

Chapter 4

Discussions

UV-Visible absorption spectroscopy

Electronic absorption spectroscopy is a very useful technique to study complex formation. Curcumin and curcuminoid derivatives display absorption in the ultraviolet and visible regions. The UV visible absorption of curcumin in 50% methanol displays an intense band at 420 nm due to the neutral molecule corresponding to the absorption of conjugated π -bond system and symmetrical structure unit, respectively, and a shoulder peak at 360 nm, corresponding to symmetrical structure unit (Wang et al., 2007). In sodium hydroxide, the maximum band was observed at 260 nm while the intensity of the band at 420 nm decreases.

Curcumin belongs to a group of naturally occurring 1,3-diketones in which the carbonyl groups are directly linked to olefinic carbons, conferring complexing properties to the molecule. Curcumin has been known for its ability to form colored complexes with other molecules. Several studies have been carried out with the curcumin-Fe(III) in non-aqueous and aqueous media that refer to the formation of complexes (Bernabe'-Pineda, et al., 2004). Moreover, curcumin forms strong colored chelates with transition metals ions Cu^{2+} , Ni^{2+} , Zn^{2+} , Pd^{2+} and Fe^{3+} determination by visible absorption spectrometry (Sundaryono, et al., 2003).

In this study, the composition of curcumin and some metal ions were studied in aqueous (Fe^{3+} , Pb^{2+} and Cd^{2+}) and non-aqueous system (Fe^{3+} , Pb^{2+} , Cd^{2+} , Mn^{2+} , Bi^{2+} and Cr^{3+}) by mean of spectrophotometric study. The stoichiometry of metal complexes which shows characteristic absorption in the visible or UV region were determined by Job's method. In this method, a series of solution is prepared by changing the concentration of the two components wherein their sum was constant. This method is

based on plotting measured absorbances against mole fractions of the two constituents of a complex. If a stable complex is formed, the resulting curve will show a maximum (or minimum) at the mole fraction corresponding to that in the complex (Ogura et al., 1979).

In this study, Job's method could not be applied to determine the stoichiometry of the complex because absorption spectra of pure curcumin and of mixed solution of metal ion and curcumin were in the same region indicating the system is a weak complex which is difficult to obtain a sharp break or straight line.

Electrochemical study

The stoichiometry of the complex is determined generally by spectrophotometric method. However, the use of this method is limited to those systems that are able to absorb the light in the UV or visible region. In addition when parent substances, that is, solvated ions of metal or of ligand, as well as their complex compounds absorb the light in the same wavelength region, the accurate estimation of stoichiometry becomes impossible.

In this experiment, the voltammetric technique was employed instead of the spectroscopic one. This method is based on the titration of ligand with metal ions or vice versa and observed the change of the reduction or oxidation current before and after equivalent point. The current peak must be first decrease with an increase of molar ratio, and eventually becomes zero. In this plot gives a sharp break at the equivalent amount of metal and ligand (Ogura et al., 1979).

Electrochemical study of curcumin in aqueous system for CPE and GCE was performed at pH 3 and 10. Only one peak was formed at pH 10 for CPE. The oxidation peak occurred at potential of 0.40 V indicating that phenolic groups were indeed responsible for the first oxidation step. No reaction of reduction during reverse scans were detected (Born et al., 1996). The anticipated chemical structure of curcumin

molecule and main possible reactions involved in the electro-oxidation of the curcumin molecule were depicted in Figure 70. In strong alkaline solution, phenol is converted into phenolate ion I which is readily oxidized and generates free radical II. The presence of methoxy group in the monomer makes the reaction possible. The methanol molecule can be eliminated from radical II by alkaline hydrolysis giving an anion radical III, which is converted to a highly reactive *O*-quinone (IV) another one-electron reaction. Then *O*-quinone species was adsorbed on the electrode surface (Majdi et al., 2007).

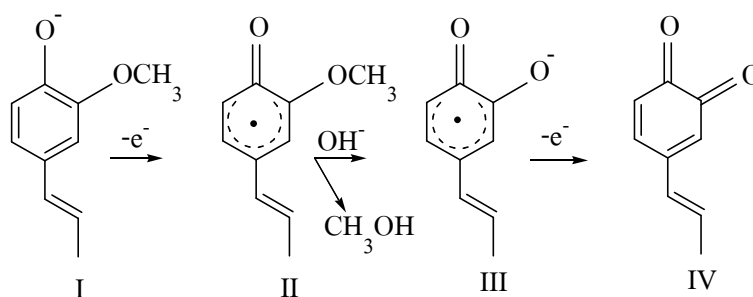


Figure 70 Electro-oxidation of the curcumin molecule

For electrochemical study of metal ions (Hg^{2+} , Pb^{2+} and Cu^{2+}) in ammonium acetate buffer, the oxidation and reduction peak occurs at -0.51 V and -0.62 V vs Ag/AgCl, respectively, and can be assigned to the process $\text{Pb}^{2+} + 2\text{e}^- \rightleftharpoons \text{Pb}$. The cyclic voltammogram of Hg^{2+} and Cu^{2+} have only oxidation peaks at 0.40 V and 0.02 V vs Ag/AgCl which correspond to the processes $\text{Hg} \rightarrow \text{Hg}^{2+} + 2\text{e}^-$ and $\text{Cu}^0 \rightarrow \text{Cu}^+ + \text{e}^-$, respectively.

