

CHAPTER 4: CHARACTERIZATION AND SPECTROSCOPY STUDY OF INCLUSION COMPLEXES

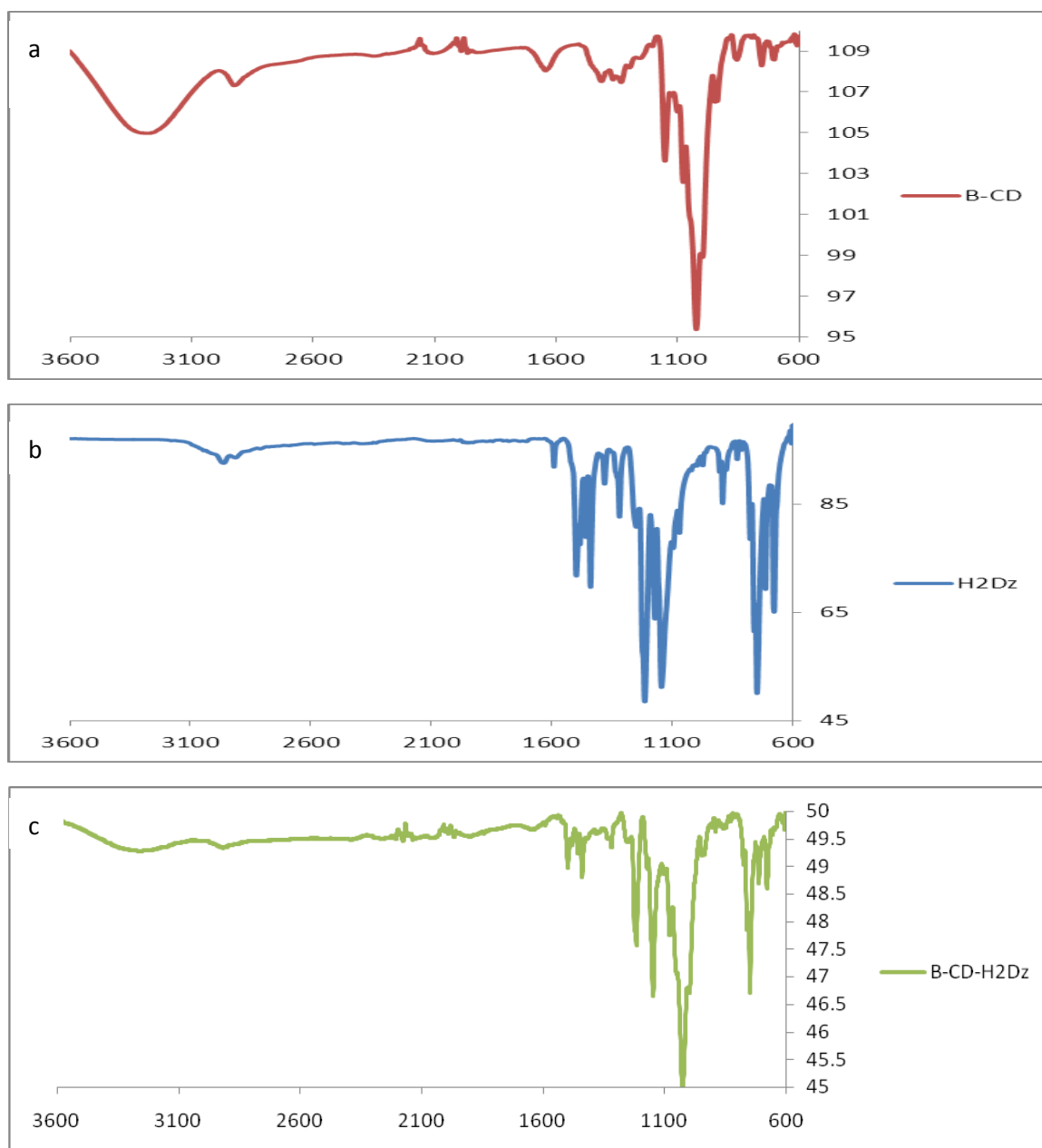
4.1 Characterizations of inclusion complexes

4.1.1 Fourier Transform Infra Red (FTIR)

FTIR is widely used to determine the functional groups of a compound. It is also used to prove the presence of component of inclusion complex which belongs to the host and guest molecules (Zheng *et al.*, 2007). Figure 4.1 (a-d) shows the FTIR spectrum for β -cyclodextrin, dithizone, inclusion complex of β -CD-H₂Dz and inclusion complex of β -CD-H₂Dz-Zn. It can be seen that the major peak in β -CD spectra at 3281 cm⁻¹ which belongs to the hydroxyl group (Suh *et al.*, 2001 & Xie *et al.*, 2007) has shifted to a lower frequency in both of the inclusion complexes due to the interactions between the two parent molecules.

Peak at 2925 cm⁻¹ in β -CD and 2961 cm⁻¹ in dithizone belongs to C-H vibration (Wen *et al.*, 2004) and N-H adjacent to C=S functional group (Denizli *et al.*, 1998) respectively. It can be seen that in both inclusion complexes, C-H vibration peak has shifted to a lower frequency; 2918 cm⁻¹ in inclusion complex of β -CD-H₂Dz and 2903 cm⁻¹ in inclusion complex of β -CD-H₂Dz-Zn, and it is overlapped with N-H from dithizone. An Overlapping of C-O peak at 1152 cm⁻¹ from β -CD with N-C-S peak from dithizone at 1144 cm⁻¹ was observed in both of the inclusion complexes and the shifting was towards lower field. Nevertheless, the 1414 cm⁻¹ absorption peak, assigned to the C-H deformation in β -CD was absent in both of the inclusion complexes.

The S=C-NH vibration peak at 1457 cm^{-1} (Denizli *et al.*, 1998) did not change in inclusion complex of β -CD-H₂Dz but shifted to a higher frequency and decreased its intensity in inclusion complex of β -CD-H₂Dz-Zn. This may be due to the attachment of zinc to the S and N that changed the S=C-NH to S-C=N which in turn changed the structure of dithizone from keto to enol form. The interpretation of FTIR spectrums were tabulated in Table 4.1.



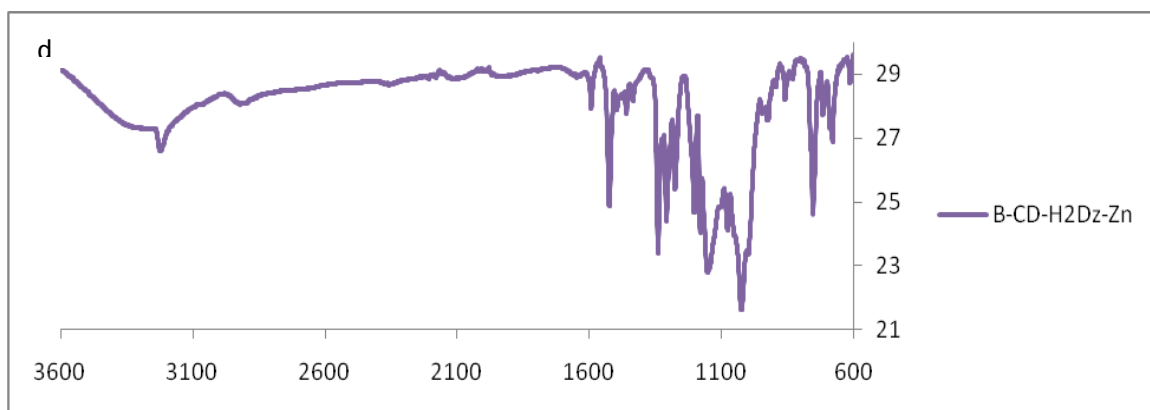


Figure 4.1: FTIR spectra for (a) β -cyclodextrin; (b) dithizone; (c) inclusion complex of β -CD- H_2Dz ; (d) inclusion complex of β -CD- H_2Dz -Zn.

Table 4.1: Interpretation of FTIR spectrum of β -CD, dithizone, inclusion complex of β -CD- H_2Dz and inclusion complex of β -CD- H_2Dz -Zn.

Wavelength (cm ⁻¹)	β -CD	H_2DZ	β -CD- H_2DZ	β -CD- H_2DZ -Zn
3281	OH group		3256	3210
2925	C-H		2918	2903
1644	O-H bend		1639 (decreased)	1638
1414	C-H deformation		disappeared	disappeared
1152	C-O		(1147) overlapped with N-C-S	1143 (overlapped with N-C-S)
1078 & 946	CO/CC		1079 & 946	1074 & 935
856	$\alpha(1,4)$ -glycosidic bond		disappeared	857
2961		N-H adj. to C=S	overlapped with C-H	overlapped with C-H
1590		N=N stretching	1596 (decreased)	1591
1497		N-H bending	1497	1523
1457		S=C-NH vib	1457	1463 (decreased)
1213 & 1171		C=S Stretching vib	1215 & 1169 (decreased)	1201 & 1179 (decreased)
1144		N-C-S stretching	1147 (decreased)	1145

4.1.2 Solid State ^{13}C CP/MAS NMR Spectroscopy

Figure 4.2 (a-c) shows a ^{13}C CP/MAS NMR spectra of β -cyclodextrin, inclusion complex of β -CD- H_2Dz and inclusion complex β -CD- H_2Dz -Zn.

