

CHAPTER 4

Discussions

4.1 UV-Vis spectral studies

4.1.1 Curcumin

Curcumin in methanolic-aqueous solution shows a broad characteristic UV-Vis absorption around 300-500 nm with maximum absorption band at wavelength 430 nm and shoulder near 360 and 460 nm, and a weak absorption band at 260-280 nm as shown in Figure 80. Since there is electrostatic interaction between polar solvent (methanol) and polar chromophores in curcumin molecule, this solvent tends to stabilize both the bonding electronic ground states and the π^* excited states. This interaction causes the $n-\pi^*$ transition which occurs at lower energy than the $\pi-\pi^*$ transitions to move to higher energy and $\pi-\pi^*$ transition to move to lower energy. Thus, the $\pi-\pi^*$ and $n-\pi^*$ absorptions of curcumin move close to each other (Figure 82) (Christian and O'Reilly, 1986).

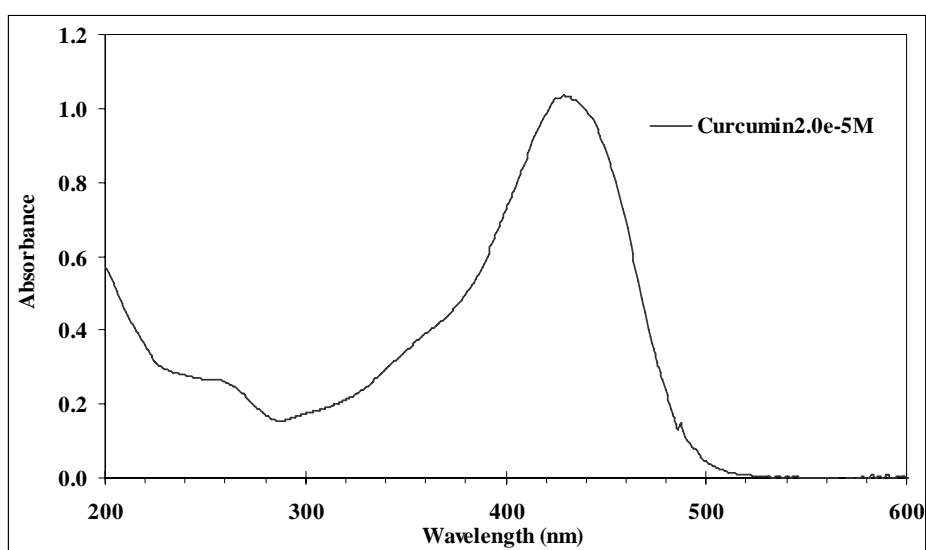


Figure 81 UV-Vis absorption spectrum of curcumin in 50% MeOH

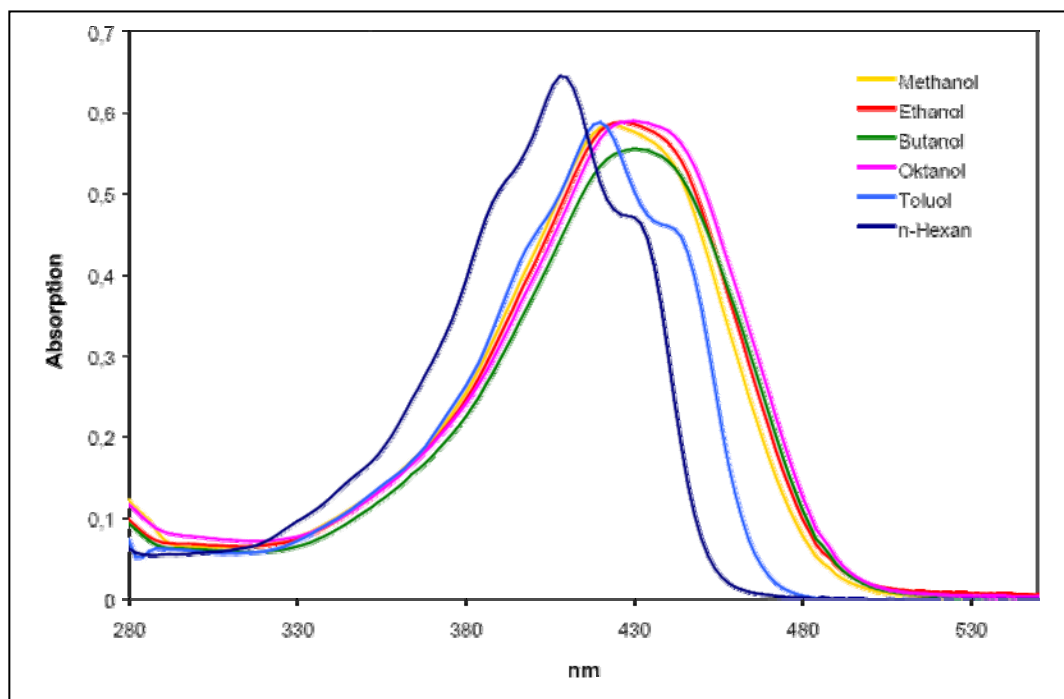


Figure 82 UV-Vis spectra of curcumin in various solvents (Kruse, 2003)

The maximum absorption is due to the electronic dipole allowed $\pi-\pi^*$ type excitation of its extended π -conjugation system. Upon light absorption a π electron is excited from the ground state to the first excited state and oscillates from one end of the chromophore to the other (Zsila, *et al.*, 2003a). Most likely, the weak, electronic dipole forbidden $n-\pi^*$ band is located somewhere under the main absorption band (Zsila, *et al.*, 2003b). The large conjugated framework of curcumin molecule predominated in the enolic curcumin, π electrons are delocalized over through whole molecule between two feruloyl parts which causes a decrease in $\pi-\pi^*$ transition energy and the absorption band appears at the lower energy (419 nm in acetonitrile) than curcumin in the ketone form (389 nm in acetonitrile) in which there is no conjugation between two feruloyl parts (Christian and O'Reilly, 1986; Zsila, *et al.*, 2003a; Zsila, *et al.*, 2003b; Zsila, *et al.*, 2004; Shen and Ji, 2007).

4.1.2 Spectrophotometric studies of curcumin-metal complexes

4.1.2.1 *Curcumin-Hg(II)*

