constants of species $[\text{FeDCur}(\text{OH})_2]$ ($\log K = 30.1$) is greater than $[\text{FeH}_2\text{Cur}(\text{OH})_2]$ ($\log K = 29.1$), these value are near to that of desferrioxamine ($\log K = 30.99$), which is currently the only iron-chelating agent for clinical use (Borsari, *et al.*, 2002).

Bernabé-Pineda and co-worker studied the formation of curcumin-Fe(II) and curcumin-Fe(III) complexes in aqueous media within 5-11 pH range by means of spectrophotometry and cyclic voltammetry. The spectrophotometric data processing with the SQUAD program was used to calculate the formation constants of the complex curcumin-Fe(III) corresponding to the species $[\text{FeCur}]$ ($\log \beta_{110} = 22.25 \pm 0.03$) and $[\text{FeCur}(\text{OH})]^-$ ($\log \beta_{11-1} = 12.14 \pm 0.03$), while for the complexes curcumin-Fe(II) the formation constants corresponding to the species $[\text{FeCur}]^-$ ($\log \beta_{110} = 9.20 \pm 0.04$), $[\text{FeHCur}]$ ($\log \beta_{111} = 19.76 \pm 0.03$), and $[\text{FeH}_2\text{Cur}]^+$ ($\log \beta_{112} = 28.11 \pm 0.02$) (Bernabé-Pineda, *et al.*, 2004).

1.2.3.3 Curcumin-copper complex

In recent years, curcumin-Cu complexes have been synthesized, characterized, and evaluated for various biological activities such as antitumour (John, *et al.*, 2002), superoxide dismutase, antioxidant activity, free radical scavengers (Barik, *et al.*, 2005; Barik, *et al.*, 2007), and potential agent to combat Alzheimer's disease (Shen, *et al.*, 2005).

John and co-workers synthesized curcumin, piperonyl curcumin, 2-hydroxy naphthyl curcumin, and cinnamyl curcumin, and their Cu(II) complexes and investigated their possible cytotoxic and antitumor activities. Cu chelates of synthetic curcuminoids showed enhanced antitumor activity. All compounds were found to be cytotoxic to cultured L929 cells, concentration needed for 50% inhibition being around 10 $\mu\text{g/mL}$ for curcuminoids and 1 $\mu\text{g/mL}$ for their Cu complexes. Cu complexes of curcuminoids with a hydroxyl group on the ring such as 2-hydroxy naphthyl curcumin were found to be most active (1 $\mu\text{g/mL}$ produced 60.45% cell death). Cu complex of cinnamyl curcumin which has an extended conjugation showed considerable activity in increasing the life span (ILS = 78.6%) of ascites tumor bearing animals whereas complex of piperonyl curcumin was found to show least antitumor activity (John, *et al.*, 2002).

Annaraj and co-workers synthesized six new Cu(II) complexes by reaction of the non-enolisable Knoevenagel condensate, 4-salicylidene-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (from curcumin) with 4-X-anilines (when X is H, OCH₃, Br, CH₃, Cl, or NO₂) to form Schiff bases. These Schiff bases are expected to form stable complex with Cu(II) ion. These complexes were characterized their structure, spectral and redox properties (by NMR, IR, UV-Vis, ESR and CV). These complexes were characterized as a high degree of distortion, which can mimic the functional properties of blue copper proteins (Annaraj, *et al.*, 2004).

The cytotoxic and antitumour activity of four synthetic curcuminoid analogues, 1,7-diphenyl-1,6-heptadiene-3,5-dione (HL¹), 1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (HL²), 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (HL³), 1,7-bis(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione (HL⁴) and their Cu(II) complexes were investigated by John and Krishnankutty. They found that the Cu(II) complexes were more active as antitumour agents compared to the free curcuminoid, in both *in vitro* studies and in increasing the life of tumour bearing mice (John and Krishnankutty, 2005).

