CHAPTER 5: APPLICATION OF INCLUSION COMPLEX IN DETERMINATION OF ZINC USING SPECTROPHOTOMETRIC TECHNIQUE

In chapter 5, the application of inclusion complex in determination of zinc using spectrophotometric technique will be discussed. A spectrophotometry method was developed based on the enhancement of the absorbance of dithizone-zinc complex produced through complex formation in the presence of β-CD.

5.1 Effect of solvents

Due to low solubility of dithizone in most solvents, only five best solvents were chosen for this study. The results are shown in Table 5.1. From the result, it was found that acetone gave the highest absorbance for the inclusion complex formation. Acetone is a less polar solvent compared to acetonitrile, DMF, ethanol and methanol, and for this reason, it is easier for it to enter the cavity of β-CD (Connors, 1997). The absorbance for the effect of solvents decreases as the polarity of solvents increases. Since acetone has the highest absorbance compared to other solvents, thus, acetone was chosen as the solvent throughout the experiment. 15% of acetone was added throughout the study to overcome precipitate during the formation of the inclusion complex due to lack of solvents.

Table 5.1: Effect of solvents on inclusion complex of β-CD-H_2Dz-Zn at 522 nm.

SOLVENTS	ABSORBANCE
Acetone	0.103
Acetonitrile	0.076
Dimethylformamide (DMF)	0.078
Ethanol	0.069
Methanol	0.066

5.2 Absorption spectra

The absorption spectra of β-CD, dithizone, the complex of dithizone-zinc (H_2Dz-Zn), the inclusion complex of β-CD-H_2Dz and the inclusion complex of β-CD-H_2Dz-Zn were recorded and shown in Figure 5.1. It could be seen that there was no absorption spectra observed for β-CD in the range of 350 – 700 nm. While for H_2Dz, there were two maximum absorption points at 450 and 620 nm which indicates the enol and keto form respectively (Pemberton & Buck, 1982). As for the inclusion complex of β-CD-H_2Dz, there was only one absorption peak at 426 nm that shows the stable condition of the complex formed. For H_2Dz-Zn complex, the absorption peak shifted to 511 nm due to the formation of a metal-ligand complex. Formation of the inclusion complex of β-CD-H_2Dz-Zn shifts the absorption spectra to 522 nm and increases the sensitivity of the absorbance. This phenomenon indicates that there is a formation of an inclusion complex. Since the absorbance of the inclusion complex of β-CD-H_2Dz-Zn increased, hence, it is suitable to be detected by UV-spectrophotometry technique.