Maximum absorption band of curcumin in the presence of Hg(II) ion decreased in intensity and a new peak appeared at 362 nm (Figure 46). When fixed the concentration of curcumin and increased the amount of Hg(II) ion, an increase of the new peak at 362 nm accompanied by a decrease of the absorbance at 428 nm could be detected. These made an isosbestic point between them at wavelength 395 nm. The presence of an isosbestic point indicated that two species were in equilibrium of each other at this wavelength (395 nm) (Drago, 1977). The increase of new peak at 362 nm is due to the formation of Hg(II)-curcumin complex as a result from addition of Hg(II) ion. These could be compared to the comment of Zsila and co-worker that if a bulk substituent is present on the central methylene carbon atom and sterically prevents the molecule from adopting a planar geometry, the maximum of light absorption shifts to the near-ultraviolet (354-366 nm) region and the molecule lost its color since there is no conjugation between the two feruloyl parts (Zsila, *et al.*, 2003a). Moreover, the C-bonded acac ion was found in $\text{Hg}_2\text{Cl}_2(\text{acac})$ by Bonati and Minghetti in 1968 (Kazuo, 1986). All of this could imply that curcumin bound with Hg(II) at the central methylene carbon atom.

4.1.2.2 *Curcumin-Cu(II)*

A change in maximum absorption spectra of curcumin when added Cu(II) ion was detected (Figure 44). The intense characteristic band of curcumin splitted at the maximum of the peak with shoulder at 460 nm. The increase in absorption intensity could be observed when increased the Cu(II) concentration. The change can be assigned to the interaction between curcumin and Cu(II) ion.

4.1.2.3 Curcumin-Ni(II)

The addition of Ni(II) ion to curcumin solution caused slightly change in curcumin absorption band, and a split of maximum peak and its shoulder at 460 nm occurred (Figure 48). These indicated the interaction between curcumin and Ni(II) ion.

Addition of Cu(II) and Ni(II) ions affected the absorption spectra of curcumin differently from the case of Hg(II) addition. In the cases of Cu(II) and Ni(II), the intense color in curcumin solution and their absorption peaks were observed which indicated that the π -conjugation system between two feruloyl parts of curcumin in the complex was still exist.

