CHAPTER 1: INTRODUCTION

1.1: Introduction

Cyclodextrin, one of the most important supramolecular compounds, has been widely used for the development of analytical chemistry. There are three types of cyclodextrin (α, β and γ) which are widely used in many industrial areas such as pharmaceutical, food, textiles, cosmetics, chemical and biotechnology and in analytical chemistry as well (Suh *et al*., 2001, Tang *et al*., 2002, Wang *et al*., 2007, Jin *et al*., 2008). β-cyclodextrin was chosen in this study since it is widely available and low cost.

β-CD is widely known for forming a host-guest type inclusion complex because of their hydrophobic cavity. Inclusion complex is formed when a molecule of a guest compound is inserted into the cavity of the host compound as shown in Figure 1.1. Inclusion complexation with a variety of organic or inorganic guest molecules can occur through various interaction such as hydrogen bonding, van der Waals, hydrophobic and also dipole-dipole interaction without any covalent bond. Water molecule will leave the β-CDs cavity and this will contribute to the stability of the β-CD complexes with these interactions (Connors, 1997). In this study, β-CD will act as a host compound while dithizone will act as a guest molecule.

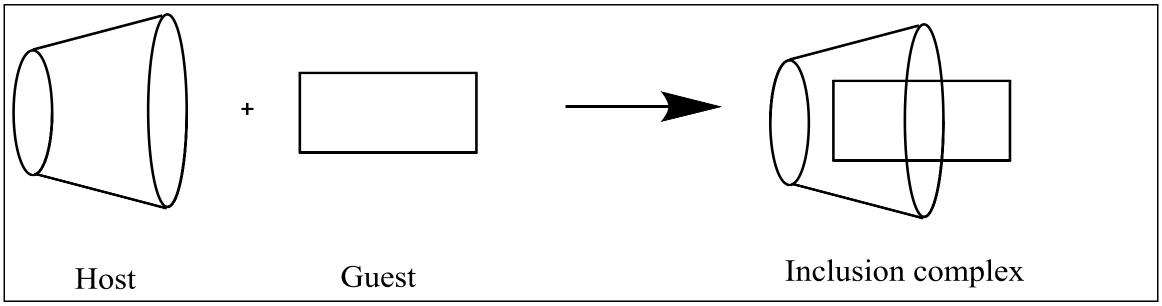

Figure 1.1: Host-guest type inclusion complex

Diphenylthiocarbazone also known as dithizone (H_2Dz) was first introduced by Hellmuth Fischer in 1925 (Sandell, 1959). It is one of the most important chelating reagents that are used to form complex with various metals to form metal dithizonates. Dithizone is widely used in analytical chemistry (Schönherr *et al*., 1999). The violet-black solid dithizone is insoluble in water but dissolves in most of the organic solvents and was also used to detect a trace amount of heavy metals (Burger, 1973).

The idea of tautomeric equilibrium between keto and enol form of dithizone (Figure 1.2) in its solution was introduced by Fischer. He assumed that keto form of dithizone belongs to the longwave band (620 nm) in the UV-Visible spectroscopy while enol form belongs to the shortwave band (450 nm) (Spěváček & Spěváčková, 1976). Dithizone is extremely sensitive towards oxidation. Due to its ability to be both readily oxidized and reduced, the chemistry of dithizone is known to be quite complicated. Dithizone is commonly represented as H_2Dz in its neutral form because it has two exchangeable protons (Pemberton & Buck, 1982).

