CHAPTER 2

Methodology

2.1 Chemicals

2.1.1 Materials from APS Finechem, Australia.

Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), Code no. 2802, A.R. grade

2.1.2 Materials from BDH, England.

Silver nitrate ($AgNO_3$), Code no. 1493, A.R. grade

2.1.3 Materials from Carlo Erba, Italy.

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), Code no. 451877, A.R. grade

Mercuric chloride ($HgCl_2$), Code no. 461005, A.R. grade

Manganous sulfate monohydrate ($MnSO_4 \cdot H_2O$), Code no. 460306, A.R. grade

2.1.4 Materials from Fluka, USA.

Acetylacetone ($CH_3COCH_2COCH_3$), Code no. 00900, GC grade (US)

Chromium chloride haxahydrate ($CrCl_3 \cdot 6H_2O$), Code no. 27096, A.R. grade

Ferric nitrate nanohydrate ($Fe(NO_3)_3 \cdot 9H_2O$), Code no. 44949, A.R. grade

Ferulic acid ($C_{10}H_{10}O_4$), Code no. 46280, A.R. grade

Lanthanum acetate hydrate ($La(CH_3COO)_3 \cdot xH_2O$), Code no. 61461, A.R. grade

Lanthanum chloride hydrate ($LaCl_3 \cdot xH_2O$), Code no. 61490, A.R. grade

Lead nitrate ($Pb(NO_3)_2$), Code no. 15335, A.R. grade

Nickel sulfate haxahydrate ($NiSO_4 \cdot 6H_2O$), Code no. 146398, A.R. grade

Vanillin ($C_8H_8O_3$), Code no. 94750, A.R. grade

Zinc sulfate haptahydrate ($ZnSO_4 \cdot 7H_2O$), Code no. 96500, A.R. grade

2.1.5 Materials from Lab-scan, Thailand

Methanol (CH_3OH), Code no. A 3513, A.R. grade

2.1.6 Materials from Merck, Germany

Acetone (C_2H_6O), Code no. 1.00014.2500, A.R. grade

Ethanol (C_2H_5O), Code no. 1.00983.2500, A.R. grade

Methanol (CH_3OH), Code no. 1.06009.2500, A.R. grade

Nickel chloride haxahydrate ($NiCl_2 \cdot 6H_2O$), Code no. 1.06717.0250, A.R. grade

2.1.7 Materials from M&B, England.

Cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2} H_2O$), Code no. Lot 06829, A.R. grade

2.1.8 Materials from Riedel-de Hean, Germany.

Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$), Code no. 11714, A.R. grade

2.1.9 Materials from Sigma, USA

Curcumin ($C_{21}H_{20}O_6$), Code no. C-1386, A.R. grade

2.1.10 Materials from Department of Chemistry, PSU

Deionized water

2.1.11 10% Nitric was used to wash glasses

2.1.12 KOH in alcohol was used to wash glasses

2.2 Instruments

2.2.1 Department of Chemistry, PSU

1. UV-Visible spectrophotometer, Specord® S100, Analytik Jena GmbH; Germany.
2. Themostat, Lauda® A100; Germany.
3. pH meter, Hanna® instruments 1589; USA.
4. Stirrer, Heidolph® MR3000, Heidolp Instruments GmbH&Co.KG; Germany.
5. Micropippet 100-1000 μL, Biohit Proline®, Biohit Plc., Finland.
6. Micropippet 20-200 μL, Lab Mate® L200, High tech lab, Poland.

7. Volumetric flask amber glassware Pyrex®, USA.

2.3 Software

2.3.1. Microsoft® Office Excel 2003 part of Microsoft Professional Edition 2003 Microsoft Corporation.

2.4 Methods

2.4.1 Determination of the composition of complexes

2.4.1.1 The mole-ratio method

In the mole-ratio method, the concentration of ligand is kept constant and the concentration of metal ion is varied for many ratio of ligand to metal ion. Initially, the solution contains only the ligand (ratio = 0), no complex, so the absorbance of the solution comes solely from the ligand. As the concentration of metal is increased, absorbance will increase (due to formation of complex) until the molar ratio of metal to ligand concentration, which equals to the ratio of the complex. This ratio represents the maximum possible concentration of complex. Further increases in metal concentration will cause slightly change in absorbance (ratio = ∞).