Barik and co-workers synthesized 1:1 and 1:2 complexes between copper(II) ion and curcumin. The 1:1 Cu(II)-curcumin complex was synthesized by mixing equimolar ratio of copper acetate and curcumin in dry ethanol and refluxed for 3 h under nitrogen atmosphere. The 1:2 Cu(II)-curcumin complex was synthesized by mixing methanolic solution of curcumin and aqueous solution of CuCl₂·2H₂O in the stoichiometry of 1:2 (copper:curcumin) and refluxed for 3 h. The precipitated complexes were filtered, washed with cold water and ethanol and dried in vacuum. The stoichiometry of both complexes were estimated by cycle voltammetric method (Barik, *et al.*, 2007). The UV-Vis spectra of curcumin in DMSO exhibited absorption maximum at 432 nm with extinction coefficients 55,400 M⁻¹ cm⁻¹ (Baril, *et al.*, 2005), the 1:1 Cu(II)-curcumin complex in DMSO at 426 nm with two shoulders at 410 and 450 nm with extinction coefficients 96700 M⁻¹ cm⁻¹ at 426 nm, and the 1:2 Cu(II)-curcumin complex at 370 nm (Barik, *et al.*, 2007). The 1:1 Cu(II)-curcumin complex showed higher SOD activity nearly 10 times of 1:2 complex, and rate constant for the scavenging of superoxide radical found to be 7 times higher than 1:2 complex, and greater ability to inhibit radiation induced lipid peroxidation in liposomes than 1:2 complex. The ESR spectra of both complexes in DMSO at 77 K suggested that the 1:2 complex should conform to square planar and the 1:1 complex is to the distorted orthorhombic. From this it was concluded that 1:1 complex would be able to undergo

and sustain the distortion from square planer geometry to the distorted tetrahedral one during its reaction with superoxide radical. The 1:2 complex, on the other hand, is planer but rigid and hence cannot undergo the disortion and therefore is less powerful antioxidant (Barik, *et al.*, 2007).

Four new curcuminoids analogues and their Cu(II) chelates of ML_2 were synthesized and characterized by UV-Vis, IR, 1H NMR, ESR and mass spectral data. The *in vitro* and *in vivo* antitumour activities of these curcuminoids and their Cu(II) chelates showed the chelation remarkably enhanced cytotoxic activity and other antitumour properties (Krishnankutty and John, 2003).

Sundaryono and co-worker synthesized new curcuminoids ligand. One interesting was bis-curcuminoid bearing a diphenylmethylethane bridge. Complexation of this ligand revealed a poor selectivity to transition metals. The formation constant of this synthetic ligand with Cu(II) ion ($\log K = 8.7 \pm 0.17$) is lower than $Cu(Cur)_2$ complex ($\log K = 10.88 \pm 0.22$) (Sundaryono, *et al.*, 2003).

1.2.3.4 Curcumin-other metal complexes

Several metallocomplexes of curcumin and curcuminoids have been synthesized, characterized and evaluated for various biological activities. Several metal-curcumin complexes showed greter biological activity compared to curcumin alone.

Anti-arthritis of a curcumin gold complex, $[Au(Cur)_2]Cl$, in which curcumin is bidentate were assessed in an adjuvant-induced rat polyarthritic model. Greatly reduced paw swelling was seen after three weeks of treatment with $[Au(Cur)_2]Cl$, 30 mg/kg/day, by injection (Sharma, *et al.*, 1987).

Krishnankutty and John synthesized four new curcuminoids and their VO(IV), Co(II) Ni(II) and Cu(II) complexes. These metal-curcuminoid complexes were prepared by refluxing a methanolic solution of the metal acetate and curcuminoid

for 1 hour, and the precipitated complexes were recrystallised from hot methanol (Krishnankutty and John, 2003).

Three manganese complexes with curcumin or one of two related compounds, diacetylcurcumin and 4-(4-hydroxy-3-methoxy-phenyl)-1-[7-(4-hydroxy-3-methoxy-phenyl)-[1,4]diazepam-5-ylidene]but-3-en-2-one, were evaluated for *in vitro* antioxidant properties and superoxide dismutase activity with IC₅₀ values for the former in the range 6.3-25.3 μ M and for the later 8.9-29.99 μ M. All the three complexes were also tested *in vivo* for their potential as neuroprotective agents in vascular dementia. [Mn(Cur)(OAc)] showed significant protective effects in a transient ischemia/reperfusion mouse model of neuronal damage (Vajragupta, *et al.*, 2003).