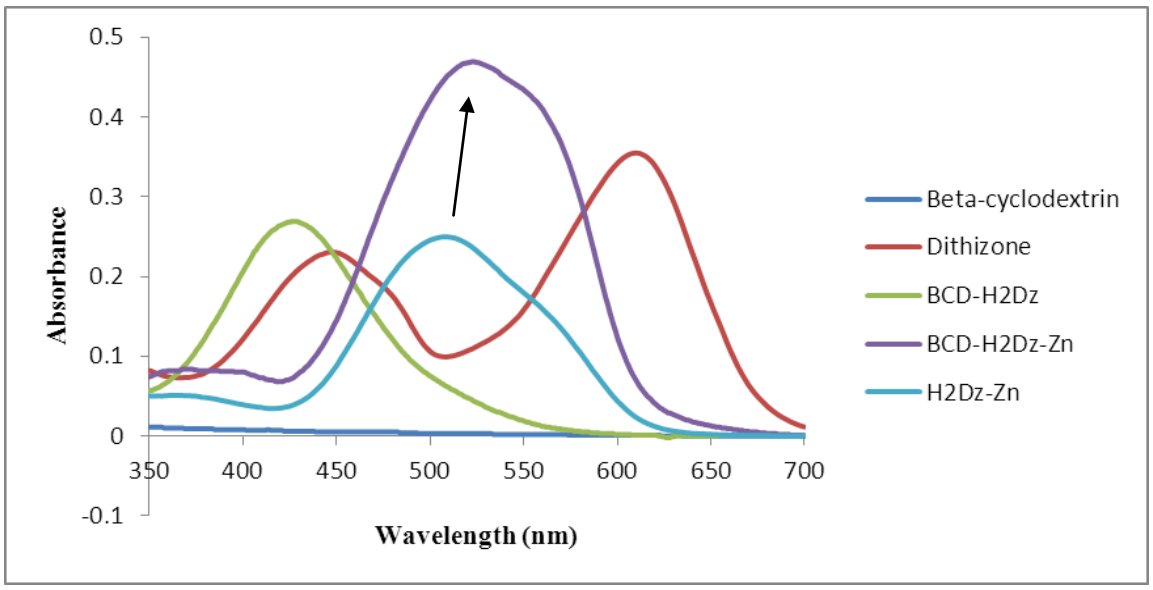

Figure 5.1: Absorption spectra of β-cyclodextrin, dithizone, complex of H_2Dz-Zn, inclusion complexes of β-CD-H_2Dz and β-CD-H_2Dz-Zn.

5.3 Effect of amount of dithizone

The effect on amount of H_2Dz solution on the absorbance of the inclusion complex was studied. The results obtained in Figure 5.2 indicates that the absorbance of inclusion complex increased with an increasing amount of H_2Dz until 1.5×10^{-5} M and decreased and remained constant thereafter. Therefore, 1.5×10^{-5} M of H_2Dz was selected throughout the experiment to be an optimum condition for dithizone.

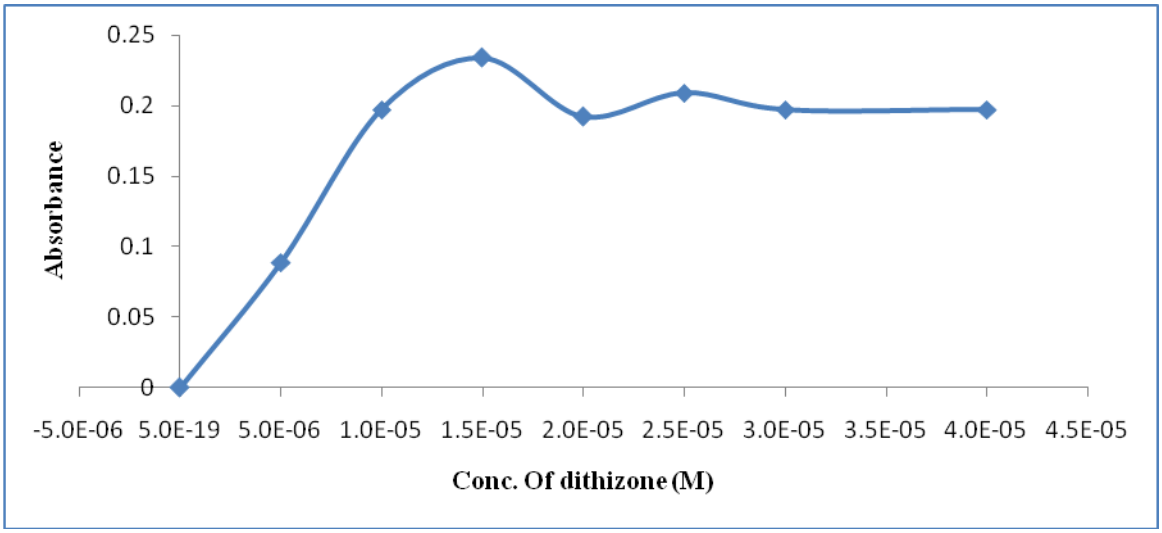

Figure 5.2: Effect of the amount of dithizone at 522 nm, pH 8.

