Chapter 2

Materials and methods

2.1 Chemicals

Chemicals from Sigma

Curcumin, $C_{21}H_{20}O_6$, A.R. grade

Chemicals from Lab-scan

Sodium hydroxide, NaOH, A.R. grade

Chemicals from Merck

Sodium chloride, NaCl, A.R. grade

Standard solution in HNO_3 0.5 M

Lead (II) nitrate, $Pb(NO_3)_2$, A.R. grade

Iron (III) nitrate, $Fe(NO_3)_3$, A.R. grade

Copper (II) nitrate, $Cu(NO_3)_2$, A.R. grade

Arsenic acid, H_3AsO_4, A.R. grade

Chromium (II) nitrate, $Cr(NO_3)_2$, A.R. grade

Manganese (II) nitrate, $Mn(NO_3)_2$, A.R. grade

Nickel (II) nitrate, $Ni(NO_3)_2$, A.R. grade

Mercury (II) nitrate, $Hg(NO_3)_2$, A.R. grade

Chemicals from BDH

Nickel (II) sulphate hexahydrate, $NiSO_4.6H_2O$, A.R. grade

Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$, A.R. grade

Cadmium (II) nitrate tetrahydrate, $Cd(NO_3)_2.4H_2O$, A.R. grade

Chemicals from Fluka

Ammonium chloride, NH_4Cl, A.R. grade

Lead (II) nitrate, $Pb(NO_3)_2$, A.R. grade

Mercury (II) acetate, $Hg(CH_3COO)_2$, A.R. grade

Chemicals from Carlo Erba

Manganese sulphate monohydrate, $MnSO_4.H_2O$, A.R. grade

Lead acetate trihydrate, $Pb(CH_3COO)_2.3H_2O$, A.R. grade

Manganese (II) chloride tetrahydrate, $MnCl_2.4H_2O$, A.R. grade

Bismuth (II) nitrate pentahydrate, $Bi(NO_3)_2.5H_2O$, A.R. grade

2.2 Solvents

Solvents from Merck

d-Chloroform, $CDCl_3$, A.R. grade

d_6-Dimethyl sulfoxide, d_6-DMSO, A.R. grade

Hydrochloric acid, HCl, A.R. grade

Ethanol, CH_3CH_2OH, A.R. grade

Solvents from J.T.Baker

Ammonium hydroxide, NH_4OH, A.R. grade

Acetic acid, CH_3COOH, A.R. grade

Nitric acid, HNO_3, A.R. grade

Solvents from Lab-scan

Acetone, C_2H_6, A.R. grade

Methanol, CH_3OH, A.R. grade

2.3 Instruments

Department of Chemistry, PSU

1. UV-Visible spectrophotometer, SPECORD S100, Analytik Jena GmbH, Germany.
2. Cyclic voltammetry; AUTOLAB PGSTAT 100, Metrohm, Switzerland.
3. Fourier-transformed infrared spectrophotometer, FT-IR, EQUINOX55, Bruker, Germany.

Scientific Equipment Center, PSU

1. Fourier-Transformed NMR spectrometer 500MHz, Model UNITY INOVA, Varian, Germany.
2. X-ray fluorescence spectrometer, PHILIPS PW 2400, The Netherlands.
3. pH meter, model 8519, HANNA Instruments, U.S.A.
4. pH electrode, model 300731.1 with wetting cap removed, Denver Instrument Company.

2.4 Principles of techniques in this study

2.4.1 UV-Vis spectroscopy

Stoichiometries of ligand-metal ion complexes are calculated based on data obtained by using Job's method. This method is based on plotting measured absorbances against mole fractions of the two constituents of the complex. A series of solutions is prepared. The sum of the number of moles of the metal, n_M and the number of moles of ligand, n_L, in the samples is kept constant. The mole fraction (X) for the metal and the ligand is defined as follows:

mole fraction of ligand = $X_L = n_L/(n_L+n_M)$

mole fraction of metal = $X_M = n_M/(n_L+n_M)$

$X_M + X_L = 1$

A series of solutions of equal volume is prepared so that the mole fractions of metal ion and ligand are varied in increments between zero and one. This plot will show a maximum where the ratio represents the composition of the complex (Barnard, et al., 1990).

2.4.2 Cyclic voltammetry

Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of CV results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reactions or adsorption processes.

Figure 15 illustrates the expected response of a reversible redox couple during a single potential cycle. Here it is assumed that only the oxidized form O is present initially. Thus, a negative-going potential scan is chosen for the first half cycle, starting from a value where no reduction occurs. As the applied potential approaches the characteristic E° for the redox process, a cathodic current begins to increase, until a peak is reached. After traversing the potential region in which the reduction process takes place, the direction of the potential sweep is reversed.