It is shown that β -CD molecule gained a less symmetrical conformation in the crystalline condition. The resolved carbon resonance from each of the glucose units was reflected by splitting from all $\text{C}_1 - \text{C}_6$ resonance in the spectrum of β -CD. However, the resolved carbon resonance (C_1 , C_4 and C_6) disappeared in the spectrum of inclusion complex of β -CD- H_2Dz and appeared as a single peak. This might be due to the formation of 2:1 host-guest inclusion complex of β -CD- H_2Dz (refer to section 4.2.1.3) where both of the aromatic rings of dithizone has entered the cavity of β -CD making it a more stable complex. These results indicate that β -CD molecules adopt a more symmetrical conformation during the complex formation and each glucose unit of β -CD is in a similar environment (Zheng *et al.*, 2007 & Puvaneswary *et al.*, 2010).

However, for inclusion complex of β -CD- H_2Dz -Zn, the β -CD molecules adopt a less symmetrical conformation upon complexation. This might be due to the formation of 1:1 host-guest inclusion complex of β -CD- H_2Dz -Zn (refer to section 4.2.2.2) where only one of the aromatic rings of dithizone entered the cavity of β -CD. Most probably, the presence of zinc is also the cause of the less symmetrical conformation of β -CD where it changes the environment of β -CD cavity. The results obtained therefore support the formation of inclusion complex between β -CD with H_2Dz and H_2Dz -Zn.

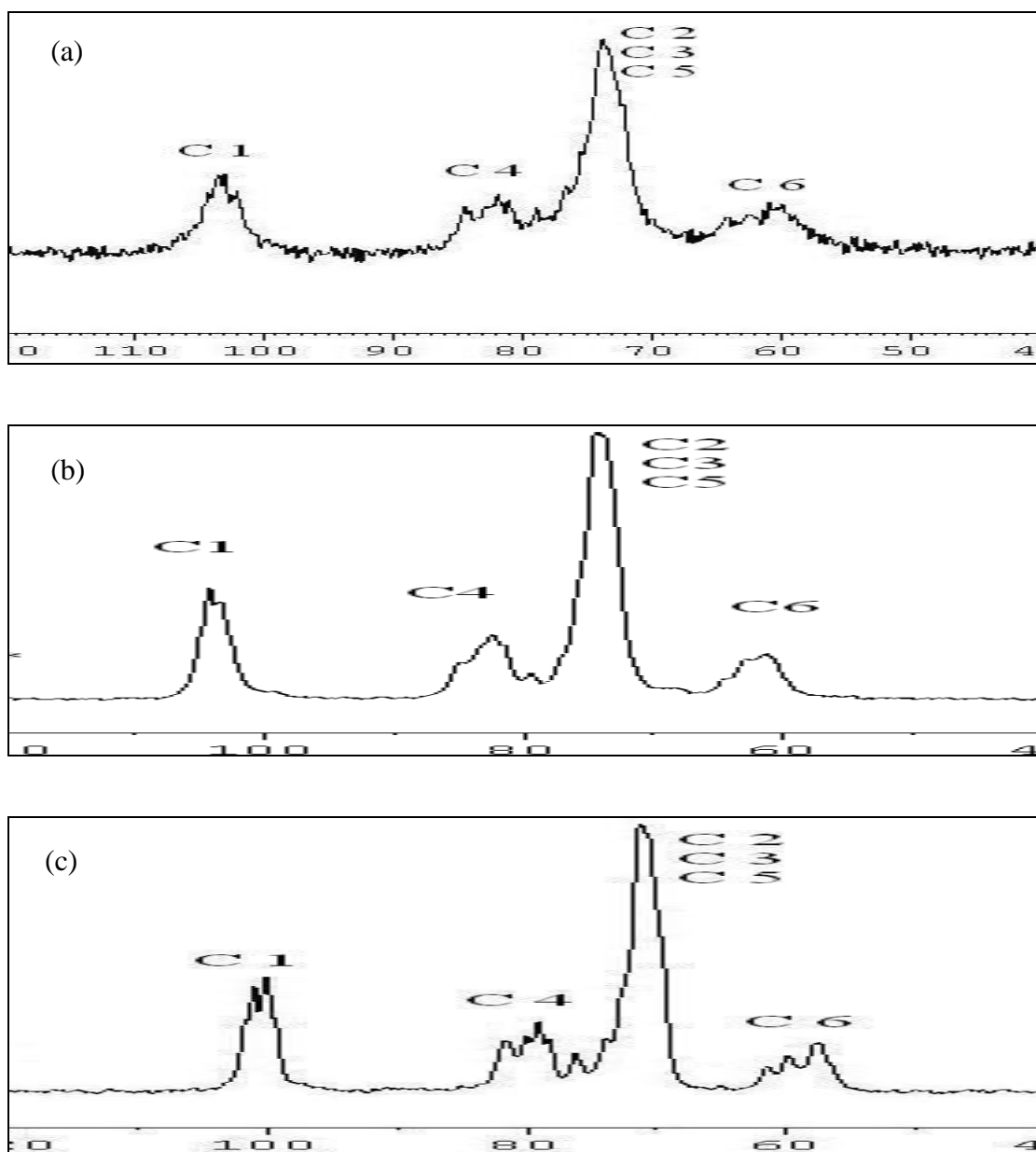


Figure 4.2: ^{13}C CP/MAS NMR spectra for (a) β -cyclodextrin; (b) Inclusion complex of β -CD- H_2Dz ; (c) Inclusion complex of β -CD- H_2Dz -Zn.

4.1.3 Thermal Gravimetric Analysis (TGA)

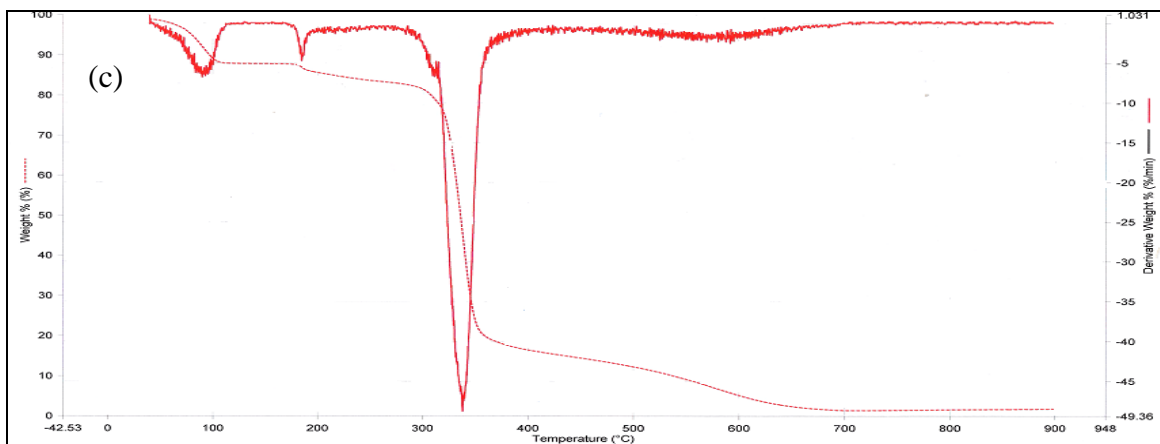
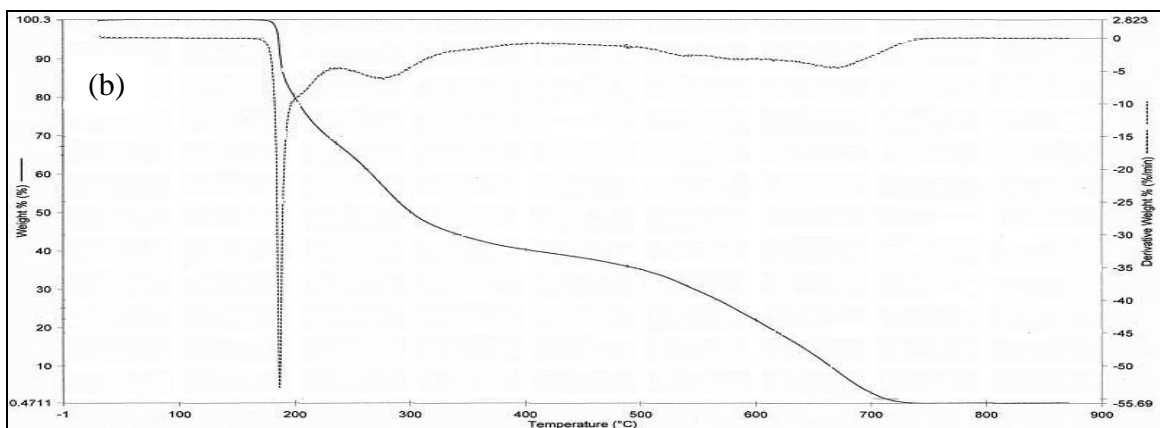
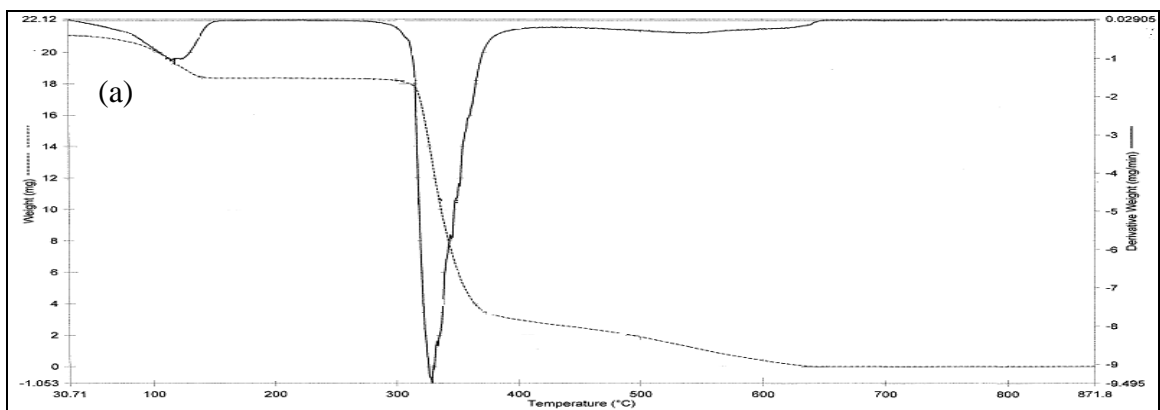
Thermal gravimetric analysis (TGA) has been widely used in investigation of thermal behavior of inclusion compound and its precursors (Abderrahim *et al.*, 2010).