4.1.3 Curcumin with Fe ions (Fe(II) and Fe(III))

Upon addition of Fe(II) solution to curcumin, the absorption profile remained the same but decreased in intensity at the maximum absorption (430 nm). This is indicative of a perturbation of curcumin by the metal cation. In this work, the complex formation of curcumin-Fe(II) was not pursued further due to their complicated behavior. However, the formation constant of complex curcumin-Fe(II) in aqueous media has been reported by Bernabé-Pineda and co-workers, by feeding the UV-Vis data into the SQUAD refining software, three complexes of curcumin-Fe(II) species were proposed with their corresponding constant; FeCur^- ($\log \beta_{110} = 9.20 \pm 0.04$), FeHCur ($\log \beta_{111} = 19.76 \pm 0.03$), FeH_2Cur^+ ($\log \beta_{112} = 28.11 \pm 0.02$). It is notable that the unsteadiness of absorption intensity in this system was not mentioned or discussed.

The decrease in curcumin maximum absorption (430 nm) and following with bathochromic shift to 510 nm, were obtained when added the solution of Fe(III) ion, concurrent with new appearance peak was observed at 510 nm. This is indicative of a strong perturbation of curcumin chromophore by the Fe(III) ion, similar to what

previously found. Tønnesen and Greenhill have indicated the appearance of absorption at 510 nm as an evident for the existence of curcumin-Fe(II) complex. The existence of Fe(II) in curcumin-Fe(III) system was demonstrated by phenantroline-complex method (Tønnesen and Greenhill, 1992), and electrochemical behaviour (Borsari, *et al.*, 2002; Bernabé-Pineda, *et al.*, 2004), attributed to the reducing ability of curcumin.

According to the unsteady UV-Vis absorption of these mixed solution, these may be due to many species were formed in the system. Nevertheless, the complex formation of these complexes have been estimated by more suitable process as corrected in Table 6 (CHAPTER 1).

4.1.4 Curcumin analogues

4.1.4.1 Ferulic acid and vanillin

Ferulic acid in methanolic-aqueous media has an intense absorption band at 320 nm with a less intense shoulder near 280 nm. In the same media, vanillin has a maximum absorption at 275 nm and a slightly different in intensity at wavelength 320 nm. The UV-vis spectrum of dehydrozingerone in ethanolic solution having an absorption at 340 nm with a shoulder around 300 nm was reported by Zsila and co-worker (Zsila, *et al.*, 2003b). Dehydrozingerone, ferulic acid, and vanillin, analogous to the half molecule of curcumin, were colorless and lack of an absorption in the visible region due to the narrow conjugation systems of them. These molecules were classified in polyphenolics as 3-methoxyl-4-phenyl phenolic. The polyphenolics were recognized as an antioxidants by radical scavenging, singlet oxygen quenching and metal chelation (Andjelković, *et al.*, 2006).

The chelating ability, or lack of, of both ligands to Cu(II) and Hg(II) were studied by means of spectrophotometric method. The spectra of both ligands in the presence of Cu(II) and Hg(II) did not show any significant change, indicating no interaction with metal ions. These may be a functionality of these ligands (*ortho-*

hydroxymethyl phenol) that are different with catechol and galloyl polyphenols groups (*ortho*-dihydroxyl phenols) which have reported as an iron chelator (Andjelković, *et al.*, 2006).

4.1.4.2 Acetylacetone spectra

Acetylacetone in methanolic-aqueous media showed an intense absorption band at 275 nm which was assigned to the intraligand $\pi - \pi^*$ transition (Lintvedt and Kernitsky, 1970). The increase in intensity at the maximum absorption and the presence of a new peak at wavelength 230 nm were observed in the presence of Hg(II) ion indicating a Hg(II)-acac complex was formed. The bathochromic shift of acetylacetone to wavelength 300 nm and new absorption peak at 230 were observed in the presence of Cu(II) ion indicating a Cu(II)-acac complex was formed. The bathochromic shift of acetylacetone absorption to wavelength 298 nm was observed in the presence of Ni(II) ion indicating the Ni(II)-acac complex was formed. The shift of maximum absorption band of acetylacetone in complexation with addition of these metals is due to the decrease in $\pi - \pi^*$ energy of ligand.