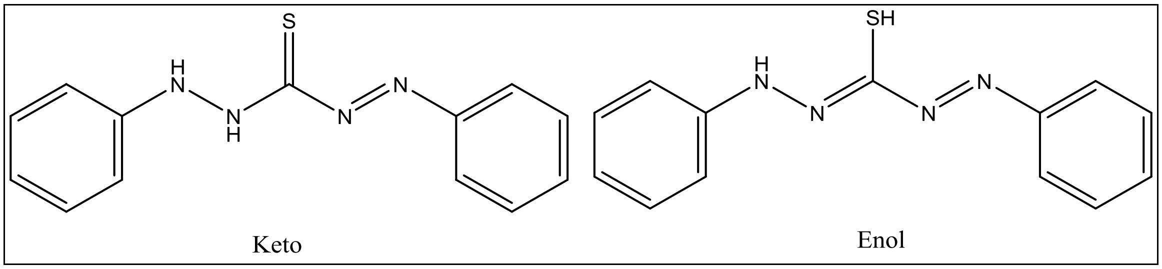

Figure 1.2: Keto and enol form of dithizone

Dithizone gives a strongly colored complex when it reacts with metal ions and is easily detected through UV-Vis spectrophotometry. Besides form metal complexes, dithizone has also been used in many applications such as in pharmaceutical preparations, biological materials and forensic specimens (Pemberton & Buck, 1982). In this study, the inclusion complex of β-cyclodextrin with dithizone was synthesized and the inclusion complex was applied to determine the presence of zinc in real samples.

Zinc (Zn) which has the oxidation number of +2 is the 24th most abundant transition metal in earth crust and second most abundant in humans and also mammals after Fe(II) and Fe(III). Zinc is widely distributed in the environment because it is used in household products, paints, pharmaceuticals, electroplating and also in chemical industries (Chandra, 2010). Zinc also is an important essential microelement in living organism. It plays an important role in various biological systems such as in protein synthesis, wound healing, DNA synthesis and cell division (Salgueiro *et al.*, 2000). Excessive intake of zinc may cause nausea, vomiting, loss of appetite, abdominal cramps and also headache. Zinc is usually detected by spectrophotometric determination and also atomic absorption spectrometry.

Since spectrophotometric determination is low in cost and easy to be handled, it has been chosen for this study. A spectrophotometry technique was developed based on the enhancement of the absorbance of dithizone-zinc complex produced through complex formation with β-CD. Furthermore, the presence of β-CD increased the solubility of the metal complex in aqueous system.

The discussion section will be divided into two major parts. The first part will be on the synthesis and characterization of the inclusion complex of β-CD-H_2Dz and β-CD-H_2Dz-Zn. The second part will focus on the application of the inclusion complex for determination of zinc using spectrophotometric technique.

1.2: Objectives

The objectives of this research are as follows:

a) To synthesis and study the inclusion complex of β-CD-H_2Dz and β-CD-H_2Dz-Zn.
b) To characterize the physical and chemical properties of the inclusion complexes.
c) To determine the stoichiometric ratio of the inclusion complexes.
d) To apply the inclusion complex for determination of zinc by spectrophotometric technique.

CHAPTER 2: LITERATURE REVIEW

2.1 Cyclodextrins

2.1.1 Properties of cyclodextrins

Cyclodextrin is a family of macrocyclic oligosaccharides which is industrially well-known and comprises of three major forms which are crystalline, homogeneous and non-hygroscopic. They are composed from six, seven and eight (D)-+-glucopyranose units which are linked with α-1,4-glycosidic bonds and known as α, β, and γ-cyclodextrin respectively (Figure 2.1).