Figure 4.3 (a-d) shows the thermal stability of β -cyclodextrin, dithizone and its inclusion complexes. Thermal analysis of β -CD in Figure 4.3a shows a small weight loss at around 110°C due to loss of moisture in the β -CD cavity (He *et al.*, 2008), while at 330°C, the weight loss was due to the decomposition of the β -CD structure itself (Puvaneswary *et al.*, 2010).

Figure 4.3b of H₂Dz shows a thermal stability up to 180°C and later shows a major weight loss which occur rapidly due to the combustion of the compound. The second and third slight decomposition occurred at 280°C and 660°C respectively without any formation of carbonaceous residue (Chahud *et al.*, 2000). Thermal analysis of inclusion complex between β -CD-H₂Dz was investigated and shown in Figure 4.3c. In the spectrum, it can be seen that three major peaks of parent compounds were observed. The first weight loss is at 90°C due to the water dehydration of β -CD cavity while the second decomposition is at 190°C due to the degradation of dithizone and the major weight loss in the inclusion compound which occurs at 340°C due to the decomposition of β -CD molecule.

The last TGA spectrum in Figure 4.3d refers to the thermal stability of the inclusion complex of β -CD-H₂Dz-Zn. In this spectrum, three major weight losses were also observed. Furthermore, more interesting finding is that, it could be seen that the second degradation occurred at a higher temperature.

Thus, from this result, it revealed that thermal stability of β -CD-H₂Dz-Zn was improved due to the formation of the inclusion complex. This result of TGA is similar to that in DSC thermogram which shall be discussed later.



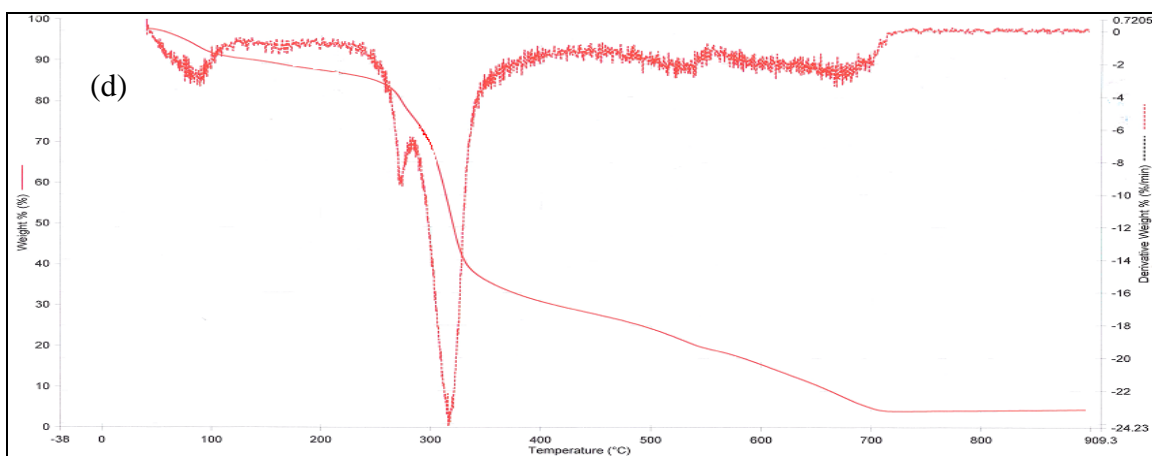


Figure 4.3: Thermal Gravimetric Analysis (TGA) for (a) β -cyclodextrin; (b) Dithizone; (c) Inclusion complex of β -CD- H_2Dz ; (d) Inclusion complex of β -CD- H_2Dz -Zn.

4.1.4 Differential Scanning Calorimetric (DSC)

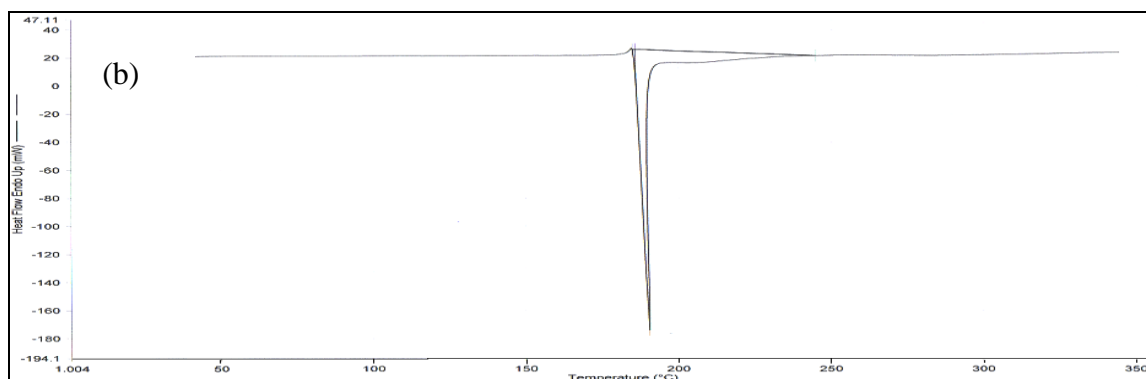
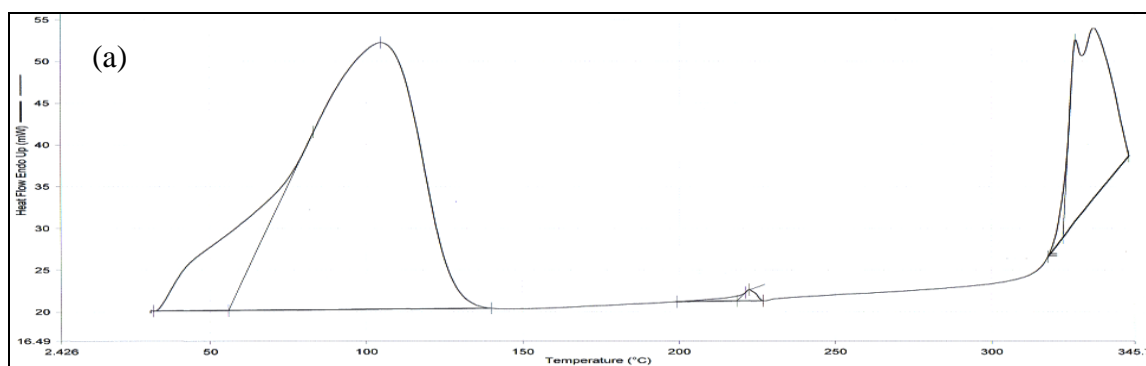
Figure 4.4a shows DSC thermogram of β -CD. The spectrum shows three endothermic peaks that appear between 30-140°C with the highest peak at 104.6°C due to removal of water from the cavity of β -CD (Mielcarek, 1998). The second peak at 222.5°C is due to the irreversible solid-liquid phase transition of β -CD. The last peak for β -CD which occurs at 326.6°C is due to the decomposition of β -CD molecule (Liu and Zhu, 2007).

The DSC thermogram for dithizone is shown in Figure 4.4b. Differing from β -CD, H_2Dz only showed a single major exothermic peak at 190.6°C which indicates the rapid decomposition of H_2Dz starting from 185.8°C.

For inclusion complex of β -CD- H_2Dz , the DSC thermogram is shown in Figure 4.4c with four major peaks from both parent molecules. The first curve between 40-110°C with the highest peak at 67.9°C is due to evaporation of water from β -CD cavity, while the second peak at 187.7°C is due to the melting and decomposition of

H₂Dz. The third curve with highest peak at 220.3°C is due to the transition state originated from β-CD, while the last peak at 329.5°C is due to the decomposition of β-CD molecule.

DSC thermogram for the inclusion complex of β-CD-H₂Dz-Zn only shows three peaks in Figure 4.4d. It could also be seen that the second degradation occurred at a higher temperature, similar to that in the previous results of TGA. Thus, it can be concluded that the presence of zinc influence the stability of the inclusion complex.