The results from this comparative studies of the reaction patterns of curcumin and its analogues (acetylacetone, ferulic acid, and vanillin) showed that the terminal functional groups of curcumin have no chelating abilities with Cu(II), Hg(II) and Ni(II) ions. To the best of our knowledge, the binding abilities of ferulic acid, vanillin and dehydrozingerone with any metals ions have never been reported. But, in this study, acetylacetone exhibited binding capacity with Cu(II), Hg(II) and Ni(II) ions, in agreement with the fact that acetylacetone formed complexes with many metal ions and so far numerous reports have been published. Therefore, from the comparative studies, they concluded that when curcumin reacts with metal ion the most likely site is by using its central β -diketone part rather than the terminal functional groups.

4.2 Stability constant of the complex formation

The stoichiometric ratios of curcumin-metal complexes were determined using the continuous variation method. Solutions of metal ions and curcumin of the same concentration were prepared and then mixed in the volume ratio from 1:9 to 9:1. The continuous variation plot between molar fraction versus their new absorbance (Cu(II) = 425 nm, Hg(II) = 368 nm, and Ni(II) = 429) gave a near linear curve without inflection portion due to the heavily overlapped absorption band of the new complex species and the residual free ligand. In this case, the spectral responses from more than one components overlap extensively and analysis by continuous variation is no longer straightforward.

The composition of the complex was also determined by applying the mole-ratio method. A series of solution were prepared with a constant concentration of ligand curcumin and variable metal ion (Cu(II), Hg(II), and Ni(II)). The mole-ratio plot between the concentration of metal ions versus its absorbance, a steep curve was obtained, but reading the intersection of the slopes of the two linear parts was difficult to determine, due to the strong overlapped absorption of residual free curcumin and its metal complex. The stoichiometric ratios of the curcumin-metal (Cu(II), Hg(II), and Ni(II)) complexes could be estimated as 2:1, 1:1, and 2:1, respectively.

Due to some free curcumin was still present in solution, the absorbance spectra of the mixed system appeared as curve (a) in Figure 83 (example for curcumin-Hg system) where the new peak of complex at 368 nm appeared as the shoulder of the curcumin peak which centered at 430 nm. The overlapping of two peaks made it difficult to obtain accurate concentrations related to the two peaks. For further calculations were to be possible the overlap must be resolved. There are quite a few commercial softwares that help to get around this problem but in some cases the experimental data are not suitable with the program operations. Instead of purchasing

expensive software, the Microsoft Excel that usually comes with the Windows-based PC was used in this work.

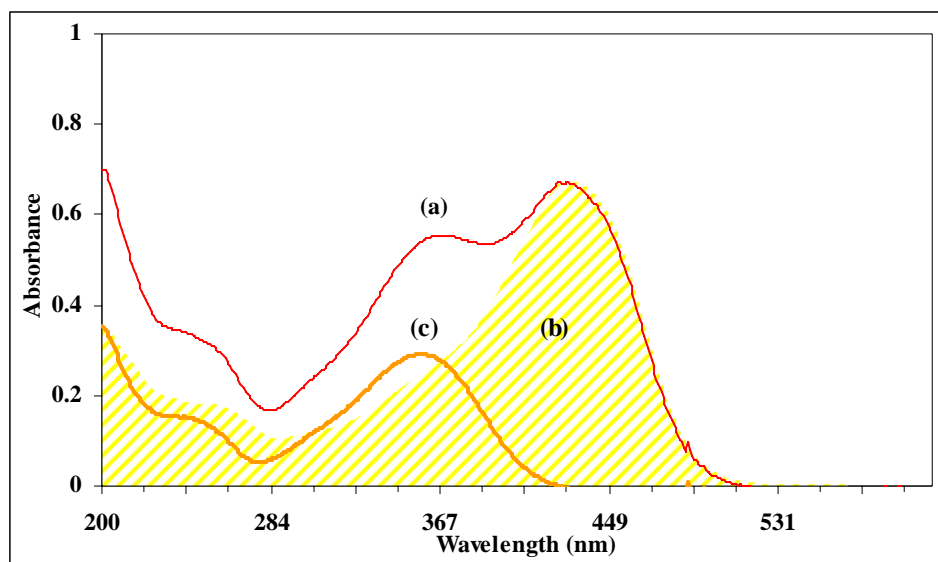


Figure 83 (a) Spectrum of mixed curcumin and Hg(II) ion, (b) estimated spectrum of residual unreacted curcumin, and (c) the clean spectrum of the complex (after subtraction (b))