Daniel and co-worker studied the interaction between curcumin and some metal ions (Cd(II) and Pb(II)) in aqueous ethanolic media by means of spectrophotometry. Lead acetate and cadmium chloride were solubilised in Milli-Q water, curcumin was solubilised in 50% ethanol. By comparing the absorption spectra of curcumin in solution alone and then upon addition of equimolar concentration of metal solutions in Millipore water; 7.8×10^{-6} M of curcumin, $\lambda_{\max} = 428$, abs. 0.96; curcumin-Fe(II), $\lambda_{\max} = 430$, abs. 0.47; curcumin-Pb(II), $\lambda_{\max} = 430$, abs. 0.79; curcumin-Cd(II), $\lambda_{\max} = 429$, abs. 0.37; clearly implied the direct interaction between curcumin and the metals (Daniel, *et al.*, 2004)

A novel vanadyl curcumin complex ([VO(cur)₂]) has been synthesized and characterized for its physicochemical properties. Biological characterization included *in vitro* testing for anti-rheumatic activity in synoviocytes, angiogenesis inhibition in smooth muscle cells and anti-cancer potential in mouse lymphoma cells; as well as *in vivo* testing for hypoglycemic activity by oral gavage in streptozotocin (STZ)-diabetic rats. [VO(cur)₂] was more effective as an anti-cancer agent, compared to uncomplexed curcumin or vanadyl ion alone, was more than twice as effective as curcumin alone as an anti-arthritic agent, and was more than four times as effective as

curcumin alone in inhibiting smooth muscle cell proliferation. In both acute and chronic screening tests, [VO(cur)₂] was ineffective as an insulin mimetic agent; however, it also proved to be exceptionally non-toxic, with no evidence of negative symptomatology during a month-long treatment period, at doses up to and including 2.0 mmol kg⁻¹ day⁻¹ (Thompson, *et al.*, 2004).

The UV-Vis and IR spectral data of some curcuminoid and curcuminoid-metal complexes are list in Tables 3-5.

Table 3 UV-Vis and IR spectral data of some synthetic curcuminoid analogues and their Cu(II) complexes (Krishnankutty and John, 2003)

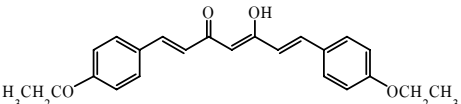
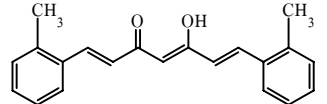
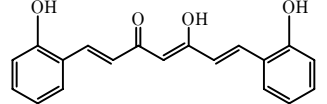
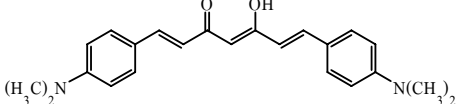
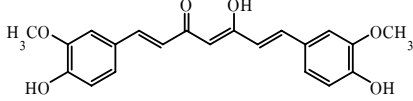
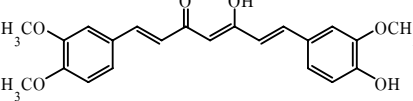
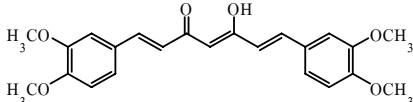
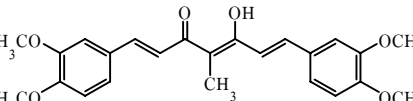
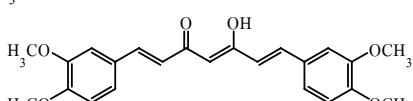
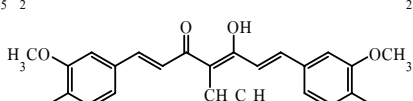
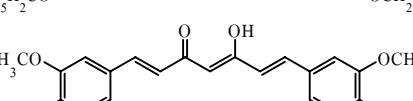
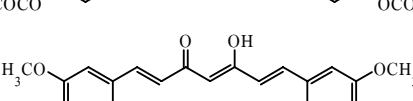
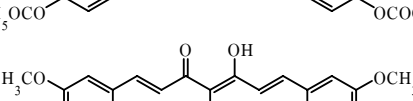
Structure	UV-Vis		IR (cm ⁻¹)										
	λ_{\max} (nm); (log ϵ)	Free ligand					Cu(L) ₂						
		$\nu_{\text{(C=O)}}$	$\nu_{\text{as (C-C-C)}}$	$\nu_{\text{(CH=CH)}}$ <i>trans</i>	$\nu_{\text{(C=O)}}$ chelate	$\nu_{\text{(C=C)}}$ phenyl	$\nu_{\text{(C=O)}}$ alkenyl	$\nu_{\text{as (C-C-C)}}$ chelate	$\nu_{\text{st(C-C-C)}}$ chelate	$\beta_{\text{(C-H)}}$ chelate	$\nu_{\text{(CH=CH)}}$ <i>trans</i>	$\nu_{\text{(C-H)}}$ chelate	$\nu_{\text{(M-O)}}$ chelate
	404 (4.70)	1628	1518	970	1588s	1580s	1550m	1515m	1465m	1101m	977m	735m	464m
	397 (4.59)	1625	1516	970	1597s	1586s	1545m	1517m	1466m	1102m	972m	734m	470m
	390 (4.67)	1630	1510	978	1590s	1578s	1540m	1510m	1464m	1112m	970m	740m	455m
	385 (4.71)	1628	1529	980	1600s	1588s	1549m	1512m	1471m	1100m	970m	729m	480m
						1565s				1065m			418m