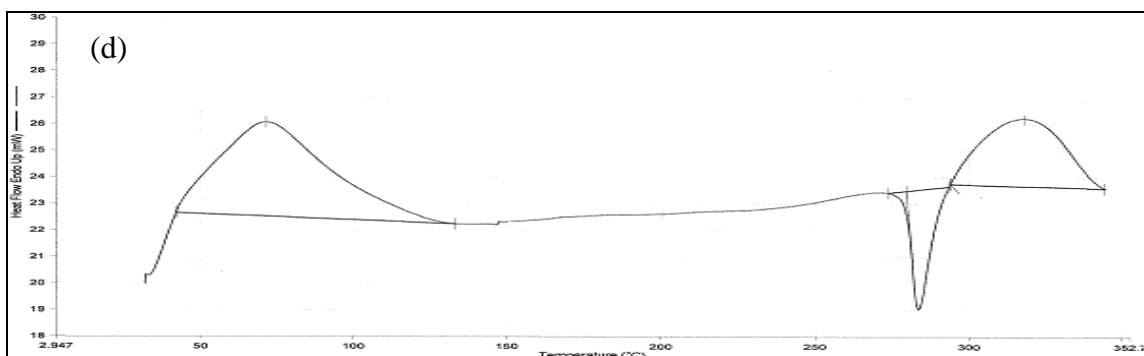
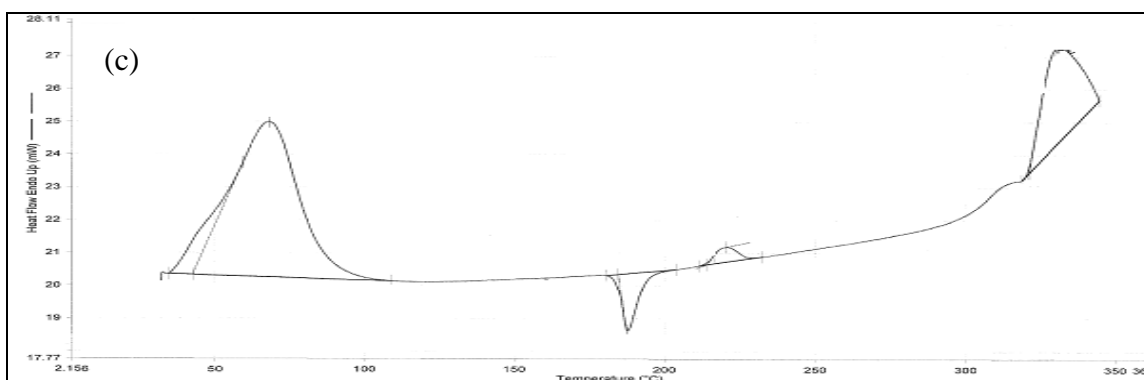


Figure 4.4: DSC for (a) β -cyclodextrin; (b) Dithizone; (c) Inclusion complex of β -CD- H_2Dz ; (d) Inclusion complex of β -CD- H_2Dz -Zn.

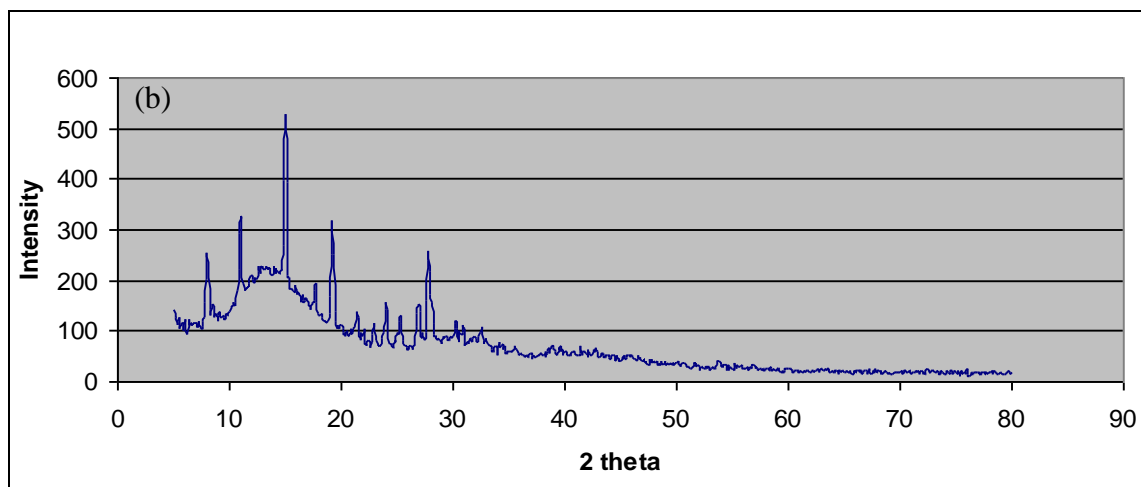
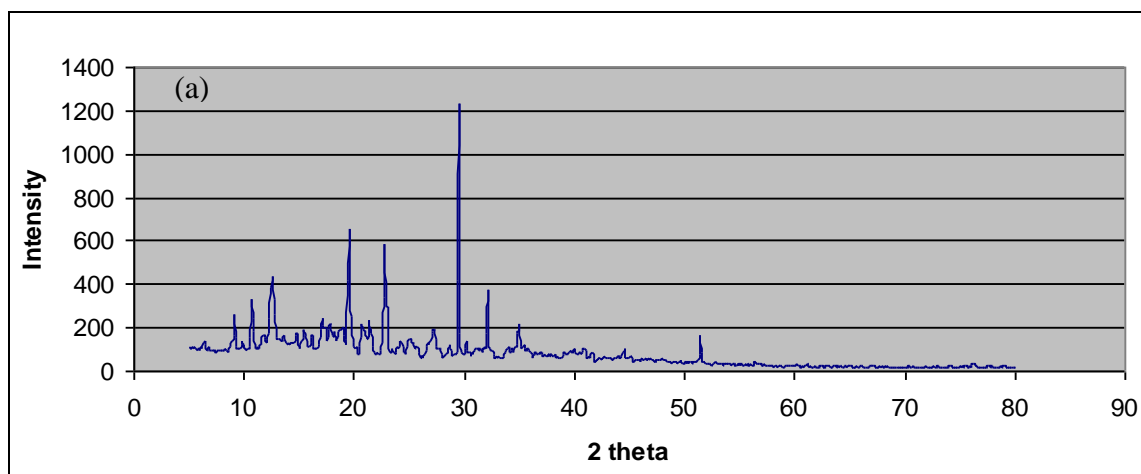
4.1.5 Powder X-Ray Diffraction (XRD)

X-Ray diffraction (XRD) is one of the most useful methods used for detection of inclusion complexes in powder or microcrystalline state and to determine the structure whether the compound has been invented from the parent molecules (Han *et al.*, 2005 & Zhang *et al.*, 2005). The crystal structure of β -CD can be assigned mainly into three types; cage, channel and layer (Han *et al.*, 2005, Liu *et al.*, 2007 and Zheng *et al.*, 2007).

Figure 4.5 shows the powder XRD pattern for β -cyclodextrin, dithizone and both of the inclusion complexes. We could see that β -CD form a cage type structure while H_2Dz is composed of a channel type structure (Han *et al.*, 2005, Diao *et al.*, 2006 & Xie *et al.*, 2007). Both of the inclusion complexes obtained suggests that it is a fine crystalline

powder and the XRD pattern of the inclusion complexes shows that the inclusion complex forms a channel type structure (Zheng *et al.*, 2007 and Puvaneswary *et al.*, 2010).

The intense peak for XRD spectrum of β -CD is at $2\theta = 29.4^\circ$, while for H_2Dz is at $2\theta = 15^\circ$ and for the inclusion complexes of β -CD- H_2Dz and β -CD- H_2Dz -Zn is at $2\theta = 12.5^\circ$ and 10.4° , respectively. The diffraction peak shows some disappearance between β -CD and dithizone that confirms the formation of inclusion complexes (Veiga and Ahsan, 2000).



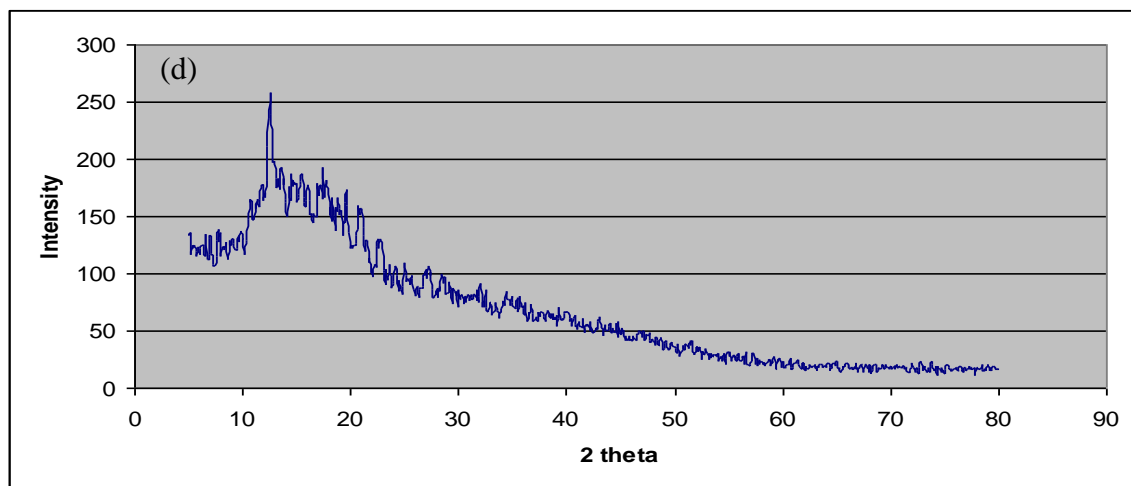
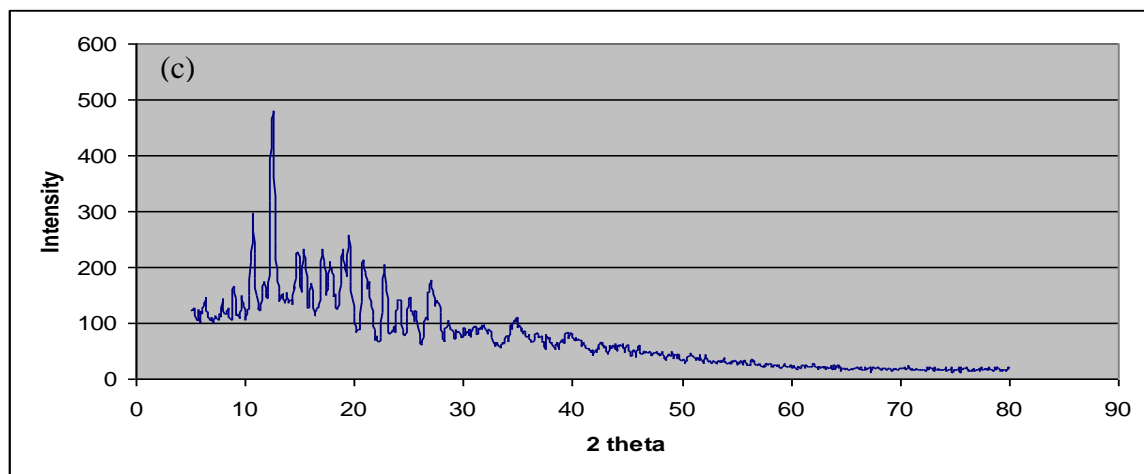