5.4 Effect of amount of β-cyclodextrin

Effect of amount of β-CD on the absorbance of the inclusion complex was also investigated. The result is shown in Figure 5.3. The absorbance of the inclusion complex was constant with increasing concentration of β-CD until 1.5 x10^{-4} M but then decreased and remained constant again. This may be due to the release of high energy water from the cavity of β-CD upon complex formation (Connors, 1997)

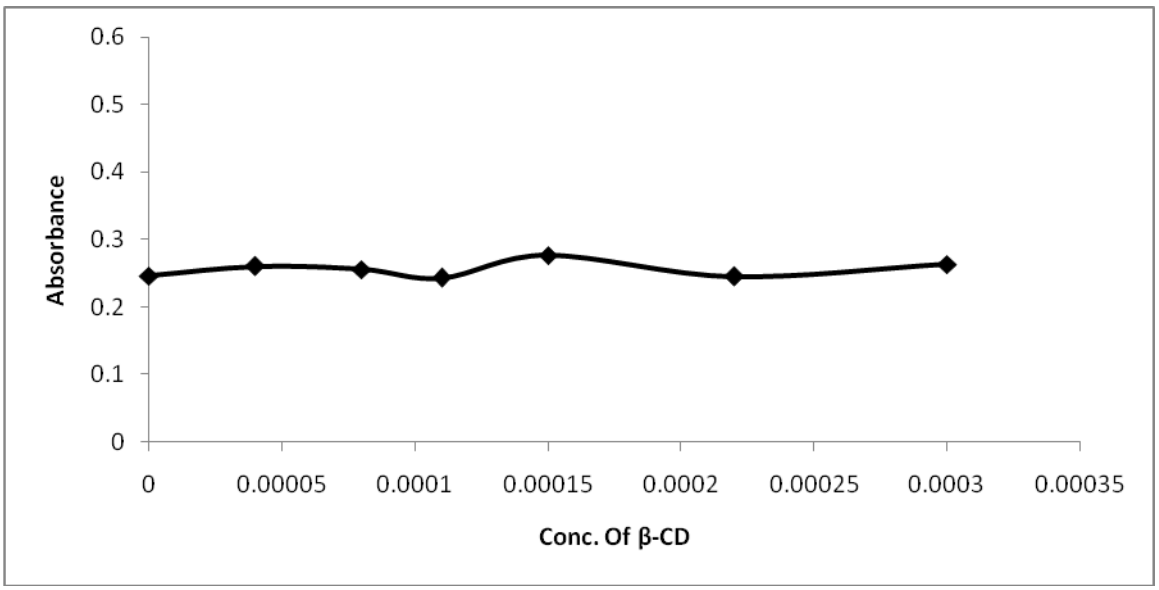

Figure 5.3: Effect of the amount of β-cyclodextrin at 522 nm and pH 8.

5.5 Effect of foreign ions

Before the proposed method was applied to real samples, a systematic study was carried out on the effect of various foreign ions on the determination of 1 mg/L of zinc (Zn). In this study, various amounts of different foreign ions were investigated by initially adding 3000 mg/L of each foreign ion into 1 mg/L of zinc. The criterion for interference was fixed at ±5.0% variation of the average absorbance intensity calculated for the established level of zinc. If interference occurred, the ratio was reduced gradually until the interference halted. From the results shown in Table 5.2, it can be seen that most of the foreign ions have no detrimental effect on the determination of zinc except for metal ions. This is because dithizone itself can form chelate complexes with these metal ions (Burger, 1973).

It could be seen from the results in Table 5.2, most of the interference occurred was a positive interference. An increasing of absorption intensity occurs when the inclusion complex form complex with foreign ions and form a positive interference. While a negative interference occurs when zinc reacts with interfering ions and the absorbance reduces.

Since there is not much interference from these foreign ions, therefore, the results indicate that the proposed method had good selectivity towards foreign ions.