Table 4 UV-Vis and IR spectral data of curcumin and dimethoxycurcumin and their metal chelated complexes (Kühlwein, *et al.*, 1997)

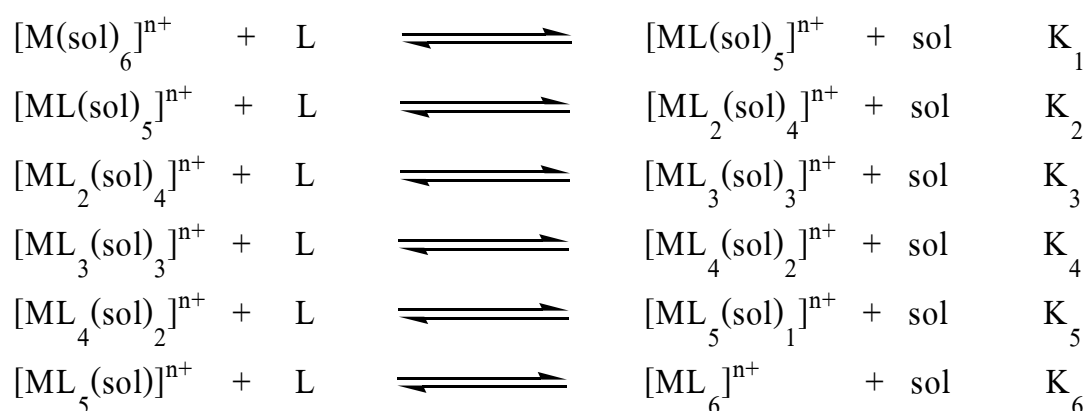
Complex	UV-Vis		IR (cm ⁻¹)		
	Solvent	λ_{\max} (nm); (log ϵ)	OH _{phenol} /OH _{chelate}	C=O _{chelate} /C=O _{ring} /C=C _{olefin}	CH=CH _{trans} /sonstige
Curcumin	EtOH	268 (4.09), 430 (4.74)	3508, 3415, 2844	1627, 1602, 1589, 1573 sh, 1511 vs	964
DMCU	EtOH	262 (4.09), 420 (4.66)	-	-	-
[Pd(Cur) ₂]	DMF	413 (4.77), 436 (4.84), 462 (4.83)	-	-	-
[Ni(DMCU) ₂]	DMF	350 (4.35), 419 (4.89), 447 (sh)	-	1627, 1599, 1581, 1547, 1509 vs, br	-
[Cu(DMCU) ₂]	Methylenchloride	264 (4.52), 421 (5.04), 444 (sh)	-	1625, 1598, 1580, 1509 vs, br	970
[Fe(Cur) ₃]	DMF	404 (sh), 428 (4.84, breit)	3479, 3420	1622, 1599, 1591, 1510 vs, br	987, 973/ 1285,1268 (C-O)