The stoichiometry of complex compounds were studied by CV. It was found that Ni^{2+} did not form complex with curcumin at pH 10. Other metal ions could not be studied in this condition because of precipitation to metal hydroxide.

Pb^{2+} and Cd^{2+} also were studied in acid medium with HMDE electrode at pH 4. The results showed that these metal ions did not form complex with curcumin.

However, when GCE was used as working electrode for Pb^{2+} -curcumin system at pH 5, a better result was obtained that the i_p versus molar ratio plot gave a sharp break at 0.8 which indicated that curcumin form complex with Pb^{2+} in the ratio of 1:1 which Daniel et al. also found that Fe^{3+} , Pb^{2+} , and Cd^{2+} formed complexes with curcumin at GCE in pH 4 buffer solution. Furthermore, these complexes could be reduced at suitable potential.

NMR study

NMR study of curcumin

Early studies concluded that curcumin existed predominantly as a keto-enol tautomer and in several recent articles the solution structure of curcumin has been represented as a β -diketone tautomer. X-ray crystal structure analyses have established that curcumin exist as keto-enol tautomers in the solid state. In fact, there are three possible structures of curcumin: the β -diketone tautomer, **71a**, and two equivalent asymmetric keto-enol tautomers, **71b**.

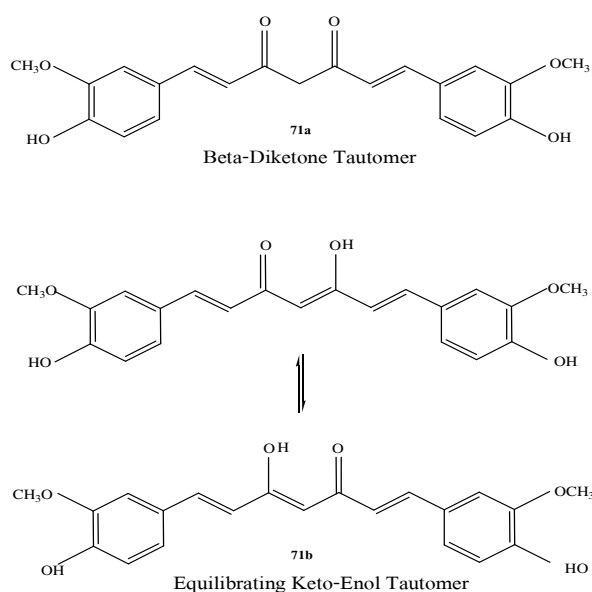


Figure 71 Potential solution structures of curcumin

Jovanovic et al. concluded that the enolate form of curcumin predominated above pH 8 and that above this pH the phenolic groups of the curcumin enolate became sites of electron donation. Other investigations of curcumin, however, had been concluded that in solution curcumin existed predominately as **71b**, at least in solvents like CDCl_3 and d_6 -DMSO. The significant difference between **71a** and **71b** lies in the hybridization of the bridging carbon at position 1, as alternatively methylene or methine (Figure 1).

The NMR experiments showed that the keto-enol tautomer, **71b**, form of curcumin was essentially the only form of this molecule present in a variety of solvents ranging from chloroform to mixtures of dimethylsulfoxide and water, and buffered aqueous d_6 -DMSO solutions varying in pH from 3 to 9 (Payton, et al., 2007).

In this study, ^1H NMR spectra of curcumin were recorded in CDCl_3 and d_6 -DMSO. It was found that curcumin existed predominantly as a keto-enol tautomer. Chemical shift (^1H and ^{13}C NMR) of curcumin (Table 8 and 9) was in accordance with reported values (Jayaprakasha, et al., 2002).

NMR study of acetylacetone

NMR spectroscopy is clear the method of choice to use in making such structural distinctions. The β -diketone core of curcumin is analogous to 2,4-pentanedione or acetylacetone. The enol form of acetylacetone was found in d_6 -DMSO while the keto tautomer was found in CDCl_3 because of the intramolecular hydrogen bonding. Thus, an increase in solvent polarity favors the more polar keto form (http://itl.chem.ufl.edu/4411L_f00/nmr/nmr.html).

