CHAPTER 3: METHODOLOGY

3.1 Reagents

All the chemicals and reagents used were of analytical grade. β-cyclodextrin hydrate, 99% (MW = 1135.01) was purchased from Acros Organic. Dithizone (MW = 256.33) was purchased from Sigma-Aldrich while $ZnCl_2$ (MW = 136.28) was purchased from Merck. Water used throughout this research was deionized water and all the chemicals were used as received without any purification.

3.2 Instruments

IR spectra were recorded on a Perkin Elmer (FTIR spectrum RX1) between wavenumbers of 400 to 4000 cm^{-1}. Samples for IR were prepared as KBr disk with 1 mg of complex in 100 mg of KBr. ^{13}C CP/MAS NMR spectra were obtained by using a Bruker AV-400 NMR spectrometer with a sample spinning rate of 8 kHz at room temperature. TGA curve was recorded on Perkin Elmer (Pyris Series TGA 6) by heating at 20°C min^{-1} over temperature range between 30°C to 900°C. DSC was performed using a Perkin Elmer DSC 6 by heating at 20°C min^{-1} over the temperature range of 35°C to 350°C. XRD spectrum was recorded on a Siemens D5000 XRD using a copper anode and energy of 25 kV and 2 mA. EDX analysis was done on FEI Quanta 200 FESEM using a silicon detector with accelerating voltage of 20 kV and 1500x magnification. UV spectra were recorded on Shimadzu UV-Vis spectrophotometer (UV-1650PC). All pH measurements were measured by Thermoline pH meter.

3.3 Synthesis and characterization of the inclusion complexes

3.3.1 Synthesis of inclusion complex of β-Cyclodextrin-dithizone (β-CD-H_2Dz)

2 mmol of β-CD was dissolved in 25 mL water and mixed with 1 mmol of dithizone which dissolved in 25 mL acetone. The mixture was then stirred and heated at 60°C until dry. The mixture was then further dried in an oven at 60°C to remove any remaining moisture. After the mixture has been dried, the black solid powder was gently ground to powder. The inclusion complex powder was analysed for further characterization.

3.3.2 Synthesis of inclusion complex of β-Cyclodextrin-dithizone-zinc (β-CD-H_2Dz-Zn)

4 mmol of β-CD was dissolved in 25 mL water and mixed with 2 mmol of dithizone which dissolved in 25 mL acetone and 1 mmol of zinc dissolved in 25 mL water. The methodology was continued as above and the inclusion complex was sent for further characterization.

3.3.3. Characterization of inclusion complex of β-CD-H_2Dz and β-CD-H_2Dz-Zn

Characterization of the inclusion complexes as conducted by Fourier Transform Infra Red (FTIR), ^{13}C CP/MAS Nuclear Magnetic Resonance (^{13}C CP/MAS NMR),

Thermal Gravimetric Analysis (TGA), Differential Scanning Calorimetric (DSC), powder X-ray Diffraction (XRD) and also Energy Dispersive X-ray (EDX).

3.4 Spectroscopy study of inclusion complex of β-CD-H_2Dz

3.4.1 Effect of pH

Effect of pH on inclusion complex of β-CD-H_2Dz was done by adjusting the pH of 10 mL inclusion complex solution to pH 3, 8 and 12. The absorbance of the inclusion complex with different pH was detected with UV-Vis spectrophotometer at 426 nm.

3.4.2 Phase Solubility Studies

The phase solubility studies were done to verify the improvement on the solubility of dithizone with the addition of β-CD. The phase solubility studies were successfully done for inclusion complex of β-CD-H_2Dz according to the Higuchi and Connors method (Higuchi & Connors, 1965). For this studies, an excess amount of dithizone were added into a series of aqueous solution of β-CD with increasing concentration from 0 to 0.02 M.

The solutions were then sealed and shaken at room temperature for 24 hours and carefully filtered through 0.45 μm Whatmann filter paper. The solutions were analyzed to their absorption recorded with UV spectrophotometer at 426 nm.

3.4.3 Formation constant

The dithizone concentration was held constant at 1.5 x 10^{-5} M while varied concentrations of β-CD were added sequentially in a 10 mL volumetric flask. 15% acetone was also added to the mixture. The mixed solution were then diluted to the calibration mark and mixed well. The absorbance spectrums were measured at 426 nm

for inclusion complex of β-CD-H_2Dz. The stoichiometry ratio of the inclusion complex was obtained through a double reciprocal plot.

3.5 Spectroscopy study of inclusion complex of β-CD-H_2Dz-Zn

3.5.1 Effect of pH

The pH of the inclusion complex of β-CD-H_2Dz-Zn was determined by adjusting the pH of the complex solution in a 10 mL volumetric flask to a basic medium ranging from pH 3, 8 and 12. The absorbance was detected at 522 nm by UV-Visible spectrophotometer.

3.5.2 Formation constant

The dithizone and zinc concentration were held constant at 1.5 x 10^{-5} M and 1 mg/L respectively while varied amounts of β-CD were added sequentially in a 10 mL volumetric flask. 15% acetone was also added to the mixture. The mixed solutions were then diluted to the calibration mark and mixed well. The absorbance spectrums were measured at 522 nm for inclusion complex of β-CD-H_2Dz-Zn. The stoichiometric ratio of the inclusion complex was obtained through the double reciprocal plot.