Table 5 UV-Vis spectral data of some synthetic curcuminoids (Bong, 2000)

Structure	UV-Vis	
	λ_{\max} (log ϵ) MeOH	λ_{\max} (log ϵ) Hexane
	428 (4.68)	409 (4.67)
	417 (4.57)	409 (4.61)
	420 (4.66)	413 (4.06)
	362 (3.95)	358 (4.15)
	349 (4.40)	346 (4.38)
	350 (4.20)	350 (4.26)
	399 (4.70)	395 (4.70)
	400 (4.69)	400 (4.67)
	367 (4.52)	366 (4.52)

1.2.4 Composition and stability constant of the complex

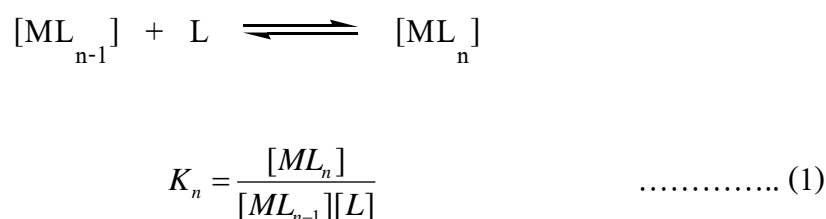
In solution the ions surrounded by the polar molecules of the solvent (sol), the ions are solvated. In general, the cations of the 3d transition metals are surrounded by six solvent molecules, they form hexasolvent-complex. A ligand added to the solution of solvated metal cations (M) may replace the solvento-ligand molecules of solvent complex.



When a complex is formed, the solvating molecules are replaced one by one by the new ligands. The complex formation takes place step by step where every step corresponds to the substitution of one solvent (sol) by ligand (L).

Every step is characterized by an equilibrium constant K_i , called the stepwise stability constant (or stepwise formation constant).

K_1, K_2, K_3, K_4, K_5 and K_6 are the stepwise stability constants. These equilibrium constants are described by a general expression



The constants decrease with increasing number of ligands.

The overall stability constant β_n describes the substitution of “n” molecules of solvent by “n” ligands.

It is easily shown that the overall constant β_n corresponds to the product of the n individual constants

$$\beta_n = K_1 \times K_2 \times \dots \times K_n$$

In general terms, the relationship between the overall constant and the individual constants is

$$\beta_n = \prod_1^n K_1 \quad \log \beta_n = \sum_1^n \log K_1 \quad \dots\dots\dots (2)$$

(University of Fribourg, 2005)

There are many solution properties (e.g. colligative, optical, kinetic reaction, calorimetry, solubility, ion-exchange, potential) which change measurably as a result of complex formation. In principle all these measurements can give information on the existence and stability of the different species. Research in the field of equilibrium chemistry is relatively inexpensive, and in most case reliable stability constant can be obtained by using simple and easily available methods and instruments (Beck, 1970).

One of the most spectacular effects of complex formation is the change of spectra properties. The reasons for light absorption by the complexes are as follows. The excitation of electrons of both the metal and the ligand(s) is influenced by their interaction. The electrons of transition metal ions are easily excited and consequently absorb in the visible region, that is these ions give colored compounds. The electron systems of non-transition metal ions and ligands are much more stable, excitation of the electrons requires much greater energy, so these species absorb radiation in the ultraviolet range of the spectrum. Owing to the interaction of the central ion and the