NMR study of curcumin and metal ions

NMR data are also useful in identifying the binding site in metal-chelate complexes; for this purpose we substituted the paramagnetic Mn^{2+} , Co^{2+} , and Cu^{2+} ions with diamagnetic Pb^{2+} , Hg^{2+} , Zn^{2+} , and Mg^{2+} ions. Each compound was studied in two solvent: CDCl_3 and d_6 -DMSO.

A characteristic feature of the ^1H NMR spectra of complex is the shift of the methine proton signal. The spectra of curcumin mixed with some metal ions (Pb^{2+} , Hg^{2+} , Zn^{2+} and Mg^{2+}) in CDCl_3 did not show the shift of this signal. This suggests that curcumin do not form complex with these metal ions in this condition. However, with Hg^{2+} and Mg^{2+} ions, there was a new but unidentifiable peak appeared at 5.0 and 5.3 ppm, respectively. The spectra are shown in Figure 48 and Figure 50.

The ^1H NMR spectra of curcumin- Co^{2+} , curcumin- Cu^{2+} and curcumin- Mn^{2+} systems are shown in Figure 55 to Figure 57. These systems are shown broad spectrum, presumably due to the paramagnetic influence from the metal ions (John, et al., 2004). It is generally known that NMR shifts are difficult to observe for paramagnetic transition metal complexes. Difficulty due to the low solubilities is expected to be overcome in the studies of nuclear-spin relaxation under the coexistence of paramagnetic transition metal ions, which shorten the nuclear relaxation times and cause line-broadening in NMR (Navorro, et al., 2005)

For curcumin- Pb^{2+} , curcumin- Mg^{2+} , and curcumin- Zn^{2+} systems the methine proton signals showed highfield shift. These data are compared with acetylacetone which showed upfield shift of this protons when coordinate to magnesium atom (Prasad, et al., 2002). In the NMR spectrum of $\text{Mg}(\text{acac})_2$ this peak shift to upfield about 0.4 ppm in comparison to the free ligand. Similar upfield shift has been reported in the acetylacetonates of the other metals too. Thus the upfield shift of this is an evidence for coordination of metal to curcumin.

For curcumin- Hg^{2+} system the methine proton signal did not shift but aromatic and olefinic protons appeared very complicate. So, this system was further characterized by ^{13}C NMR technique. It was found that, all carbon atoms were not shifted suggesting that curcumin might be in neutral enolic form with only monodentate coordination to the metal.

In the ^{13}C NMR spectrum of curcumin- Mn^{2+} system, all carbon atoms showed upfield shift, it can be concluded that curcumin was not in the form of 71a or 71b to interact with Mn^{2+} ion. The active species may be in the quinone form of one half of the curcumin molecule (Rao, et al., 1988).

Infrared spectroscopy

Infrared spectroscopy is a good indicator of metal ion and ligands in the complex system. The infrared spectrum of isolated molecule is determined by arrangement of atoms in space and by the force between atoms. When a ligand is coordinated, all of these forces change. The changes are to be expected both in the vibrational spectral features associated with the free ligand and those systems to which it attached.

Curcumin shows two possible sites of interaction with a metal ion; the hydroxyl group attached to the two rings and the β -diketone moiety bridging the two rings. Both sites have been identified as being important to biological activity. Four major and other minor forms of metal interaction are known for the β -diketo moiety, which undergoes keto-enol tautomerism, as shown in Figure 72 (NaKamoto, et al., 1970).

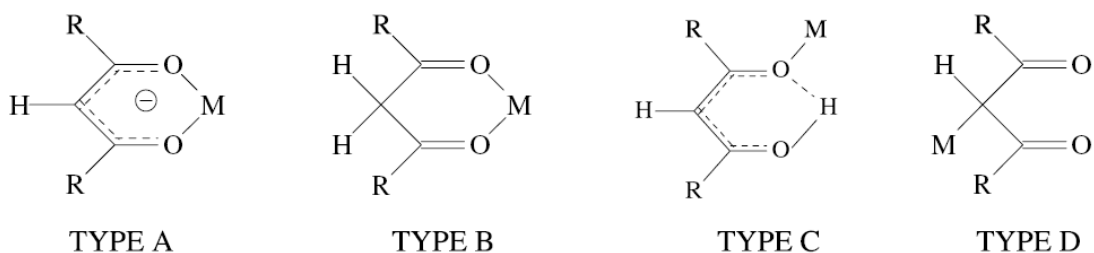


Figure 72 Four forms of metal interaction known for the β -diketo moiety

Type A is associated with chelation in the ionic enolic form. It is characterised by two strong bands between 1570 and 1525 cm^{-1} and two bands between 1400 and 1280 cm^{-1} , associated with the coupled $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{C})$ stretches, respectively. Type B is associated with chelation in the neutral ketonic form. It is characterised by higher wavenumbers of the carbonyls found between 1700 and 1650 cm^{-1} . Type C is associated with the neutral enolic form with only monodentate coordination to the metal. It is characterised by two carbonyl bands at 1630 cm^{-1} (between type A and B) and a lower band at 1560 cm^{-1} . Type D is associated with the metal binding to the central γ -carbon of the ionic ketonic form. It is characterized by two $\nu(\text{C}=\text{O})$ stretches between 1650 and 1600 cm^{-1} and two $\nu(\text{C}=\text{C})$ between 1350 and 1200 cm^{-1} , lower than that in type A (Daniel et al., 2004).