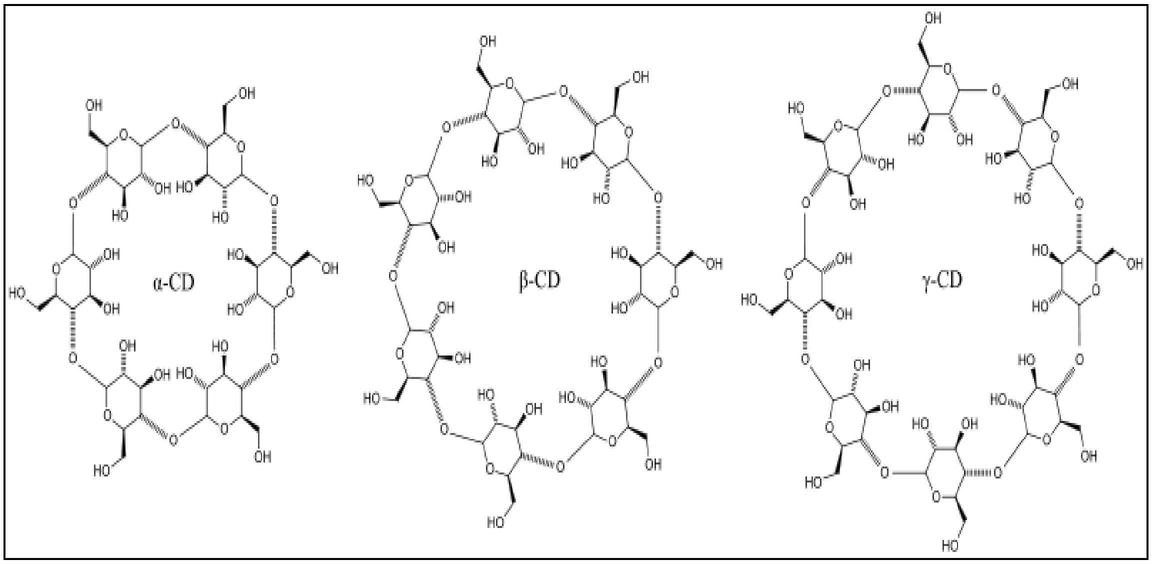

Figure 2.1: Structure of α, β and γ-cyclodextrins

Cyclodextrin is also called doughnut-shaped molecule because it comprises of a truncated conical structure and has a hollow in the center (Figure 2.2). It also comprises of primary hydroxyl group on the narrow side and secondary hydroxyl group at the wide side of the ring. This characteristics result in the formation of hydrophobic inner cavity and hydrophilic outside surface of cyclodextrin.

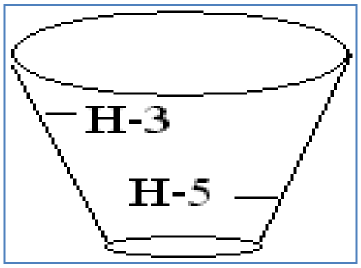

Figure 2.2: Truncated structure of β-cyclodextrin

Cyclodextrin is a supramolecule where the intermolecular interactions between the interacting species do not involve covalent bonds. Therefore, it is a potential host in a host-guest type complexation. They are a seminatural products produced from starch thus a renewable natural material. Other than that, the production of cyclodextrins annually is high and for this reason, their initially high prices have dropped. Other important characteristic of the cyclodextrins is the ability to eliminate the toxic effect of cyclodextrins itself by selecting the appropriate derivatives and as a results, cyclodextrin can be consumed by humans in a form of drugs, foods or cosmetics. Due to all of the properties and characterization that has been stated above, cyclodextrin has been chosen to form the host-guest type inclusion complex and act as a host molecule.

2.1.2 History of cyclodextrins

In 1891, Villiers first published a reference to a substance called “cellulosine”. “Cellulosine” was later proved to be α and β-cyclodextrin because he observed that two distinct crystallines were formed. After 12 years of Villiers discovery, Schardinger, an Austrian microbiologist published a report of a substance identical to “cellulosine” resulted from digestion of starch with a microorganism named *Bacillus macerans*. He then continued the study and observed that there was an iodine characterization at the crystalline dextrin due to the addition of iodine-iodide solution. He also reported that the 25 – 30% of starch could be converted to crystalline dextrin. The major crystalline products in all of his experiments were the so-called α-dextrin.

After 24 years of Schardinger's last work on cyclodextrin published in 1911, Pringsheim took the lead in cyclodextrin research. He and his co-workers discovered that the crystalline dextrin and their acetates have a high ability to form complexes with various organic compounds.

In 1930, based on the experiments of enzymatic hydrolysis, acetolysis with acetyl bromide and hydrolysis of the permethylated dextrins, Freudenberg and co-worker concluded that the crystalline dextrins that was discovered by Schardinger was built from maltose and contained only α-1,4-glycosidic linkages. γ-cyclodextrin however was discovered and its structure had been elucidated in 1948 to 1950.