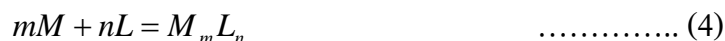
ligand a charge transfer from the ligand to the metal ion and vice versa may occur on irradiation. This phenomenon is the reason for the so-called charge transfer spectra in the visible of the near-ultraviolet region. As the matter of course, complex formation results in changes in the vibrational-rotational characteristics of the ligands, lead to the shift of peaks in the infrared spectrum. The relationship between the absorbance and the composition of a given solution is given in the ideal case by the Beer-Lambert law:

$$\log \frac{I_0}{I} = A = d \sum \varepsilon_i c_i \quad \dots\dots\dots (3)$$

where d is the cell length, A is the absorbance of the solution, ε_i the molar absorptivity of the i^{th} species (which has concentration c_i); A and ε_i refer to the given wavelength. The intensive factor, the molar absorptivity, depends on the medium and on the temperature. The molar absorptivity of the metal ion, of the ligand and - in most cases - of the co-ordinatively saturated complex, can be determined directly, but those of the intermediary complexes are just as unknown as their stability constants. If two species in the solution have at a certain wavelength the same molar absorptivity, the series of the spectra of solutions, when the sum of the concentrations is constant but the ratio of their concentrations are changed, exhibits a common crossing point, the so-called isosbestic point. Because it is very unlikely that a third species has the very same molar absorptivity at this wavelength, the occurrence of the isosbestic point(s) in the formation of a series of complexes gives evidence for the simultaneous existence of two absorbing species. It is a great evidence for the spectrophotometric method that the measurements can be made at many different wavelengths. In selecting the wavelengths it is helpful to use different bands of the spectrum. The agreement of stability constants obtained from measurements at different wavelengths is strong evidence for their reliability (Beck, 1970).

The continuous variation method or Job's method was very popular between the mid-forties and mid-fifties and is still frequently applied, although its

limitations are quite clear. The principle of the method is simple. Suppose that the metal ion and the ligand form one complex



A series of solutions is prepared in which the sum of the total concentrations of metal (M) and ligand (L) is constant, but their proportions are continuously varied:

$$T_M + T_L = C \quad \dots\dots\dots (5)$$

$$x = C \frac{TL}{T_M + T_L} \quad \dots\dots\dots (6)$$

The equilibrium concentrations of the species M, L and M_mL_n are given by the equations

$$[M] = C(1 - x) - m[M_mL_n] \quad \dots\dots\dots (7)$$

$$[L] = Cx - n[M_mL_n] \quad \dots\dots\dots (8)$$

$$[M_mL_n] = \beta_{m,n} [M]^m [L]^n \quad \dots\dots\dots (9)$$

Evidently, the concentration of the complex reaches a maximum at a certain value of x . This value (x_{\max}) can be obtained by differentiating Eqs.7-9 with respect to x , setting $d[M_mL_n]/dx=0$ and eliminating $[M]$ and $[L]$:

$$m(Cx - n[M_mL_n]) = n(C(1 - x) - m[M_mL_n]) \quad \dots\dots\dots (10)$$

$$mx_{\max} = n(1 - x_{\max}) \quad \dots\dots\dots (11)$$

$$\frac{n}{m} = \frac{x_{\max}}{1 - x_{\max}} \quad \dots\dots\dots (12)$$

If any property of the solution which varies linearly with $[M_mL_n]$ is measured and plotted as a function of x , the ratio of the stoichiometric coefficients (n/m) can be obtained. The method of continuous variation is most frequently applied in the evaluation of spectrophotometric data (Beck, 1970).

If only one complex is formed, the method can furnish some further information. Klausen and Langmyhr have shown that if $x_{\max} = 0.5$, i.e. $m = n$, and the curve exhibits inflections and parabolic portions for value of x near zero and one, this is an indication that $m = n > 1$. On the other hand the absence of inflections and

parabolic portion indicates the formation of a complex with $m = n = 1$. It must be borne in mind, however, that case of complexes of high stability the inflections may not be appreciable even when $m = n > 1$ (Beck, 1970).

If the complex is very stable, the curve of absorbance versus x consists of two strictly linear intersecting portions. In the case of moderately stable complexes there is no such linearity, and from the slopes near the extreme values of x , the value of the stability constant can be calculated (Beck, 1970).