Recently there have been many reports in the literatures on the metal-chelating properties of curcumin, employing technique like potentiometry and absorption spectroscopy. Curcumin forms complexes of type 1:1 and 1:2 with copper, iron, and other metals. The previous studies of infrared absorption spectra for curcumin derivatives and some metal curcumin complexes are summarized as follows.

Sharma, et al., (1986) synthesized and characterized curcumin gold complex, $\text{Au}(\text{CUR})_2\text{Cl}$ (Figure 3) by infrared spectroscopic technique. The results showed that on complexation the position of the O-H band remained unaltered suggesting no coordination takes place through the OH group. The position of the C=O band was

shifted towards lower wave numbers which suggested that the ligand coordinated with the central metal ion through the C=O group.

Thompson, et al., (2004) synthesized vanadyl curcumin complex (VO(cur)₂) (Figure 9) and identified structures of compounds by FT-IR. A broad medium intensity IR band at $\sim 3400\text{ cm}^{-1}$ ($\nu_{\text{O-H}}$) strongly suggested that the phenolic OH group was not coordinated to the vanadyl ion. A band at 1628 cm^{-1} , present in the IR spectrum of curcumin, was shifted downfield (to $\sim 1600\text{ cm}^{-1}$) in the vanadyl complex spectrum, indicative of coordinated carbonyl moieties.

Danie, et al., (2004) examined the precipitates formed between metal (Pb and Cd) and ligand using infrared spectroscopic technique. IR analysis inferred that both the hydroxyl groups and the β -diketone moiety of curcumin were involved in metal–ligand complexation, either directly bonding to the metal or in intermolecular hydrogen bonding.

Barik, et al. synthesized and characterized a new copper(II)-curcumin complex (Figure 10). The result showed that the C=O frequency in curcumin which appeared at 1630 cm^{-1} was shifted by 10 cm^{-1} on copper complexation indicating its involvement in complexation

Mohammadi, et al., (2005) studied curcumin complexes of the formula, ML_3 , where M was Ga(III) or In(III), or of the formula, ML_2 where M was $[\text{VO}]^{2+}$ (Figure 11) and characterized by infrared. Vanadyl complexes had no broad band in the $2600\text{--}3400\text{ cm}^{-1}$ range, related to the stretching of intramolecular H in the enol function.

The reddish-brown precipitate of curcumin-Pb²⁺ and greenish-brown of curcumin-Cu²⁺ were analyzed by FT-IR spectroscopy and the results are shown in Table 11. The vibrational modes of these products compared with curcumin are summarized in Table 12. The metal derivatives show OH bands with shifts to lower wavenumber. This indicates that these sites are involved in the complex, either in direct bonding to the metal or in intermolecular hydrogen bonding. The presence of

two carbonyls shifts to lower wavelengths about 10 cm^{-1} for both complexes suggest a type A bonding (Figure 72) in the solid state. In addition, several other bands in the infrared spectra are shown slightly shifted to lower wavenumber, an indicator for complexation. These spectral changes are possibly due to symmetry changes associated with the benzene rings on complexation. IR analysis inferred that both the hydroxyl groups and the β -diketone moiety of curcumin are involved in metal-ligand complexation, either directly bonding to the metal or in intermolecular hydrogen bonding. So the IR data in combination of XRF result which showed that the Pb and Cu are present in these products lead to the conclusion that these precipitates should be curcumin complexes with the two metal ions.

X-ray Fluorescence Spectrometry

This method was used to prove the presence or absence of the elemental composition of compounds, such as, the added metals. It was only fundamentally qualitative analysis. In this research, the complexes of Pb^{2+} -curcumin and Cu^{2+} -curcumin were analyzed by XRF technique. For Pb^{2+} -curcumin complex, the spectrum of Pb was detected in which it showed that Pb was present in the complex, as the L_{α} and L_{β} lines of Pb appeared at 10.6 and 12.6 keV. For Cu^{2+} -curcumin complex, the spectrum of Cu was found in the complex, as the K_{α} lines of Cu appeared at 8.8 keV. This indicated that the elements were present in the products. We can say that Pb and Cu interacted with curcumin. The XRF spectra of these complexes are illustrated in Figures 68-69.