In the beginning of 1950, French et al. and Cramer et al. began their work on enzymic production of cyclodextrin and the first fundamental review of cyclodextrins published in 1957 by French. The method for the laboratory-scale preparation of cyclodextrins, their structure, their physical and chemical properties as well as their inclusion complex forming properties was clarified by the end of 1960s. In 1981, the first International Symposium on Cyclodextrin was held and beginning from 1984 onwards, the International Cyclodextrin Symposium has been held every 2 years (Szetli, 1998).

2.2 Inclusion Complex

2.2.1 General overview of inclusion complex

Inclusion complex is a complex when one chemical compound ("host") forms a cavity in which molecules from the second "guest" compound are located or trapped. The inclusion process of organic molecules with cyclodextrins usually results in modulation of the physicochemical properties of guest molecules, such as increased solubility, improved chemical stability, reduced toxicity and so on (Stalin *et al*., 2010). From an analytical point of view, formation of inclusion complexes in certain cases allows improvement in the performance of the methods used for the determination of different analytes using spectrophotometry technique (Manzoori *et al*., 2005).

2.2.2 Application of inclusion complex of β-CD in spectrophotometric determination

The application of inclusion complex of β-CD by using UV-Vis spectrophotometer was done by Li *et al*. (1993) where they determined trace copper with a Cu-diethyldithiocarbamate-β-cyclodextrin inclusion complex. In this study, they used ammonia solution as the medium in forming the inclusion complex. The maximum absorption of the inclusion complex was determined at 436 nm. There was no influence to the inclusion complex in the temperature range of 20 to 100 ˚C. The molar absorptivity calculated was 1.3×10^4 mol^{-1} cm^{-1} and the detection limit determined was 4.38×10^{-7} M. They also used a suitable masking agents such as ammonium citrate (2 M) and 1,10-phenanthroline (0.01 M) to ensure that the proposed method for determination of copper(II) is free from interfering elements. The proposed method has been successfully applied to the determination of copper in aluminium alloys, soils, millet, wheat flour, herbs, vegetables and some traditional Chinese herbal medicines with satisfactory results (Li *et al*., 1993).

In 2002, Tang *et al*. studied a supramolecular interaction of curcumin and β-cyclodextrin by spectrophotometry. In this study, the mechanism of the inclusion complex was studied and discussed based on the variations of pK_a, absorption intensity and infrared spectrograms. A spectrophotometric method for the determination of curcumin in bulk aqueous solution in the presence of β-CD was developed based on the enhancement of the absorbance of curcumin produced through complex formation. From the results, it is shown that β-CD and curcumin form inclusion complex in a ratio of 2:1 with an apparent formation constant of 5.53×10^5 mol^{-2} L^2. Tri-sodium citrate-HCl buffer system was selected to control the pH formation of the complex. The results also showed that most of the foreign ions have no detrimental effect on determination of curcumin. The linear relationship between the absorbance and curcumin concentration was obtained in the range of 0 – 15 µg/mL with a correlation coefficient of 0.9991. The detection limit was 0.076 µg/mL. The proposed method was successfully applied in determination of curcumin in curry and mustard with a recovery of 97.28 – 101.81% (Tang *et al*., 2002).