In the case of successive complex formation, more reliable information can be obtained by the mole-ratio methods. Here the total concentration of the metal or ligand is kept constant while the total concentration of the other is increased. The absorbance is plotted as a function of the total concentration of the variable species. If only one complex of high stability is formed, the graph consists of two linear intersecting parts. The ratio of total concentrations of metal and ligand at the intersection gives directly the ligand to metal ion ratio in the complex. The stability constant in this can not be obtained. If the complex is moderately stable a steep curve is obtained and the intersection of the slopes of the two linear parts of the curve gives the ratio. However, there is an increasing uncertainty with decreasing stability. In favorable case the stability constant can be simply derived from the difference between the measured curve and that constructed from the slopes. In the case of weak complexes this simple method fails to give any reliable information (Beck, 1970).

The stability constant $\log \beta'$ of 1:1 complex was determined using the Benesi-Hildebrand method. A series of solutions with a constant concentration of curcumin and varied concentrations of metal ion were prepared and recorded. A straight line was obtained from plotting $C_L C_M / A$ as a function of C_M from Benesi-Hildebrand equation:

$$\frac{C_M d}{A} = \frac{1}{\beta C_L \cdot \epsilon_{ML}} + \frac{1}{\epsilon_{ML}} \dots\dots\dots (13)$$

which is also written as follows:

$$\frac{C_M C_L}{A} = \frac{1}{\beta \cdot \epsilon_{ML}} + \frac{1}{\epsilon_{ML}} \cdot C_M \quad \dots\dots\dots (14)$$

which is valid for complexes where the metal:ligand ratio is 1:1. In Eq.13, ϵ_{ML} is the molar absorption coefficient of the complex, which can be determined from the slope ($1/\epsilon_{ML}$) of the straight line and β is the relative stability constant $\beta'_{m,n} = [M_m L_n]/[M]^m [L]^n$, calculated from the intercept (Perkampus, 1992).

The complex formation constant of some curcumin-metal complexes are list in Table 6.

Table 6 Complex formation constant of some metal-curcumin complexes and relatives

Complex	Media	Method	Complex formation constant	Referance
[FeH ₂ Cur(OH) ₂]	Water:MeOH (1/1)	Potentiometry	log <i>K</i> 29.1	Bosari, <i>et al.</i> 2002
[FeDCur(OH) ₂]	Water:MeOH (1/1)	Potentiometry	log <i>K</i> 30.1	Bosari, <i>et al.</i> , 2002
[FeCur]	Water	Spectrophotometry-SQUAD	log β ₁₁₀ =22.25(25°C)	Bernabé-Pineda, <i>et al.</i> , 2004
[FeCur(OH)] ⁻	Water	Spectrophotometry-SQUAD	log β ₁₁₋₁ =12.14 (25°C)	Bernabé-Pineda, <i>et al.</i> , 2004
[FeCur] ⁻	Water	Spectrophotometry-SQUAD	log β ₁₁₀ =9.20 (25°C)	Bernabé-Pineda, <i>et al.</i> , 2004
[FeHCur]	Water	Spectrophotometry-SQUAD	log β ₁₁₁ =19.76 (25°C)	Bernabé-Pineda, <i>et al.</i> , 2004
[FeH ₂ Cur] ⁺	Water	Spectrophotometry-SQUAD	log β ₁₁₂ =28.11 (25°C)	Bernabé-Pineda, <i>et al.</i> , 2004
[CuCur]	DMSO	Spectrophotometry	log <i>K</i> 2.21	Barik, <i>et al.</i> , 2005
[Cu(H ₂ Cur)(OAc)(OH)]	DMSO	Electrochemical (CV)	log <i>K</i> 14.57 (25°C)	Barik, <i>et al.</i> , 2007
[Cu(H ₂ Cur) ₂]	DMSO	Electrochemical (CV)	log <i>K</i> 15.59 (25°C)	Barik, <i>et al.</i> , 2007
[Cu(Cur) ₂]	DMF	Spectrophotometry- LETAGROP-SPEFO	log <i>K</i> 10.88 (20°C)	Sundaryono, <i>et al.</i> , 2003

1.3 Objectives

The objectives of this work are:

1. To study complex formation of curcumin, using spectrophotometric method, with as many metal ions as possible especially those showing toxicity *in vivo*.
2. Some of those in 1. that show sign of complexation will be studied further to obtain the stoichiometric binding ratio and, if possible, determine the value of stability constant (or complex formation constant).
3. The possible binding position of curcumin (Figure 12) with metals will be determined by the comparative studies of the reaction patterns of curcumin and its analogues (acetylacetone for β -diketone moiety, and, ferulic acid and vanillin for the terminal methoxyphenyl ring (Figure 13)).