2.4.1.2 The continuous variation method (Job's method)

In this method, the total molar concentration of metal ion plus ligand is kept constant and the ratio varied from zero to infinity. Initially in a solution containing no metal ion (ratio = 0), no complex is present and the absorbance of the solution is due solely to ligand. As the concentration of metal is increased, absorbance will increase *(due to formation of complex)* until the molar ratio of metal to ligand equals the ratio in the complex. Under the Job conditions, i.e. total molar concentrations of the

two species are constant that represents the maximum possible concentration of complex. Further increasing in metal concentration (with corresponding decreases in ligand concentration) will cause a decrease in absorbance until a solution containing no ligand (ratio = ∞) has an absorbance due to metal ions alone. A plot of absorbance against ratio of molar concentration of metal ion to ligand will show the maximum where the ratio represents the complex.

2.4.2 Spectrophotometric determination of the overlapped absorptions

In the case of overlapped absorption between the corresponding peak and other adjacent absorption, the absorption intensity of the corresponding peak will be inexactly depending on how adjacent they are. Several examples of overlap are illustrated in the following computer generated 'spectral' plots (Figure 14). When the pink absorption is an interested absorption, and when the green absorption is other adjacent absorption around the corresponding one, and the dash line is their overlay absorption. The corresponding intensity was disturbed by the adjacent one, and the error will increase as they move closer together.

In this work, the corresponding peak is the peak of the complex which appears close to the ligand curcumin absorption. When curcumin usually existed in the equilibrium, this effect the overlapped of their absorptions. Then the absorption intensity is read from absorption spectra of their mixture which will bear some error. In this thesis, we tried to improve the method of evaluating the concentration of residual curcumin by establishing relationship between concentration and absorbance of all wavelengths in the curcumin absorption range (200-550 nm). The obtained spectrum of residual curcumin is then subtracted from the original spectrum.

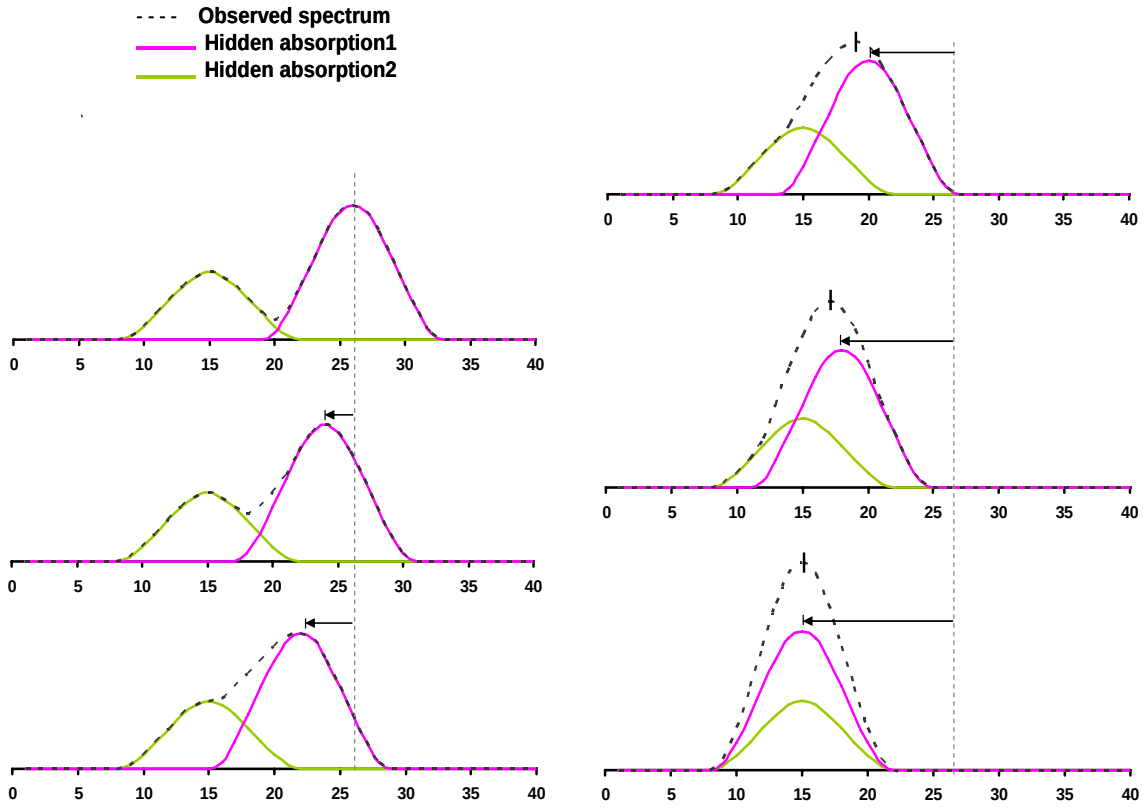
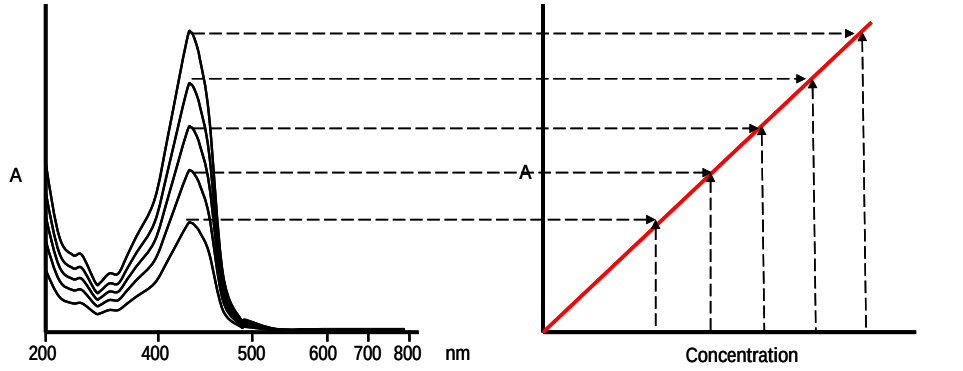