The spectrum of free curcumin of unknown concentration was estimated by using the correction of calibration curve (as explained in CHAPTER 2; 2.4.2), with these we could estimate the concentration of the residual unreacted curcumin. The original spectrum was subtracted with the absorption of residual free curcumin numerically and the resulting complex spectrum was without residual free ligand disturbance. Subsequently, the complex formations were studied using the continuous variation and mole-ratio methods. The continuous variation plot show the parabolic portion with maximum at mole fractions 0.33, 0.50, and 0.32 which correspond to the stoichiometric ratios 2:1, 1:1, and 2:1 for curcumin-Cu(II), curcumin-Hg(II), and curcumin-Ni(II), respectively. For the mole-ratio plot, the intercept of the two linear parts were more distinct than the original mole-ratio plot. The intersection in

the mole-ratio plots of curcumin-Cu(II), curcumin-Hg(II), and curcumin-Ni(II) gave equivalent concentrations $2.0 \times 10^{-5} : 9.3 \times 10^{-6}$, $2.0 \times 10^{-5} : 2.0 \times 10^{-5}$, and $2.0 \times 10^{-5} : 1.0 \times 10^{-5}$ or ratios 2:1, 1:1, and 2:1, respectively. The compositions that were estimated from continuous variation method were in agreement with the result from mole-ratio method. By these ratios, the complexes can be divided into two groups, the 1:1 and 2:1 complexes.

4.2.1 Curcumin-Hg(II) complex (1:1 complex)

Curcumin and Hg(II) formed a 1:1 complex in methonolic-aqueous media. The complex formation constant of curcumin-Hg(II) complex was calculated using general equation (Eq. 1) and compared with that obtained from the Benesi-Hildebrand method (Eq. 15). These results are summarized in Table 16.

4.2.2 Curcumin-Cu(II) and curcumin-Ni(II) complexes (2:1 complexes)

Curcumin formed complexes with Cu(II) and Ni(II) with stoichiometry 2:1 in methonolic-aqueous media and the complex formation constants of both complexes were calculated by using the general equation (Eq. 2). The results are summarized in Table 16.

Table 16 The complex formation constants of curcumin complexes

Complex	Stoichiometry	Method	Complex formation constant	Temperature (°C)
Curcumin-Hg	1:1	General equation	$\log \beta = 4.60$	25
Curcumin-Hg	1:1	Benesi-Hildebrand	$\log \beta = 4.82$	25
Curcumin-Cu	2:1	General equation	$\log \beta = 9.83$	25
Curcumin-Ni	2:1	General equation	$\log \beta = 8.83$	25

The curcumin-Cu(II) complex formation found in this work 9.83 is close to the value reported by Sundaryano and co-worker 10.88 (20°C), for the stoichiometric ratio of curcumin to Cu(II) 2:1. Sundaryano and co-worker could not evaluate the stoichiometry of the complex by continuous variation method because of the difficulty to analyzed curves. Instead they used the LENTAGROP-SPEFO software to determined the stoichiometry of the complex and its binding constants. In other work, the different binding constant values were also reported by Barik and his co-worker.

The complex of curcumin-Cu(II) (1:1) was reported in 2005 and an attempt to estimate the binding constant was made by following the absorption changes at 450 nm, after mixing curcumin solutions with stiochiometric amounts of copper acetate. The binding constant of this 1:1 complex was estimated to be $\sim 1.1 \pm 0.3 \times 10^5 \text{ M}^{-1}$ as similar to the values reported by Baum and Ng (Barik, *et al.*, 2005). Nevertheless, they (Barik and co-workers) expected that the estimated value from the synthesized complex should be greater than the value obtained by mixing the two reagents in solution (Barik, *et al.*, 2005). However, in 2007 the same researchers reported the formation constants of 1:1 and 2:1 complexes of curcumin and Cu(II) as 3.7×10^{14} and 3.9×10^{15} , respectively. In this later work they used electrochemical method and DMSO was used as solvent (Barik, *et al.*, 2007). There was so much different in the value of 1:1 complex between the first and the second report but the authors did not even mention this discrepancy.