Figure 4.5: Powder XRD for (a) β -cyclodextrin; (b) Dithizone; (c) Inclusion complex of β -CD- H_2Dz ; (d) Inclusion complex of β -CD- H_2Dz -Zn.

4.1.6: Energy Dispersive X-ray (EDX)

Energy Dispersive X-ray (EDX) analysis is an analytical technique used for the analysis of elements or chemical characterization of a compound. In this study, the EDX analysis has been conducted to observe the presence of zinc in the inclusion complex of β -CD- H_2Dz -Zn. From the result obtained, zinc was found in the inclusion complex of β -CD- H_2Dz -Zn (Table 4.2). Therefore, this result proved the formation of inclusion complex of β -CD- H_2Dz -Zn.

Table 4.2: Elements present in inclusion complex of β -CD-H₂Dz-Zn

Elements	Percentage
C	44.40%
N	30.09%
O	18.14%
S	3.30%
Zn	3.71%

4.2 Spectroscopy study of inclusion complex

4.2.1 Spectroscopy study of inclusion complex of β -CD-H₂Dz

4.2.1.1 Effect of pH

The effect of pH was studied by randomly choosing pH 3, 8 and 12. From the result in Figure 4.6, it shows that the inclusion complex formation occurred in basic medium shows the highest absorbance. Therefore, pH 12 has been chosen as the optimum pH for the formation of inclusion complex of β -CD-H₂Dz at 426 nm.

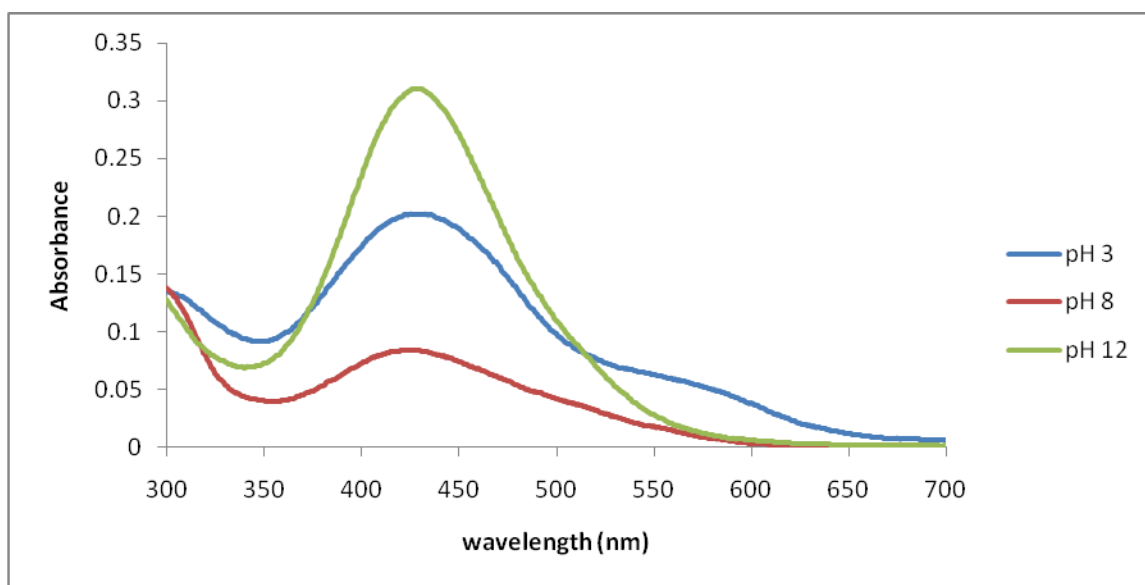


Figure 4.6: Formation of inclusion complex of β -CD-H₂Dz at pH 3, 8 and 12 at 426 nm.

4.2.1.2 Phase Solubility Studies

The low solubility of H₂Dz in water was improved by β -CD where investigation of the solubility of H₂Dz in aqueous solution containing different concentration of β -CD was studied in this work. The phase solubility study was done according to the Higuchi and Connors method (Higuchi & Connors, 1965). Phase solubility diagram is shown in Figure 4.7 and it is an Ap-type. It can be seen that the solubility of dithizone increased with an increase in concentration of β -CD, indicating that dithizone can be more soluble in aqueous region in the presence of β -CD. This confirms that the formation of the inclusion complex improved the solubility of dithizone in the aqueous system thus at the same time reducing the usage of organic solvents.

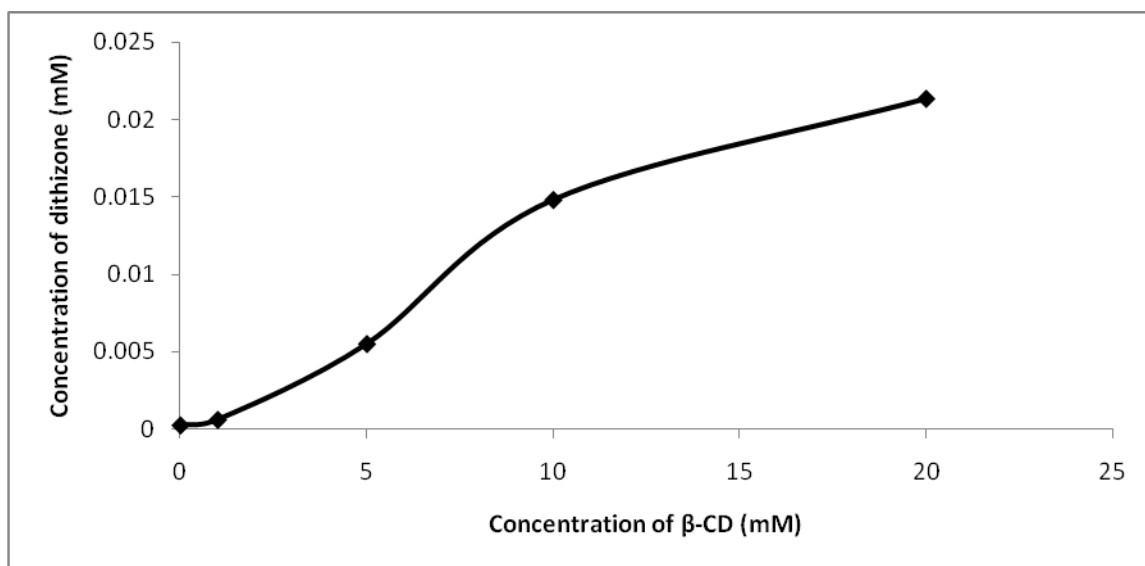


Figure 4.7: Phase solubility diagram of β -CD-H₂Dz system in water

4.2.1.3 Formation constant

Formation constant (K) is an important parameter to be investigated since it is an inclusion interaction. The formation constant study was done for the inclusion complex of β -CD- H_2Dz and the inclusion complex of β -CD- H_2Dz -Zn in order to investigate their formation constant as well as their mole ratio in the complex. The results for the formation constant might show an increase or decrease in the absorbance which suggests that there is a formation of inclusion complex (Diao *et al.*, 2006, He *et al.*, 2008).

For inclusion complex of β -CD- H_2Dz , the concentration of dithizone was held constant at 1.5×10^{-5} M while the concentration of β -CD was varied from 0 to 2.4×10^{-3} M. The absorption spectrum of inclusion complex of β -CD- H_2Dz is shown in Figure 4.8 and it is measured at 426 nm.