Figure 14 Illustration of two overlapped absorptions and their overlay

In the normal single calibration curve, the linear equation of calibration curve was processed to determine the relationship between absorbance and concentration (Figure 15).

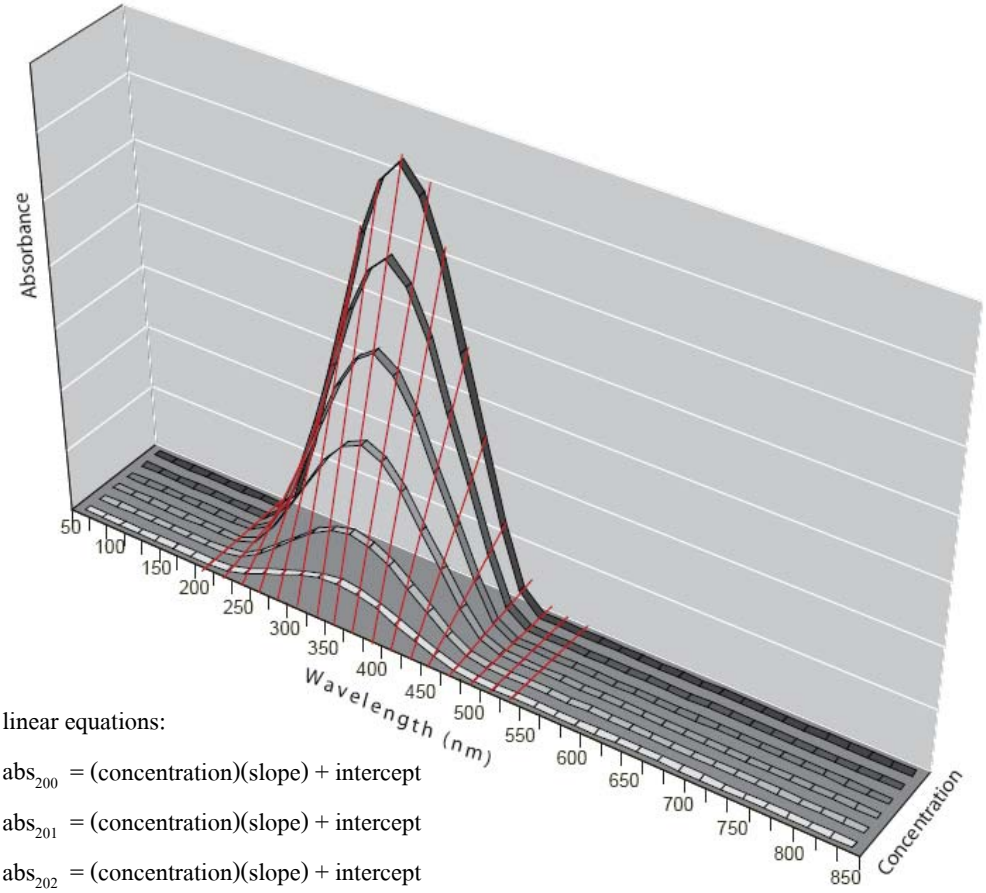


linear equation:

abs_{430} = (concentration)(slope) + intercept

Figure 15 Single calibration curve

For the corrected calibration curves, the linear equations of all calibration curves through 200-550 nm were obtained. The absorbance at every wavelength through 200-550 nm was obtained for a concentration. Each absorbance was plotted against each wavelength to obtain the absorption spectrum (Figure 16).



linear equations:

abs_{200} = (concentration)(slope) + intercept

abs_{201} = (concentration)(slope) + intercept

abs_{202} = (concentration)(slope) + intercept

:

:

abs_{550} = (concentration)(slope) + intercept

Figure 16 Model of corrected calibration curves

The estimated residual curcumin spectrum obtained from the corrected calibration curve will be used to subtract from the original spectra as depicted by the flowchart in Figure 17.