3.5.3 Stoichiometry study

In order to confirm the ratio of the inclusion complex, stoichiometry study or Job's method was applied for metal complex of H_2Dz-Zn where a series of solution of dithizone and zinc were prepared with the same number of moles but at different ratios. The absorption of the solutions was then obtained from UV spectrophotometer at 522 nm. The experimental conditions for determination of H_2Dz-Zn ratio are tabulated in

Table 3.1 while the experimental conditions for determination of inclusion complex of β-CD-H_2Dz-Zn are tabulated in Table 3.2.

Table 3.1: Experimental conditions for the determination of H_2Dz-Zn ratio (Job's method)

H_2Dz (mole)	Zinc (mole)
0.9	0.1
0.8	0.2
0.7	0.3
0.6	0.4
0.5	0.5
0.4	0.6
0.3	0.7
0.2	0.8
0.1	0.9

Table 3.2: Experimental conditions for the determination of inclusion complex of β-CD-H_2Dz-Zn ratio (Job's method)

H_2Dz-Zn (mole)	β-CD (mole)
0.9	0.1
0.8	0.2
0.7	0.3
0.6	0.4
0.5	0.5
0.4	0.6
0.3	0.7
0.2	0.8
0.1	0.9

3.6 Application of inclusion complex in determination of Zn by spectrophotometric technique.

3.6.1 Effect of solvents

In 10 mL volumetric flask, an inclusion complex of β-CD-H_2Dz-Zn was prepared with 15% of various types of organic solvents. The absorbance of the inclusion complex solution was then determined by UV-Vis spectrophotometer at 522 nm.

3.6.2 Standard procedure

The β-CD-H_2Dz-Zn complex solution was prepared by mixing 0.1 mL of 1.5 x 10^{-3} M of dithizone, 0.1 ml of 1.5 x 10^{-2} M of β-CD and 0.01 mL of 1000 mg/L of zinc in a 10 mL volumetric flask and 15% of acetone was added. The pH of the solution was adjusted to pH 8 and diluted to the mark with deionised water. The absorbance was detected at 522 nm by UV-Visible spectrophotometer.

3.6.3 Effect of amount of dithizone

The effect of amount of dithizone in the inclusion complex was investigated. In a 10 mL volumetric flask, 0.001 M of β-CD and 1 mg/L of zinc were mixed with a series of different concentration of dithizone ranging from 0 to 4 x 10^{-5} M. 15% of acetone was also added to the mixture. The mixed solutions were diluted with deionized water and mixed well. The absorbance was determined at 522 nm.

3.6.4 Effect of amount of β-cyclodextrin

The effect of amount of β-CD in the inclusion complex was studied. In a 10 mL volumetric flask, 0.1 mL of 1.5 x 10^{-3} M dithizone and 0.1 mL of 1000 mg/L of zinc were

mixed and were added with different concentration of β-CD ranging from 0 to 3 x 10^{-4} M. 15% of acetone was added to the mixture. The mixture were diluted with deionized water and mixed well. The absorbance was determined with UV-Visible spectrophotometer at 522 nm.

3.6.5 Effect of foreign ions

The standard procedure (3.6.2) for the determination of 1 mg/L of zinc was followed with the addition of foreign ions and diluted to the calibration mark in a 10 mL volumetric flask. A 3000 mg/L level of each foreign ion was investigated and the ratio was reduced gradually until the interference was in the range of ±5%. The absorbance was determined with UV-Visible spectrophotometer at 522 nm.

3.6.6 Dynamic range and sensitivity study

In this study, a series of solution was prepared in a 10 mL volumetric flask, where a solution of dithizone with a constant concentration of 7.5 x 10^{-5} M was mixed with a varied concentration of zinc and the addition of β-CD with a constant concentration of 1.5 x 10^{-4} M. 15% of acetone was also added to the solutions and was diluted to the calibration mark. The absorbance of the series was determined at 522 nm using UV-Visible spectrophotometer.

3.6.7 Limit of detection

10 blank samples with constant optimum concentration of β-CD and dithizone were prepared without the addition of zinc. The absorbance was detected at 522 nm using UV-Visible spectrophotometer.

3.6.8 Reproducibility

A series of 10 samples of inclusion complex of β-CD-H_2Dz-Zn was prepared using the standard procedure (3.6.2). The absorbance of all the solution was detected at 522 nm by UV-Visible spectrophotometer.

3.6.9 Application on real samples

0.2 g of Chinese herbal medicine was digested using HNO_3, 65% suprapur (8 mL) and H_2O_2 (2 mL) and was diluted to 25 mL with distilled water. 1 mL aliquot of this solution was taken and 0.5 mg/L of zinc was spiked and the standard procedure (3.6.2) was followed to analyse zinc.

1 mL of tap water was taken and used without any pre-treatment. 0.5 mg/L of zinc was spiked into the solution and the standard procedure (3.6.2) was followed to analyse zinc at 522 nm by UV-Visible spectrophotometer.