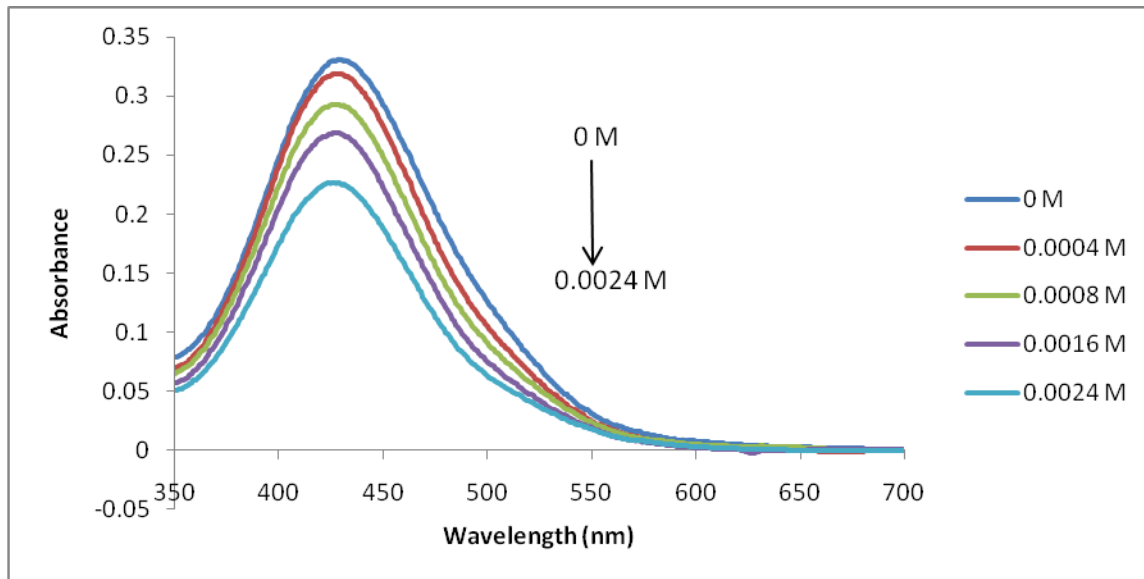


Figure 4.8: Absorption spectra for inclusion complex of β -CD- H_2Dz

It can be seen that the absorbance intensity decreased when the concentration of β -CD was increased due to the formation of inclusion complex of β -CD- H_2Dz . This suggests that guest molecules entered deep into the cavity of β -CD (He *et al.*, 2008). From these absorption spectra, a double reciprocal plot was plotted to determine the apparent formation constant (K) from the Benesi-Hildebrand equation (Benesi & Hildebrand, 1949):

$$\frac{1}{A} = \frac{1}{\epsilon[G]_0 K [CD]} + \frac{1}{\epsilon[G]_0} \quad \text{Equation 4.1}$$

$$\frac{1}{A} = \frac{1}{\epsilon[G]_0 K [CD]^2} + \frac{1}{\epsilon[G]_0} \quad \text{Equation 4.2}$$

where A is the absorbance of the dithizone solution of each β -CD concentration, $[G]_0$ is the initial concentration of dithizone, $[CD]$ is the concentration of β -CD and ϵ is the molar absorptivity.

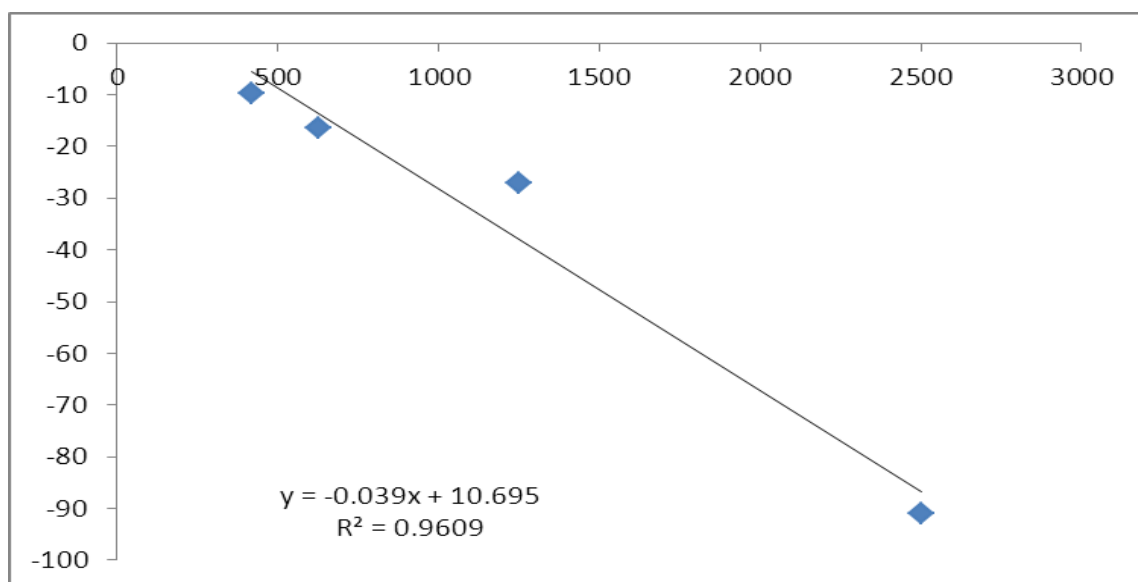


Figure 4.9: Double reciprocal plot for inclusion complex of β -CD- H_2Dz for $1/Abs$ vs $1/[CD]$.

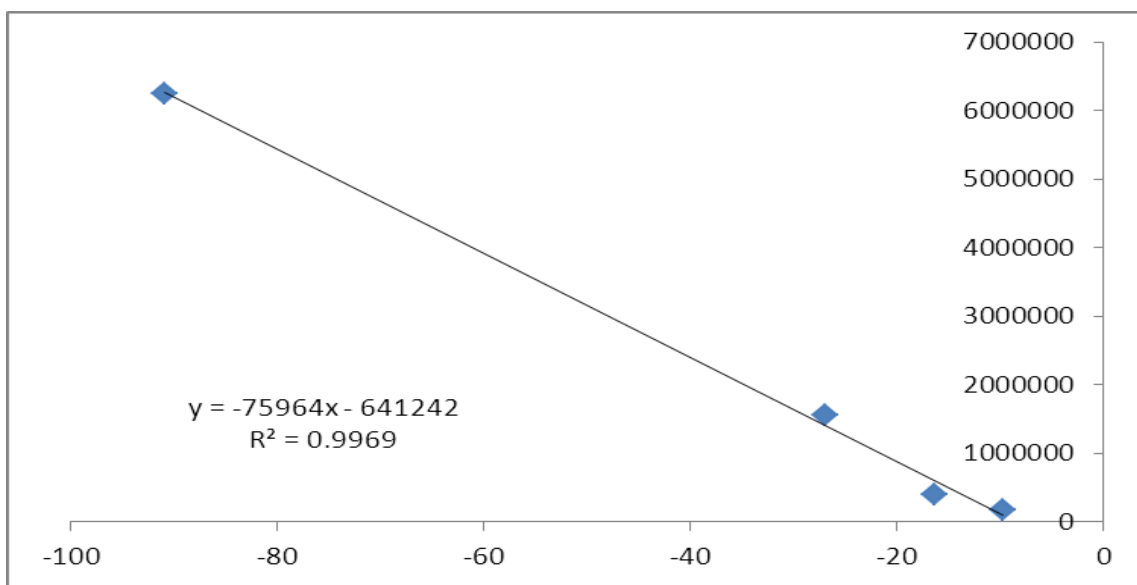


Figure 4.10: Double reciprocal plot for inclusion complex of β -CD- H_2Dz for $1/Abs$ vs $1/[CD]^2$.

Figure 4.9 and 4.10 shows a double reciprocal plot for inclusion complex of β -CD- H_2Dz for $1/Abs$ vs $1/[CD]$ which refers to 1:1 ratio inclusion complex and $1/Abs$ vs $1/[CD]^2$ which refers to 2:1 host-guest inclusion complex system. Referring to the figures above, Figure 4.10 shows a better linear relationship compared to Figure 4.9. Thus, this confirmed that inclusion complex of β -CD- H_2Dz forms a 2:1 host-guest inclusion complex system. The apparent formation constant (K) value was calculated to be $0.8441 M^{-1}$. This shows that the formation of the inclusion complex is very weak.

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

4.2.2.1 Effect of pH

Effect of pH for inclusion complex of β -CD- H_2Dz -Zn was studied at randomly chosen pH of 3, 8 and 12. Based on the result obtained on Figure 4.11, it shows three different peaks at different wavelength. pH 3 gives a maximum absorption at 583 nm, pH 8 gives a maximum absorption at 522 nm while pH 12 gives a maximum absorption at 433 nm. Therefore, pH 8 has been chosen as the optimum pH for the formation of inclusion complex of β -CD- H_2Dz -Zn throughout the experiment because it gives the highest absorption at 522 nm.

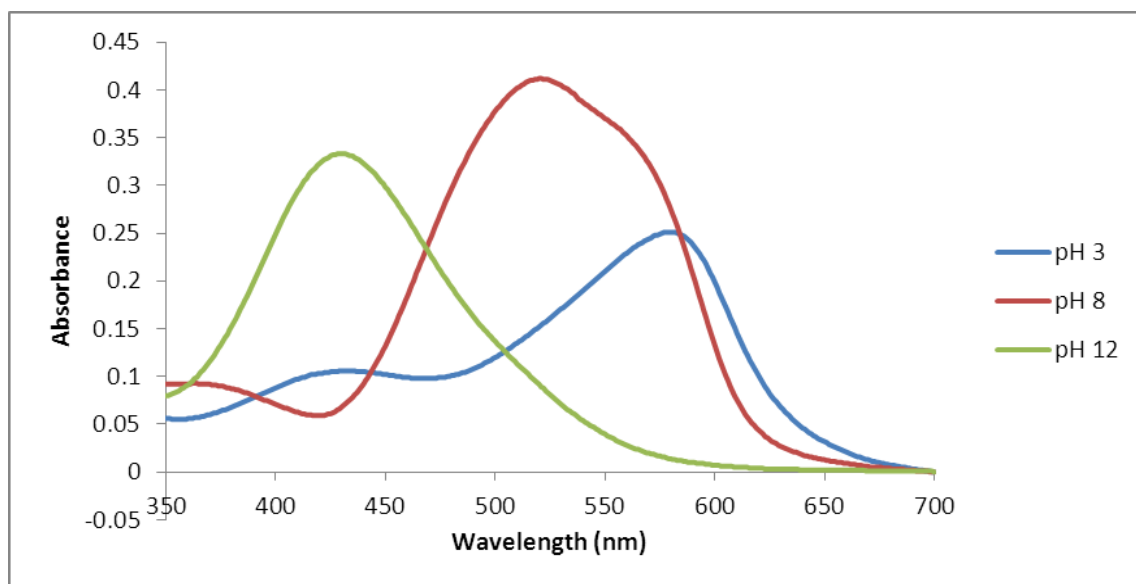


Figure 4.11: Effect of pH for inclusion complex of β -CD- H_2Dz -Zn.