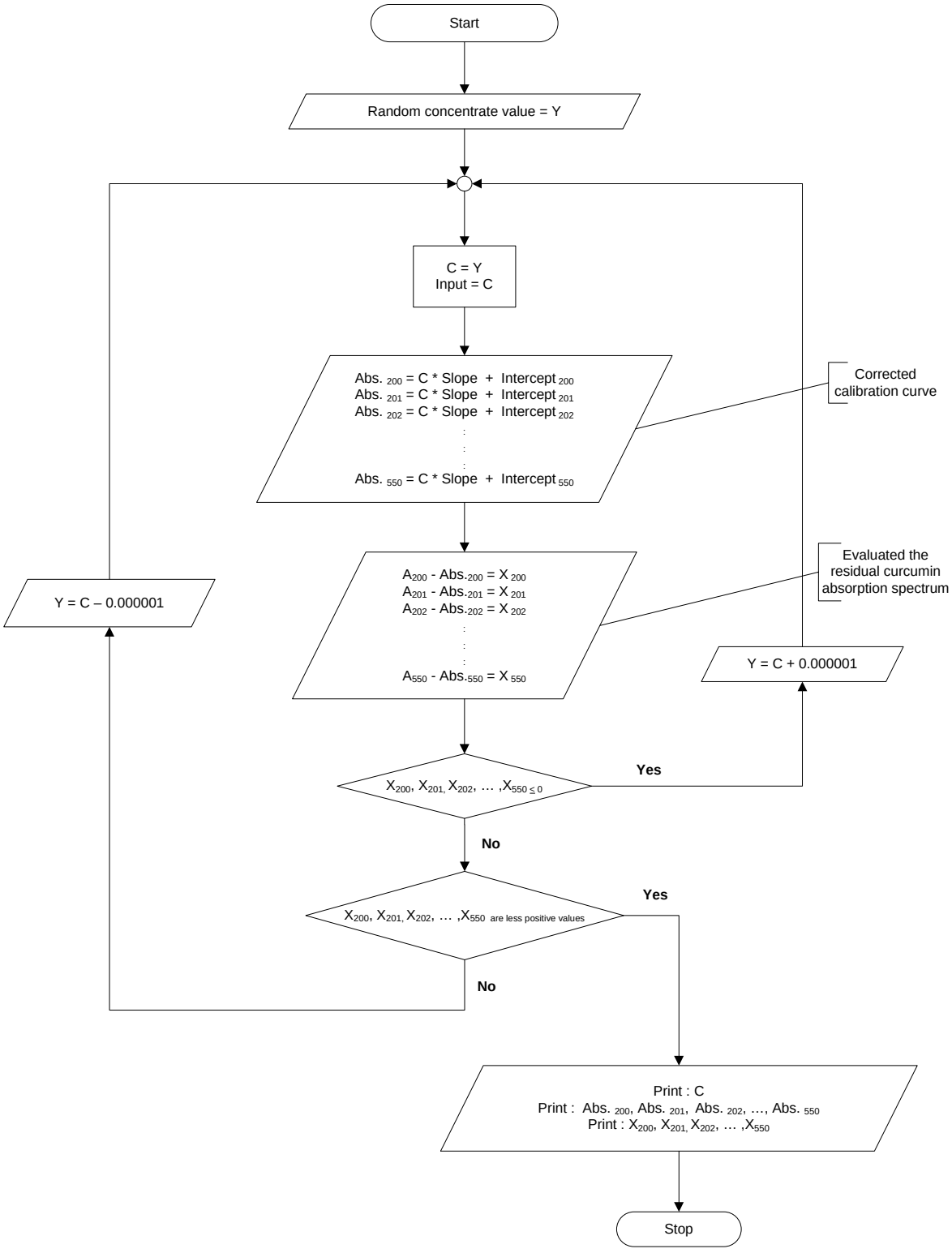


Figure 17 Flowchart of the residual free ligand subtraction

2.5 Preparation of curcumin and metal ions

2.5.1 Preparation of stock solution of curcumin

Stock solution of curcumin (2.0×10^{-4} M) in 50% MeOH (methanol: deionized water) was freshly prepared before measurement. Since the solubility of curcumin is low in water, curcumin powder was first dissolved in absolute methanol then the deionized water was added to make a final solution be 50% MeOH. In order to minimize the effects of light on these solutions, all the experiments were carried out in the dark. The temperature through out the experiment was kept constant at 25^{o} C.