O + e- --> R

During the reverse scan, R molecules (generated in the forward half cycle, and accumulated near the surface) are reoxidized back to O and an anodic peak results (Nicholson. et al., 1964).

R --> O + e-

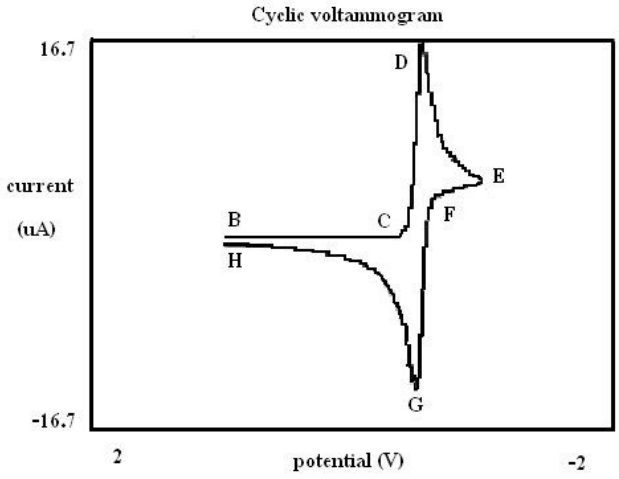

Figure 15 Typical cyclic voltammogram for a reversible redox process

If a redox system remains in equilibrium throughout the potential scan, the redox process is said to be reversible (equilibrium requires that the surface concentrations of O and R are maintained at the values requires by the Nernst equation). The following parameters are used to characterize the cyclic voltammogram of a reversible process:

1. The peak potential separation $\Delta E_p = |E_{pa} - E_{pc}| = 0.059/n$ at all scan rates $25^{\circ}C$. Thus, the peak separation cab be used to determine the number of electrons transferred, and as a criterion for a Nerntian behavior

2. The peak current ratio = $I_{pa}/ I_{pc} = 1$ at all scan rates

3. The position of the peaks on the position axis (E_p) is related to the formal potential of the redox process. The formal potential for a reversible couple is centered between E_{pa} and E_{pc} which $E^{O/} = (E_{pa} + E_{pc}) / 2$

4. The peak current function $i_p/n^{1/2}$ (n = scan rate) is independent of n (see equation for peak current)

The peak current is given by the Randle Sevcik equation $I_p = 2.69 \times 10^5 n^{3/2} A C D^{1/2} \nu^{1/2}$

Where : n = number of electron transferred/ molecule

$\nu^{1/2}$ = Square root of scan rate

A = electrode surface area (cm^2)

C = concentration (mol cm^{-3})

D = diffusion coefficient (cm^2 s^{-1})

For a reversible process, $E^{O/}$ is given by the mean of potentials.

Electrochemical irreversibility is caused by slow electron exchange of the redox species with the working electrode. Electrochemical irreversibility is characterized by a separation of peak potentials greater than indicated by equation $\Delta E_p = |E_{pa} - E_{pc}| = 0.059/n$ (Photicunapat, 2005).

The application of CV for the determination of a complex compound stoichiometry is based on the change of current peak before and after the equivalence point, and it is applicable to the system in which one component of the complex, metal ion or ligand, is electroactive. A current peak obtained in this study is attributed to the reduction or oxidation of either the metal or the complex. The current is plotted against the molar ratio of reagent to metal ion, and the molar ratio at a sharp break indicates the composition of the complex. In this study chosen systems with ligand that is not reduced on the mercury electrode in wide potential range, thus making it possible to carry on the experiments under the conditions where only metal ions are active (Ogura, et al., 1979).

2.4.3 Nuclear magnetic resonance

This technique arises from transitions between nuclear spin states, and the ^{1}H and ^{13}C isotopes are the most common targets. If 5-50 mgs of a pure sample are available, then a ^{13}C NMR spectrum can easily provide an analysis of carbon and hydrogen content. Similarly, ^{1}H NMR data used to assess hydrogen content (Crews, 1998).

2.4.4 Infrared spectroscopy

The instrument that determines the absorption spectrum for a compound provide spectra in the common range of 4000 to 400 cm^{-1}. The value of infrared spectroscopy undoubtedly lies in its contribution to elucidation of molecular structure, particularly the recognition of functional groups and their environment. The infrared spectrum, notably in the fingerprint region is supreme for establishing identity between an organic substance and a compound of unknown structure, by empirical comparison of traces (Frank, 1997).