The mechanism of the inclusion interaction of dibenzoyl peroxide and β-cyclodextrin was studied by spectrophotometry. The study done by Wang *et al*. (2005) showed that β-CD reacts with dibenzoyl peroxide to form a 2:1 host-guest complex with an apparent formation constant of 2.5×10^4 mol^{-2} L^2. Study on absorption spectra revealed that inclusion complex of β-CD-dibenzoyl peroxide formed at 237 nm. After the dibenzoyl peroxide was reduced by hydroxyl ammonium, β-CD reacts with benzoic acid to form a 1:1 host-guest complex with an apparent formation constant of 6.9×10^{2} mol^{-1} L. Inclusion complex of β-CD-benzoic acid was formed at 229 nm. A spectrophotometric method for determination of dibenzoyl peroxide in bulk aqueous solution in the presence of β-CD was developed based on the enhancement of the absorbance of dibenzoyl peroxide produced through complex formation. pH 1.0 was chosen as the best pH for complex formation that is controlled by KCl-HCl buffer solution. The formation of inclusion complex was fast at room temperature and was in a stable condition for at least 4 hours. A linear relationship between the absorbance and dibenzoyl peroxide concentration was obtained in the range of 0.3 – 50 µg/mL with a correlation coefficient of 0.9984 and a relative standard deviation (R.S.D) of 3.4%. The detection limit was 0.2 µg/mL and the recovery of the wheat flour sample was from 98 – 105% (Wang *et al*., 2005).

Due to the limited application of inclusion complex using spectrophotometric technique, therefore we are interested to extend the work with our research to obtain new findings in the study of inclusion complex.

2.3 Dithizone

2.3.1 Dithizone as a chelating reagent for metals in spectrophotometry

An intense color formation when dithizone was complexed with metals makes it an important chelating reagent in determining trace metals using UV-Vis spectrophotometer. In 1966, RÜzicka *et al*. studied on the substoichiometric separation of gold and palladium using dithizone as a chelating reagent. In this study, a 1:1 red-brown colored solution and 2:1 yellow colored solution of gold:dithizone was formed in chloroform. According to the authors, the changes of the red-brown complex of 1:1 gold:dithizone (500 nm) to yellow complex of 2:1 gold:dithizone was dependent on the pH of the aqueous solution. Their finding indicated that complex formed in alkaline solution was more stable. They also reported a formation of violet complex of dithizone in carbon tetrachloride with palladium in a 1:1 ratio (Růžička *et al*., 1965).

Tϋtem *et al*. (2000) investigated a spectrophotometric study on determination of cobalt(II) and nickel(II) by dissolving dithizone in tetrahydrofuran (THF). The sample metal was mixed with dithizone, distilled water and concentrated nitric acid and the absorbance was measured at 620 nm and 740 nm for Co(II) and Ni(II) respectively. Both complexes of cobalt and nickel formed at pH 12.0 and were stable for at least 2 hours. During the analysis, Cr(VI) and Fe(III) were noticed to be an interfering ion to the proposed method. The method was said to be more sensitive to Co(III) since the relative standard deviation (R.S.D) is lower compared to Ni(VI). The method was also successfully applied to a Ni-Cr-based dental alloy, Wiron 999. (Tϋtem *et al*., 2000).

Bismuth and zinc has been widely used in pharmaceuticals and for various industrial purposes. Gumus *et al*. (2005) studied on the determination of bismuth(III) and zinc(II) in pharmaceuticals by derivative UV-Visible spectrophotometry with dithizone prepared in acetone and n-propanol mixture. The absorbance of Bi(III) and Zn(II) complex was observed at 493 nm and 510 nm respectively. In this study, a mixture of acetone-n-propanol (1/1, v/v) solvent was chosen as it was stable for a long time and gave high absorbance in the absorption spectrum. The complexes were formed at pH 5.0 and were stable for at least 2 hours. The limit of detection for both cations was 0.05 mg/L.

The method was also applied to a pharmaceutical product and the results were compared with flame atomic absorption spectrometer (FAAS) procedure. Both procedures gave similar results with 95% of confidence level (Gumus *et al*., 2005).

In 2007, Rajesh and Gurulakshmanan developed a simple method for the pre-concentration of mercury based on the adsorption of its dithizone complex on a neutral alumina column. The concentration of mercury was detected at 520 nm using UV-Visible spectrophotometer. The limit of detection was found to be 4 µg/L and the relative standard deviation (R.S.D) was 2.5%. The method was applied for the determination of mercury in spiked water samples and city waste incineration ash (CRM 176) with a recovery of above 95% (Rajesh and Gurulakshmanan, 2007).