2.5.2 Preparation of stock solution of metal ions and other substances

Stock solution of each metal (2.0×10^{-4} M of each metal ion) was prepared in 50% MeOH at 25^{o} C.

Stock solution of ferulic acid and vanillin (2.0×10^{-4} M) were prepared in 50% MeOH. Stock solution of acetylacetone (4.0×10^{-4} M) was prepared by diluting 1M of acetylacetone with 50% MeOH. All the experiments with ferulic acid, vanillin, and acetylacetone solutions were protected from light and the temperature was kept constant at 25^{o}C.

2.6 Spectral studies by UV-Visible spectrophotometry

2.6.1 Curcumin spectra

2.6.1.1 Determining suitable concentration of curcumin

The suitable concentration of curcumin was examined by collecting series of curcumin absorption at different concentrations. The stock solution of curcumin (2.0×10^{-4} M) was diluted with 50% MeOH consecutively to achieve the concentrations of 1.0×10^{-6}, 2.0×10^{-6}, 3.0×10^{-6}, 4.0×10^{-6}, 5.0×10^{-6}, 6.0×10^{-6}, 7.0×10^{-6}, 8.0×10^{-6}, 9.0×10^{-6}, 1.0×10^{-5}, 1.5×10^{-5}, 2.0×10^{-5}, 2.5×10^{-5}, 3.0×10^{-5}, 4.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 7.0×10^{-5}, 8.0×10^{-5}, and 9.0×10^{-5} M. The UV-Vis spectra of this series were

recorded by SPECORD S100 spectrophotometer and 50% MeOH was used as blank. To keep constant the temperature (25^{o} C), a thermoelectrically temperature-controlled cell holder was used.

2.6.1.1 Calibration curve of curcumin

To prepare a calibration curve, the stock solution of curcumin (2.0×10^{-4} M) in 50% MeOH (methanol: deionized water) was freshly prepared before measurement. Stock solution of curcumin was diluted with 50% MeOH to achieve concentrations of curcumin 4.0×10^{-6}, 5.0×10^{-6}, 6.0×10^{-6}, 7.0×10^{-6}, 8.0×10^{-6}, 9.0×10^{-6}, 1.0×10^{-5}, 2.0×10^{-5}, 3.0×10^{-5}, and 4.0×10^{-5} M. The solutions were prepared in an amber volumetric flask to protect it from light. The spectra were recorded in 200-800 nm range at 25±0.1^{o}C using a thermoelectrically temperature-controlled cell holder.

The data were obtained in 5 replica and averaged by using Microsoft Excel. The average data then were used to create calibration curves of each wavelength through 200-550 nm range. From the calibration curve which is a straight line, we can get a linear equation by using the built-in function in Microsoft Excel. The linear equation correlates absorbance with concentration. By this method, the UV-Vis absorbance of curcumin for any concentrations can be obtained.

2.6.2 Effect of some metal ions on curcumin spectra

Stock solutions of metal ions were prepared at initial concentration of 2.0×10^{-4} M from the corresponding metal salts. The weighed amount of each metal salt was first dissolved with 50% MeOH then diluted to 100 mL with the same solvent. The stock solution of each metal ion was further diluted to several lower concentrations prior to mixing with curcumin solution.

The mixed solutions of curcumin with metal ions were prepared by using 2.0×10^{-5} M curcumin to which each metal ion solution (as prepared in the above

paragraph) was added. All the preparations were carried out using amber volumetric flasks to protect from light. The UV-Vis spectra of the mixed solutions were recorded in the range 200-800 nm at 25±0.1°C using 50% MeOH as blank in 1 cm quartz cell.

(The metal salts were: $AgNO_3$, $CdCl_2 \cdot 2\frac{1}{2}H_2O$, $CrCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $HgCl_2$, $La(CH_3COO)_3 \cdot xH_2O$, $LaCl_3 \cdot xH_2O$, $MnSO_4 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$, $NiSO_4 \cdot 6H_2O$, $Pb(NO_2)_3$, $ZnSO_4 \cdot 7H_2O$)

2.6.3 Studying stability of curcumin-metal ions spectra with time

Curcumin (4.0×10^{-5} M) was mixed either with metal ions of the same concentration to make solution of 1:1 ratio or with metal ions of half concentration to make 2:1 ratio. Metal ions in the case of 1:1 were Hg(II), Fe(II), and for 2:1 were Cu(II), Ni(II), Fe(III). The spectra of mixed solutions were recorded with time. The intensities at 428 nm were plotted against time to observed the stability of solutions with time.