4.2.2.2 Formation constant

Differing from the inclusion complex of β -CD- H_2Dz , Figure 4.12 shows the absorption spectra of inclusion complex of β -CD- H_2Dz -Zn which can be seen that when the concentration of β -CD increased, the absorbance also increased.

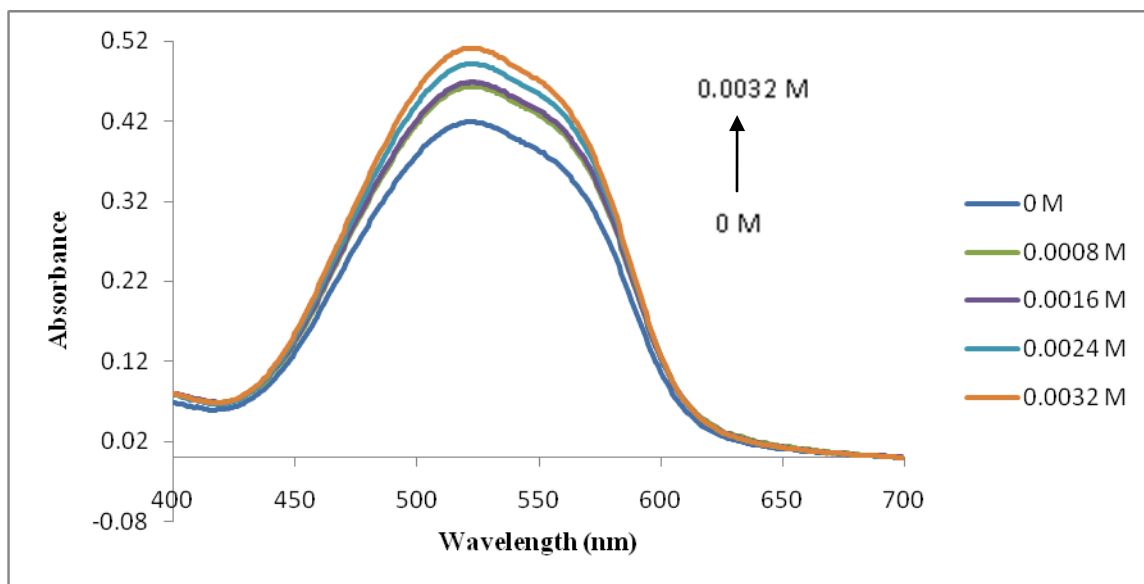


Figure 4.12: Absorption spectra of inclusion complex of β -CD- H_2Dz -Zn with varied concentration of β -CD from 0 M to 0.0032 M.

From these absorption spectra, two more double reciprocal plots were plotted to determine the apparent formation constant for inclusion complex of β -CD- H_2Dz -Zn. Figure 4.13 and 4.14 show a double reciprocal plot for $1/Abs$ vs $1/[CD]$ and $1/Abs$ vs $1/[CD]^2$, respectively for inclusion complex of β -CD- H_2Dz -Zn.

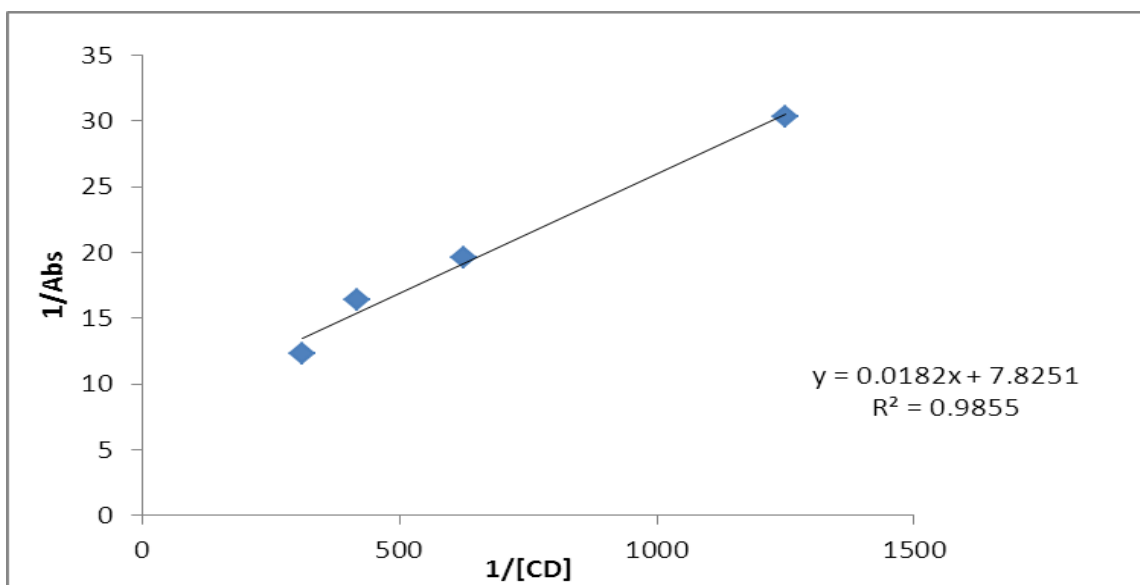


Figure 4.13: Double reciprocal plot for inclusion complex of β -CD- H_2Dz -Zn for $1/Abs$ vs $1/[CD]$.

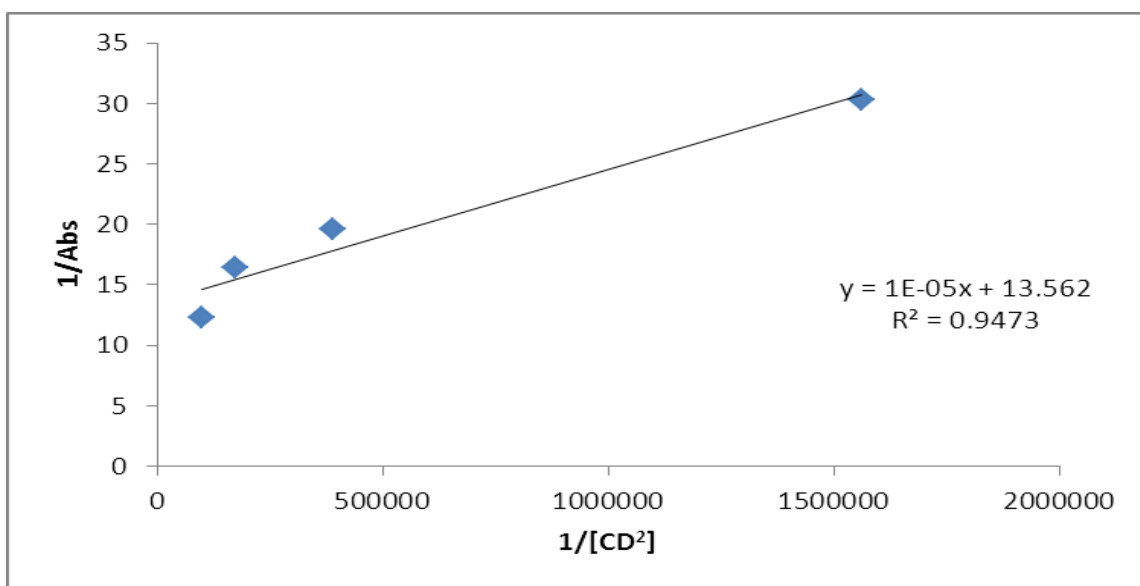


Figure 4.14: Double reciprocal plot for inclusion complex of β -CD- H_2Dz -Zn for $1/Abs$ vs $1/[CD]^2$.

It could be seen that for the inclusion complex of β -CD- H_2Dz -Zn, a good linear relationship was obtained when $1/Abs$ was plotted against $1/[CD]$ in Figure 4.13 compared to a plot of $1/Abs$ against $1/[CD]^2$ in Figure 4.14. This results confirmed that inclusion complex of β -CD- H_2Dz -Zn was in a ratio of 1:1. The calculated formation constant was 434.78 M^{-1} which suggests that the inclusion complex was a stable complex

compared with the inclusion complex of β -CD- H_2Dz with the formation constant value of 0.8441 M^{-1} . The high value of formation constant for the inclusion complex of β -CD- H_2Dz -Zn shows the stability of the complex where the addition of zinc binds β -CD and H_2Dz -Zn tightly as reported in TGA results in section 4.1.3.

4.2.2.3 Job's method

The stoichiometry for a metal-ligand complexation is usually determined by Job's method or also called the method of continuous variation. Metal-ligand complexation of H_2Dz -Zn and the inclusion complex of β -CD- H_2Dz -Zn were determined by Job's method.