Based on the reviews, it can be concluded that dithizone is an important chelating reagent especially in trace metal analysis. Thus, it is applicable for most of the analytical application analysis. Since dithizone has very low solubility in water, an inclusion complex of dithizone with β-CD is needed to improve and increase the solubility of dithizone in an aqueous system and reduce the usage of organic solvent.

2.4 Zinc

2.4.1 General overview of zinc

Zinc (Zn) is one of the metallic chemical elements that have the atomic number of 30. It is also known as an essential trace element for all living organisms including plants, animals, as well as in humans (Eisler, 1993). Zinc can be found in many forms, for example zinc dialkyldithiophosphate is mainly found in lubricating oils, zinc oxide is used in sensors, zinc phosphide is mainly used to control agricultural rodent pests, zinc sulfate is used in supplements and many more (Barnes *et al*., 2001, Leung *et al*., 2008, Rink *et al*., 2008, Gupta *et al*., 2011).

An excess of zinc can cause harm to humans and also plants. From a study that has been done, lack of zinc in child development may cause weight loss, deficiency in growth, weakened immune system, diarrhea, anemia and also early death (Salgueiro *et al*., 2002). It can also effect on reproduction system and on pregnancy leading to spontaneous abortion, premature birth and complication during delivery (Salgueiro *et al*., 2000).

In plants, an excess of zinc can cause delay in growth and root development as well as leaf chlorosis (Cherif *et al*., 2010). Deficiency of zinc in plants may cause higher level of reactive oxygen species and other relevant damages to plants (Wang *et al*., 2007).

2.4.2 Determination of Zinc by UV- spectrophotometry analysis

There are many techniques that were reported on the determination of zinc such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC), capillary electrophoresis (CE) and many more (Ong *et al*., 1988, Szpunar *et al*., 1997, Marti *et al*., 2000, Wang *et al*., 2007). In this study, UV-Visible spectrophotometry analysis was chosen due to its low cost and maintenance and it only required a simple analysis method. Since direct determination of metal using spectrophotometry is not sensitive, a chelating reagent should be added to make it more sensitive and easily detected.

In 1998, Santelli and co-workers developed a simple procedure for the determination of zinc. They used solid phase extraction (SPE) and a form of polyurethane foam as an adsorbent to pre-concentrate zinc in aqueous solution from biological materials. Zinc-thiocyanate complex was determined by spectrophotometry analysis using 4-(2-pyridylazo)-resorcinol as a chromogenic reagent. The optimum pH for zinc pre-concentration and determination was chosen at pH 3.0. There were not much interference from other species except from Cd(II) and the limit of detection was 0.9 mg/L. The method was successfully applied to pre-concentrate and determine zinc in biological matrices (Santelli *et al.*, 1998).

Benamor and co-workers performed a simple, rapid and sensitive spectrophotometric method for determination of zinc based on color reaction between the metal ion and xylenol orange in the presence of surfactant cationic cetylpyridinium chloride. Zinc reacts with the reagent and surfactant in the pH range of 5.0 – 6.0 to form a complex with an absorption maximum at 580 nm. The apparent molar absorptivity was found to be at 1.1×10^4 L mol cm^{-1}. The interfering ions were eliminated by masking with sodium fluoride and ethylenediamine tetraacetic acid. The method was successfully applied to the determination of zinc in a pharmaceutical product (Benamor *et al.*, 2000).

In 2003, Pyrzyńska and Kilian developed a sensitive method for the spectrophotometric determination of trace amount of zinc, complexed with 5, 10, 15, 20-tetrakis(4-carboxylphenyl)porphyrin. The maximum absorption of this complex was at 423 nm and the molar absorptivity was 3.5×10^5 M^{-1}. The limit of detection was 0.9 mg/L with relative standard deviation (R.S.D) of 2.7%. Fe(III) was seen to give an interference with this complex but was eliminated with the addition of 0.1% (w/v) NH_2OH solution. The method was successfully applied to the determination of zinc in some natural water samples and nutritional supplement (Pyrzyńska and Kilian, 2003).