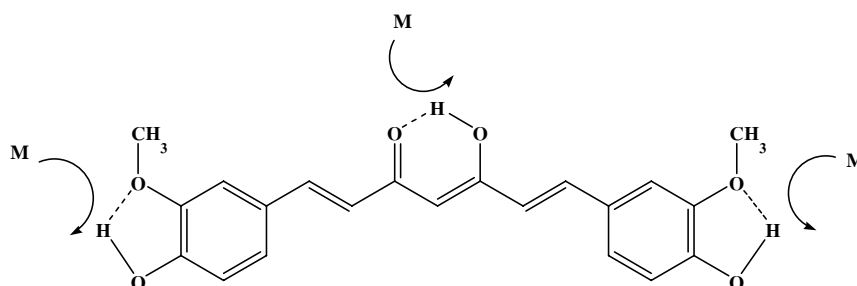


Figure 12 The three possible binding positions in curcumin molecule

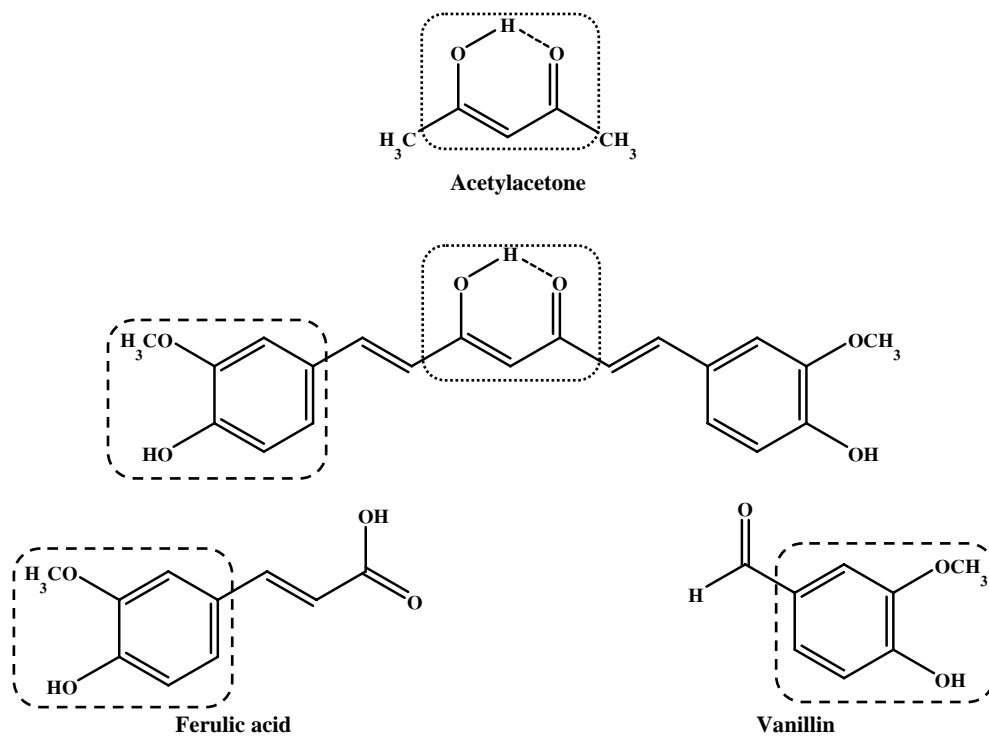


Figure 13 The analogous molecules (acetylacetone for β -diketone moiety, and, ferulic acid and vanillin for the terminal methoxyphenyl ring) of curcumin moieties