2.6.4 Studying the stoichiometry of curcumin metal ions complex

2.6.4.1 Curcumin-Hg(II) complex

2.6.4.1.1 Studying the stoichiometry of curcumin-Hg(II) complex by the mole-ratio method

A series of solutions were prepared with a constant concentration of curcumin (2.0×10^{-5} M) and variable Hg(II) ion concentrations (5.0×10^{-6}-1.8×10^{-4} M) as shown in Table 7.

Table 7 Concentrations of curcumin and Hg(II) in the mole-ratio method

Concentration (M)		**Ratio**
Curcumin	**Hg(II) ion**	**Curcumin:Hg(II)ion**
2.0×10^{-5}	0	1.0 : 0
2.0×10^{-5}	5.0×10^{-6}	4.0 : 1.0
2.0×10^{-5}	6.7×10^{-6}	3.0 : 1.0
2.0×10^{-5}	1.0×10^{-5}	2.0 : 1.0
2.0×10^{-5}	2.0×10^{-5}	1.0 : 1.0
2.0×10^{-5}	4.0×10^{-5}	1.0 : 2.0
2.0×10^{-5}	6.0×10^{-5}	1.0 : 3.0
2.0×10^{-5}	8.0×10^{-5}	1.0 : 4.0
2.0×10^{-5}	1.0×10^{-5}	1.0 : 5.0
2.0×10^{-5}	1.2×10^{-5}	1.0 : 6.0
2.0×10^{-5}	1.4×10^{-5}	1.0 : 7.0
2.0×10^{-5}	1.6×10^{-5}	1.0 : 8.0
2.0×10^{-5}	1.8×10^{-5}	1.0 : 9.0
0	2.0×10^{-5}	0.0 : 1.0

Concentrations of Hg(II) ion were plotted against the maximum absorbances and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

2.6.4.1.2 Studying the stoichiometry of curcumin-Hg(II) complex by the continuous variation method (Job's method)

The solution of curcumin and Hg(II) ion in the same concentration (2.0×10^{-5} M) were prepared and mixed in the volume ratio from 0:10 to 10:0 as shown in Table 8.

Table 8 Concentrations of curcumin and Hg(II) in the continuous variation method

Concentration (M)		**Ratio**	**Mole fraction of**
Curcumin	**Hg(II) ion**	**Curcumin:Hg(II)ion**	**curcumin**
2.0×10^{-5}	0	10:0	1
1.8×10^{-5}	2.0×10^{-6}	9:1	0.9
1.6×10^{-5}	4.0×10^{-6}	8:2	0.8
1.4×10^{-5}	6.0×10^{-6}	7:3	0.7
1.2×10^{-5}	8.0×10^{-6}	6:4	0.6
1.0×10^{-5}	1.0×10^{-5}	5:5	0.5
8.0×10^{-6}	1.2×10^{-5}	4:6	0.4
6.0×10^{-6}	1.4×10^{-5}	3:7	0.3
4.0×10^{-6}	1.6×10^{-5}	2:8	0.2
2.0×10^{-6}	1.8×10^{-5}	1:9	0.1
0	2.0×10^{-5}	0	0

The mole fractions of curcumin were plotted against its maximum absorbance and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

2.6.4.2 Curcumin-Cu(II) complex

2.6.4.2.1 Studying the stoichiometry of curcumin-Cu(II) complex by the mole-ratio method

A series of solutions were prepared with a constant concentration of curcumin (2.0×10^{-5} M) and variable Cu(II) ion concentrations (2.0×10^{-6}-4.0×10^{-5} M) as shown in Table 9.