The formation constants of curcumin-Ni(II) and curcumin-Hg(II) complexes have never been reported so far. To confirm the values obtained in this thesis, the values were compared with those of acetylacetone (acac) complexes with the same metal ion as shown in Table 17 (Stary and Liljenzin, 1982). Acac has the β -diketone functional group resembles the β -diketone moiety in the center of curcumin molecule. Acac has been known to readily form complex with variety of metal ions

and chemical and physical properties of these complexes have been well documented, such as those in Table 17.

Table 17 The complex stability of acetylacetone with some metal ions (Stary and Liljenzin, 1982)

Complex	Stoichiometry	Method	Complex formation constant	Temperature (°C)
acac-Hg(II)	1:1	-	$\log \beta_1 = 12.90$	25
acac-Hg(II)	2:1	-	$\log \beta_2 = 20.10$	25
acac-Cu(II)	1:1	-	$\log \beta_1 = 8.22$	25
acac-Cu(II)	2:1	-	$\log \beta_2 = 14.86$	25
acac-Ni(II)	1:1	Potentiometry	$\log \beta_1 = 5.71 \pm 0.04$	25
acac-Ni(II)	2:1	Potentiometry	$\log \beta_2 = 10.16 \pm 0.09$	25

The values of formation constants in Table 16 show the same trend as those in Table 17. Comparison of curcumin complexes to the acac complexes of the same stoichiometries, the value of Ni(II) complex is slightly lower than that of Cu(II) complex. Formation constants of both Cu(II) and Ni(II) complexes are much higher than Hg(II) complex. This is very encouraging for our calculated results in Table 16. The values of curcumin complex of the same metal ion with the same stoichiometry are lower than their corresponding acac complexes. This is not unexpected if we consider the bulkiness of curcumin molecule and compare to the much smaller acac molecule. Both have the same functional group chelating to the metal ion. In this case the steric factor plays a dominant role, and we should expect curcumin complex to be less stable than its corresponding acac complex. This is reflected by the lower formation constant values of curcumin complex.

4.3 Changes of pH of the reaction solutions

The pH of curcumin solution, in 50% MeOH, after addition of metal ion solutions (Cu(II), Hg(II), and Ni(II)) slightly decreased (more acidity) due to more protons (H^+) were released in the system. When the metal ion was added to the solution, it quickly formed complex with the enolate anion of curcumin and, in the process, hydrogen was expelled as H^+ as shown in Figure. 84. Hence, the solution became more acidic and the pH decreased.

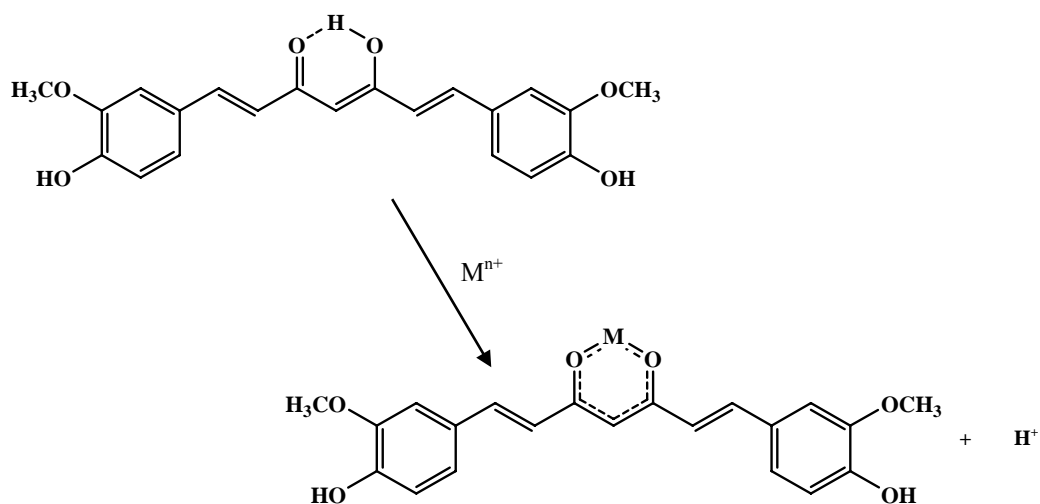


Figure 84 Replacement of metal ion on the enolic moiety of curcumin molecule