2.4.5 X-ray fluorescence spectrometry

The X-ray fluorescence principle, an inner shell electron is excited by an incident photon in the X-ray region. During the de-excitation process, an electron is moving from a higher energy level to fill the vacancy. The energy difference between the two shells appears as an X-ray, emitted by the atom. The X-ray spectrum acquired during the above process reveals a number of characteristic peaks. The energy of the peaks leads to the identification of the elements present in the sample (qualitative analysis), while the peak intensity provides the relevant or absolute elemental concentration (semi-quantitative or quantitative analysis) (http://omega.physics.uoi.gr/xrf/english/xrf_equipment.htm).

2.5 Methods

2.5.1 UV-Vis Spectrophotometric study

Preparation of curcumin-metal (Fe^{3+}, Pb^{2+} and Cd^{2+}) solutions for studying the complex stoichiometry in aqueous system

Solution of curcumin and metal ions (1×10^{-4} M) was prepared by dissolving in 5×10^{-3} M NaOH. The sum of the number of moles of the metal ions and the number of moles of curcumin in the samples were kept constant. A series of solutions of equal

volume are prepared so that the mole fractions of metal ion and curcumin were varied in increments between zero and one.

Table 1 The preparation of curcumin-metal solutions for the mole ratio study

metal (ml)	curcumin(ml)	X_{metal}	$X_{curcumin}$
1	9	0.1	0.9
2	8	0.2	0.8
3	7	0.3	0.7
4	6	0.4	0.6
5	5	0.5	0.5
6	4	0.6	0.4
7	3	0.7	0.3
8	2	0.8	0.2
9	1	0.9	0.1

Preparation of curcumin-metal (Fe^{3+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Bi^{2+} and Cr^{3+}) solutions for studying the interaction in 50% methanol media

Interactions of curcumin with some metal ions were studied by comparing the absorption spectra of curcumin in solution alone, and then upon addition of equimolar concentrations of the metal solutions in 50% methanol. A range of equimolar concentrations of curcumin and metal, from $5x10^{-5}$ to $5x10^{-4}$ M, were studied by the UV-Vis spectrophotometer for the behaviour of curcumin and mixed metal curcumin solutions.

2.5.2 Electrochemical study

Solutions were introduced in the cell and were deoxygenated by purging with N_2 for about five minutes. Then, N_2 was allowed to flow over solutions to prevent O_2 from reentering the cell during the experiment.

Preparation of curcumin solution

Solution of curcumin was prepared by dissolving 0.0386 g of curcumin in 5 x 10^{-3} M sodium hydroxide solution to keep the initial basic pH (on the basic side). Carbon Paste Electrode (CPE) and Glassy Carbon Electrode (GCE) were used as working substrate (Bernabé-Pineda et al., 2004).

Preparation of metal solutions

The metal solutions (1 x 10^{-4} M) were prepared by dissolving appropriate metal ions in ammonium acetate buffer pH 4.

Electrodes

Hanging mercury drop electrode (HMDE) and GCE were used as working substrates, silver/silver chloride (Ag/AgCl) as reference electrode, and platinum wire as auxillary electrodes. For the study of complex stoichiometry of curcumin with Ni^{2+}, Pb^{2+} and Cd^{2+}, HMDE was used. Moreover the complex stoichiometry of curcumin with Pb^{2+} was also studied by GCE. The surface of the GCE was cleaned by polishing with alumina slurry, immersing in 10% HNO_3 and was rinsed with buffer after each scan.

Preparation of curcumin-metal solutions for studying the complex stoichiometry

The three series of solutions containing curcumin and metal ions (Pb^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, As^{5+}, Hg^{2+} and Cr^{3+}) at defined molar ratio from 0.0 to any desired values were prepared. The pH was adjusted to 10.77 or 4.2 by adding ammonia-ammoniumchloride (NH_3-NH_4Cl) or ammonium acetate buffer (NH_4Ac), respectively (Piszczek et al., 1988).

- Stoichiometric study of curcumin-Ni^{2+} system

To 5 ml of Ni^{2+} 0.1 mM the appropriate amount of curcumin and 5 ml of ammonia-ammonium chloride buffer were added, then the flask was filled up with 1 M KCl to 25 ml. The working electrode was HMDE.

Table 2 The preparation of Ni^{2+}-curcumin solutions for the mole ratio study

Ni^{2+} : cur	ml				
	Ni^{2+}	curcumin	buffer	KCl	NaOH
Base line	-	-	5	15	5
0.0	5	-	5	10	5
0.2	5	5 (0.02 mM)	5	10	-
0.4	5	5 (0.04 mM)	5	10	-
0.6	5	5 (0.06 mM)	5	10	-
0.8	5	5 (0.08 mM)	5	10	-
1.0	5	5 (0.10 mM)	5	10	-
1.2	5	5 (0.12 mM)	5	10	-
1.4	5	5 (0.14 mM)	5	10	-
1.6	5	5 (0.16 mM)	5	10	-
1.8	5	5 (0.18 mM)	5	10	-
2.0	5	5 (0.20 mM)	5	10	-
2.2	5	5 (0.22 mM)	5	10	-
2.4	5	5 (0.24 mM)	5	10	-
2.6	5	5 (0.26 mM)	5	10	-
2.8	5	5 (0.28 mM)	5	10	-
3.0	5	5 (0.30 mM)	5	10	-