Table 9 Concentrations of curcumin and Cu(II) in the mole-ratio method

Concentration (M)		**Ratio**
Curcumin	**Cu(II) ion**	**Curcumin:Cu(II)ion**
2.0×10^{-5}	0	1.0 : 0
2.0×10^{-5}	2.0×10^{-6}	10 : 1.0
2.0×10^{-5}	4.0×10^{-6}	5.0 : 1.0
2.0×10^{-5}	6.0×10^{-6}	3.3 : 1.0
2.0×10^{-5}	8.0×10^{-6}	2.5 : 1.0
2.0×10^{-5}	1.0×10^{-5}	2.0 : 1.0
2.0×10^{-5}	1.5×10^{-5}	1.3 : 1.0
2.0×10^{-5}	2.0×10^{-5}	1.0 : 1.0
2.0×10^{-5}	3.0×10^{-5}	1.0 : 1.5
2.0×10^{-5}	4.0×10^{-5}	1.0 : 2.0

Concentrations of Cu(II) ion were plotted against the maximum absorbances and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

2.6.4.2.2 Studying the stoichiometry of curcumin-Cu(II) complex by the continuous variation method (Job's method)

The solution of curcumin and Cu(II) ion in the same concentration (2.0×10^{-5} M) were prepared and mixed in the volume ratio from 0:10 to 10:0 as shown in Table 10.

Table 10 Concentrations of curcumin and Cu(II) in the continuous variation method

Concentration (M)		**Ratio**	**Mole fraction**
Curcumin	**Cu(II) ion**	**Curcumin:Cu(II)ion**	**of curcumin**
2.0×10^{-5}	0	10 : 0.0	1
1.8×10^{-5}	2.0×10^{-6}	9.0 : 1.0	0.90
1.7×10^{-5}	3.0×10^{-6}	8.5 : 1.5	0.85
1.6×10^{-5}	4.0×10^{-6}	8.0 : 2.0	0.80
1.5×10^{-5}	5.0×10^{-6}	7.5 : 2.5	0.75
1.4×10^{-5}	6.0×10^{-6}	7.0 : 3.0	0.70
1.3×10^{-5}	7.0×10^{-6}	6.5 : 2.5	0.65
1.2×10^{-5}	8.0×10^{-6}	6.0 : 4.0	0.60
1.0×10^{-5}	1.0×10^{-5}	5.0 : 5.0	0.50
8.0×10^{-6}	1.2×10^{-5}	4.0 : 6.0	0.40
6.0×10^{-6}	1.4×10^{-5}	3.0 : 7.0	0.30
4.0×10^{-6}	1.6×10^{-5}	2.0 : 8.0	0.20
2.0×10^{-6}	1.8×10^{-5}	1.0 : 9.0	0.10
0	2.0×10^{-5}	0.0 : 10	0

The mole fractions of curcumin were plotted against its maximum absorbance and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

2.6.4.3 Curcumin-Ni(II) complex

2.6.4.3.1 Studying the stoichiometry of curcumin-Ni(II) complex by the mole-ratio method

A series of solutions were prepared with a constant concentration of curcumin (2.0×10^{-5} M) and variable Cu(II) ion concentrations (2.0×10^{-6}-4.0×10^{-5} M) as shown in Table 11.

Table 11 Concentrations of curcumin and Ni(II) in the mole-ratio method

Concentration (M)		**Ratio**
Curcumin	**Ni(II) ion**	**Curcumin:Ni(II)ion**
2.0×10^{-5}	0	1.0 : 0.0
2.0×10^{-5}	2.0×10^{-6}	10 : 1.0
2.0×10^{-5}	4.0×10^{-6}	5.0 : 1.0
2.0×10^{-5}	6.0×10^{-6}	3.3 : 1.0
2.0×10^{-5}	8.0×10^{-6}	2.5 : 1.0
2.0×10^{-5}	1.0×10^{-5}	2.0 : 1.0
2.0×10^{-5}	1.5×10^{-5}	1.5 : 1.0
2.0×10^{-5}	2.0×10^{-5}	1.0 : 1.0
2.0×10^{-5}	3.0×10^{-5}	1.0 : 1.5
2.0×10^{-5}	4.0×10^{-5}	1.0 : 2.0

Concentrations of Ni(II) ion were plotted against the maximum absorbances and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

2.6.4.3.2 Studying the stoichiometry of curcumin-Ni(II) complex by the continuous variation method (Job's method)

The solution of curcumin and Ni(II) ion in the same concentration (2.0×10^{-5} M) were prepared and mixed in the volume ratio from 0:10 to 10:0 as shown in Table 12.