The stoichiometry ratio for the complex of dithizone and zinc was determined by preparing a series of solution in which the total concentration of dithizone and zinc was held constant at $1 \times 10^{-4}\text{ M}$ but the ratio varied. The absorbance of each solution was measured at a wavelength of 511 nm. The maximum absorbance was determined by extrapolating the two linear portions of the plot. The result is shown in Figure 4.15 where it could be seen that the mole ratio of dithizone and zinc complex is 1:1.

The same method goes for the inclusion complex of β -CD- H_2Dz -Zn where a series of solution was prepared with the total concentration of β -CD, dithizone and zinc held constant at $1 \times 10^{-4}\text{ M}$ but their ratio varied. The absorbance was measured at 522 nm and from the result in Figure 4.16, it could be seen that the mole ratio of the inclusion complex of β -CD- H_2Dz -Zn is 1:1.

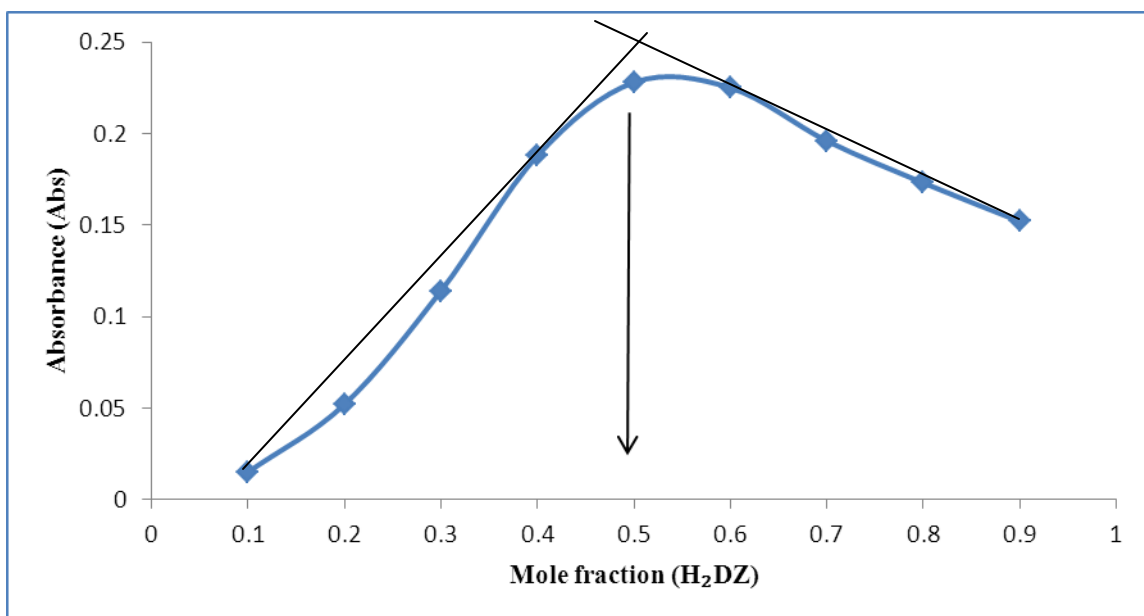


Figure 4.15: Job's plot for complex of H₂Dz-Zn

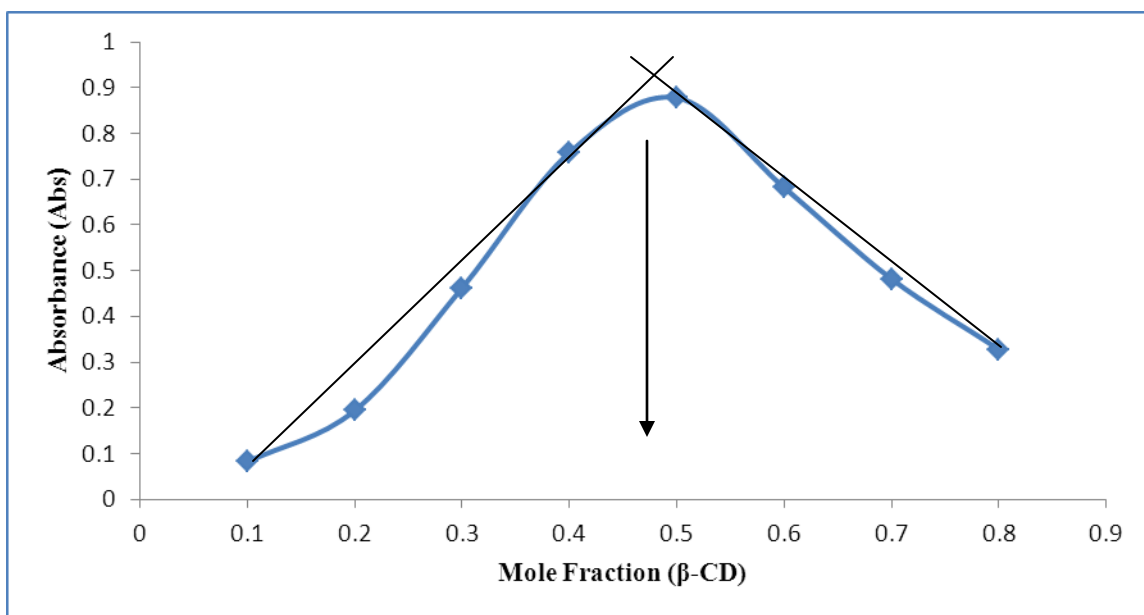


Figure 4.16: Job's plot for inclusion complex of β -CD-H₂Dz-Zn

4.3 Proposed structure for the inclusion complexes

4.3.1 Proposed structure for the inclusion complex of β -CD- H_2Dz

Based on all the results that has been discussed earlier, a structure of the inclusion complexes can be predicted and proposed. For the inclusion complex of β -CD- H_2Dz , it is known that from the formation constant study, the double reciprocal plot shows a good linear relationship for a 2:1 host-guest system complex. Since the inclusion complex forms a 2:1 ratio, thus it is known that both of the aromatic rings of dithizone have entered the cavity of β -CD. Therefore, the structure of the inclusion complex of β -CD- H_2Dz is proposed as in Figure 4.17 below.

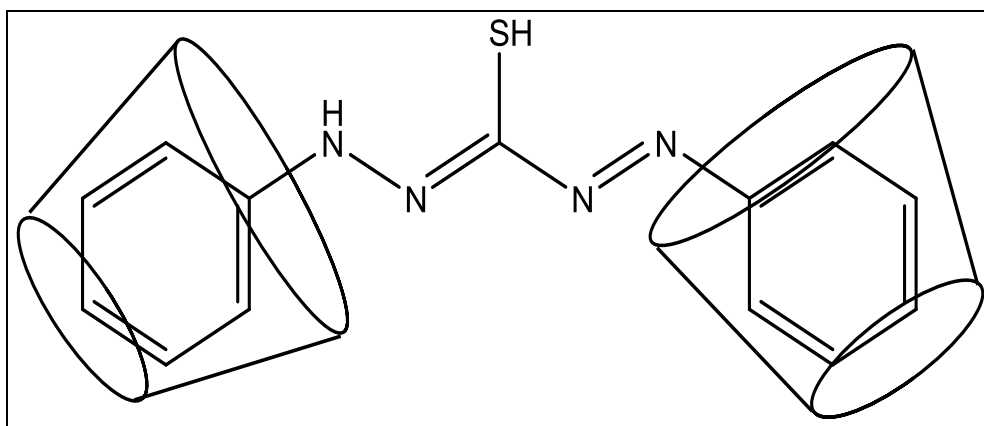


Figure 4.17: Proposed structure for inclusion complex of β -CD- H_2Dz .

4.3.2: Proposed structure for the inclusion complex of β -CD- H_2Dz -Zn

For the inclusion complex of β -CD- H_2Dz -Zn, the double reciprocal plot from the formation constant study shows that a 1:1 host-guest system complex has a better linear relationship compared to 2:1 host-guest system complex. The ratio of this complex was

also determined by Job's method that shows it is a 1:1 complex. Hence, the structure of the inclusion complex of β -CD- H_2Dz -Zn is proposed as in Figure 4.18 below.

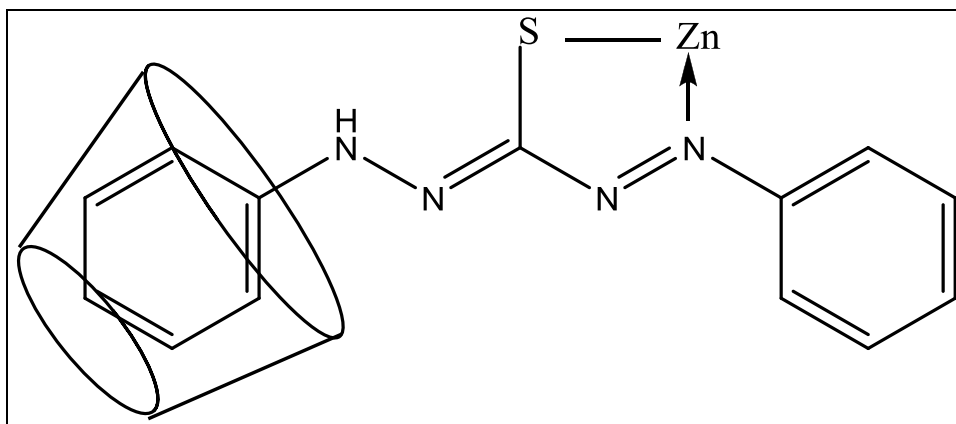


Figure 4.18: Proposed structure for inclusion complex of β -CD- H_2Dz -Zn.