- Stoichiometric study of curcumin-M^{2+} system (M = Pb and Cd)

To 5 ml of metal ion solutions (Pb^{2+} 0.5 mM and Cd^{2+} 0.25 mM) the appropriate amount of curcumin was added. The solutions were made to 25 ml with ammonium acetate buffer. HMDE was used the working electrode.

Table 3 The preparation of curcumin-M^{2+} (M = Pb, Cd) solutions for the mole ratio Study

M^{2+} : cur	ml			
	metal	curcumin (for Pb, Cd)	buffer	NaOH
base line	-	-	20	5
0.0	5	-	15	5
0.2	5	5 (0.1, 0.05 mM)	15	-
0.4	5	5 (0.2, 0.10 mM)	15	-
0.6	5	5 (0.3, 0.15 mM)	15	-
0.8	5	5 (0.4, 0.20 mM)	15	-
1.0	5	5 (0.5, 0.25 mM)	15	-
1.2	5	5 (0.6, 0.30 mM)	15	-
1.4	5	5 (0.7, 0.35 mM)	15	-
1.6	5	5 (0.8, 0.40 mM)	15	-
1.8	5	5 (0.9, 0.45 mM)	15	-
2.0	5	5 (1.1, 0.50 mM)	15	-
2.2	5	5 (1.2, 0.55 mM)	15	-
2.4	5	5 (1.3, 0.60 mM)	15	-
2.6	5	5 (1.4, 0.65 mM)	15	-
2.8	5	5 (1.5, 0.70 mM)	15	-
3.0	5	5 (1.6, 0.75 mM)	15	-

- Stoichiometry study of curcumin-Pb^{2+} system

To 5 ml of Pb^{2+} 0.1 mM the appropriate amount of curcumin and 10 ml of ammonium acetate buffer were added. Next the flask was filled up with deionized water (DI) to 25 ml. The working electrode was GCE.

Table 4 The preparation of curcumin-Pb^{2+} solutions for the mole ratio study

Pb^{2+} : cur	ml				
	Pb^{2+}	curcumin	buffer	DI	NaOH
base line	-	-	10	15	5
0.0	5	-	10	5	5
0.2	5	5 (0.02 mM)	10	5	-
0.4	5	5 (0.04 mM)	10	5	-
0.6	5	5 (0.06 mM)	10	5	-
0.8	5	5 (0.08 mM)	10	5	-
1.0	5	5 (0.10 mM)	10	5	-
2.0	5	5 (0.20 mM)	10	5	-
3.0	5	5 (0.30 mM)	10	5	-

2.5.3 NMR study

Preparation of curcumin-metal ions

Spectrum of ligand was run in $CDCl_3$/d_6-DMSO solution. Solutions of mixed metal ion and ligand were prepared by dissolving equimolar of metal ion and curcumin in the same solvent, then filled up with $CDCl_3$/d_6-DMSO to 1 ml. Solution of curcumin and mixing curcumin with metal were studied by ^1H NMR.

Solution of metals was prepared by dissolving 12 mg of metal salts ($MnCl_2.4H_2O$ and HgI_2) and added 12 mg of curcumin in 1 ml of d_6-DMSO then obtained the ^{13}C NMR spectra of curcumin and metals system containing curcumin.

Preparation of acetylacetone with metal ion solutions

Spectrum of ligand was run in d_6-DMSO solution. Solutions of mixed metal ion and ligand were prepared by dissolving equimolar of acetylacetone and metal ion in the same solvent then filled up with d_6-DMSO to 1 ml. The ^{1}H NMR spectra of acetylacetone and acetylacetone-metal ion solutions then were measured.

2.5.4 IR and XRF study of the residue

Preparation of residue from curcumin and Pb^{2+}

Pb^{2+}-curcumin complex was prepared by mixing equimolar ratios of lead(II) acetate and curcumin in methanol. The mixed solution was kept in the hood overnight and then filtered. The residue obtained was washed with methanol and deionized water.

Preparation of residue from curcumin and Cu^{2+}

Cu^{2+}-curcumin complex was synthesized by mixing equimolar ratios of copper acetate and curcumin in DMSO and refluxed for 3 h. The complex was precipitated out and the solid was separated by filtration. It was washed several times by DMSO and deionized water to remove unreacted curcumin and copper(II) acetate.