Table 12 Concentrations of curcumin and Ni(II) in the continuous variation method

Concentration (M)		**Ratio**
Curcumin	**Ni(II) ion**	**Curcumin:Ni(II)ion**
2.0×10^{-5}	0	10 : 0.0
1.9×10^{-5}	1.0×10^{-6}	9.5 : 0.5
1.8×10^{-5}	2.0×10^{-6}	9.0 : 1.0
1.7×10^{-5}	3.0×10^{-6}	8.5 : 1.5
1.6×10^{-5}	4.0×10^{-6}	8.0 : 2.0
1.5×10^{-5}	5.0×10^{-6}	7.5 : 2.5
1.4×10^{-5}	6.0×10^{-6}	7.0 : 3.0
1.3×10^{-5}	7.0×10^{-6}	6.5 : 3.5
1.2×10^{-5}	8.0×10^{-6}	6.0 : 4.0
1.0×10^{-5}	1.0×10^{-5}	5.0 : 5.0
8.0×10^{-6}	1.2×10^{-5}	4.0 : 6.0
6.0×10^{-6}	1.4×10^{-5}	3.0 : 7.0
4.0×10^{-6}	1.6×10^{-5}	2.0 : 8.0
2.0×10^{-6}	1.8×10^{-5}	1.0 : 9.0
0	2.0×10^{-5}	0.0 : 10

The mole fractions of curcumin were plotted against its maximum absorbance and the stoichiometry of the complex was read from the intersection of the two linear parts of the graph.

The absorption of reaction solutions showed broad absorption peak in the same area of curcumin causing heavily overlap peaks. The origin of these absorptions came from the complex in solution and the residual free curcumin that was still left in the same solution. The concentration of residual free curcumin could be obtained with the *correcting calibration spectra (as explained in 2.4.2)*. When the residual free curcumin absorption was eliminated from the original absorption spectra, the complex absorption spectra alone could be obtained.

2.6.5 Studying the complex formation constant of curcumin metal ions complex

2.6.5.1 Complex formation constant of 1:1 complex

The complex formation constant (K) of 1:1 complex was calculated using equilibrium data (Eq. 2) and also compared with the value calculated from Benesi-Hildebrand equation (Eq. 14).

2.6.5.2 Complex formation constant of 2:1 complex

The overall complex formation constant (β) of 1:2 complex was calculated using equilibrium data (Eq. 2).

2.6.6 Studying the absorption spectra of ferulic acid-metal ions (metal ions = Cu(II), Hg(II), and Ni(II))

Solutions of ferulic acid (3.0×10^{-5} M) were mixed with metal ion solutions of varying concentrations (for Cu(II) and Ni(II) : 6.0×10^{-5}, 2.0×10^{-5}, and 1.0×10^{-5} M and for Hg(II) : 1.4×10^{-5}, 2.0×10^{-5}, and 4.0×10^{-5} M)

Amber volumetric flasks were used in all preparations. The UV-Vis spectra were recorded at 25±0.1^{o}C, using 50% MeOH as blank, in 1 cm quartz cell.

2.6.7 Studying the absorption spectra of vanillin-metal ions (metal ions = Cu(II), Hg(II), and Ni(II))

Solutions of vanillin (5.0×10^{-5} M) were mixed with metal ion solutions of varying concentrations (for Cu(II) : 4.0×10^{-5}, and1.0×10^{-4} M; for Hg(II) : 4.0×10^{-5}, 1.2×10^{-4}, and 1.6×10^{-4} M; and for Ni(II) : 2.0×10^{-5}, 6.0×10^{-5}, and 1.8×10^{-4} M)

Amber volumetric flasks were used in all preparations. The UV-Vis spectra were recorded at 25±0.1^{o}C, using 50% MeOH as blank, in 1 cm quartz cell.

2.6.8 Studying the absorption spectra of acetylacetone-metal ions (metal ions = Cu(II), Hg(II), and Ni(II))

Solutions of acetylacetone (4.0×10^{-5} M) were mixed with metal ion solutions of varying concentrations (for Cu(II), Hg(II), Ni(II) : 5.0×10^{-6}, 1.0×10^{-5}, 2.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 1.0×10^{-4}, 1.2×10^{-4}, 1.4×10^{-4}, and 1.6×10^{-4} M).

Amber volumetric flasks were used in all preparations. The UV-Vis spectra were recorded at 25±0.1^{o}C, using 50% MeOH as blank, in 1 cm quartz cell.

2.7 Studying the neutral pH of curcumin and after addition of metal ions (Cu(II), Ni(II), and Hg(II))

The pH of the curcumin and curcumin-metal system were studied by recording the pH of curcumin solution and then with slowly addition of metal solution. First, the curcumin solution (30 mL) was stirred and its pH was measured. Small amount of the metal solution was added by using micropipette and the pH was recorded at one minute after the addition (when the pH was constant). The metal solution was added continuously with pH recorded until a change of pH of the system

was constant. The change of the pH was presented as a graph between the volume of metal solution and pH.