Table 5.2: Effect of foreign ions

Foreign ions	Tolerence limits (mg/L)	Effect
Ca(II)	3000	Positive interference
K(I)	3000	Positive interference
Ba(II)	3000	Positive interference
Na(I)	2000	Positive interference
NH_4^+	1000	Positive interference
Mg(II)	750	Positive interference
Mn(II)	5	Positive interference
Cu(II)	5	Positive interference
Al(III)	3	Positive interference
Cd(II)	3	negative interference
Ni(II)	2	Positive interference
Co(II)	2	Positive interference
Acetate	3000	Negative interference
Chloride	3000	Negative interference
Carbonate	3000	Positive interference
Flouride	3000	Positive interference
Sulfate	3000	Positive interference
Bromide	2000	Positive interference
Nitrate	2000	Positive interference
Oxalate	1000	Negative interference
Citric acid	3000	Positive interference
Oxalic acid	2000	Positive interference

5.6 Dynamic Range and sensitivity Study

The effect and sensitivity of β-CD in inclusion complex were determined by analyzing the dynamic range and sensitivity study of the complex with the presence of β-CD. The effect of zinc concentration was studied over range of 0.1 – 10 mg/L, while the concentration of β-CD and dithizone were kept constant. The absorbance was linear for concentration of zinc in the range of 0.1 – 9.0 mg/L then it decreased. The absorbance was determined at 522 nm.

In metal-ligand complex, sensitivity is often described as molar absorptivity (ε, units L mol^{-1} cm^{-1}). There are also some other ways of describing sensitivity known as specific absorptivity and Sandell sensitivity (Bode, 1991). Specific absorptivity (a) was determined from slope of each calibration curve (absorbance versus concentration n of zinc, Figure 5.4 and 5.5). Molar absorptivity (ε) and Sandell sensitivity (S) values were calculated from the specific absorptivity (a), where,

$$a = \varepsilon / \text{atomic weight of zinc} = 65.39 \times 1000 \qquad \text{Equation 5.1}$$

and Sandell sensitivity (S) is the concentration of analyte (µg/ml) which will give an absorbance of 0.001 in a cell of path length 1 cm and is expressed as µg/cm^2,

$$S = 10^{-3} / a = \mu g/cm^2 \qquad \text{Equation 5.2}$$

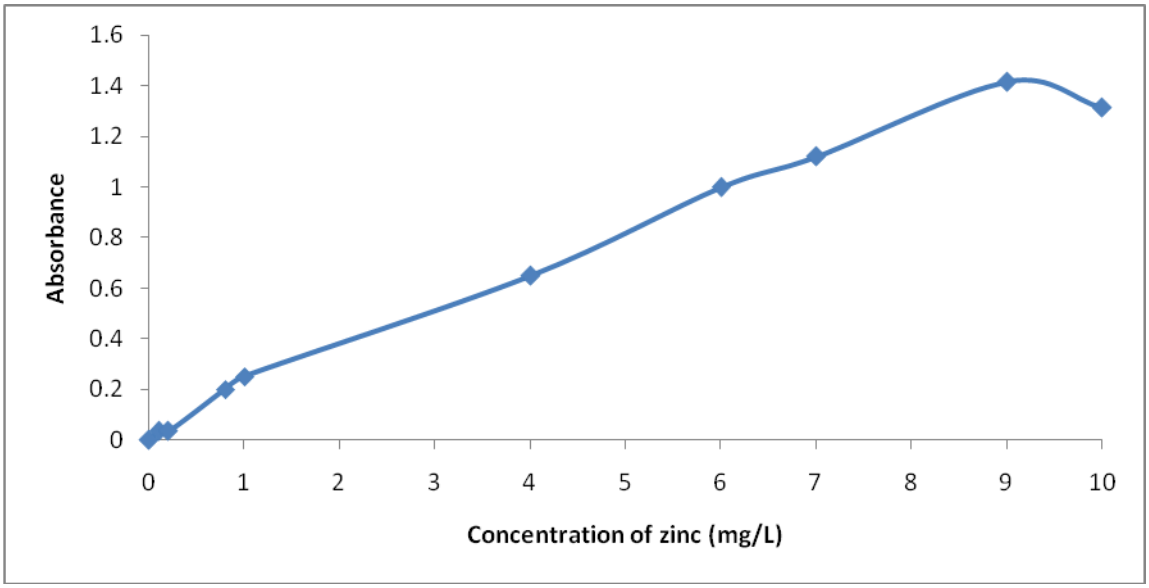

Figure 5.4: Response curve of inclusion complex with β-CD towards different concentration of Zn.

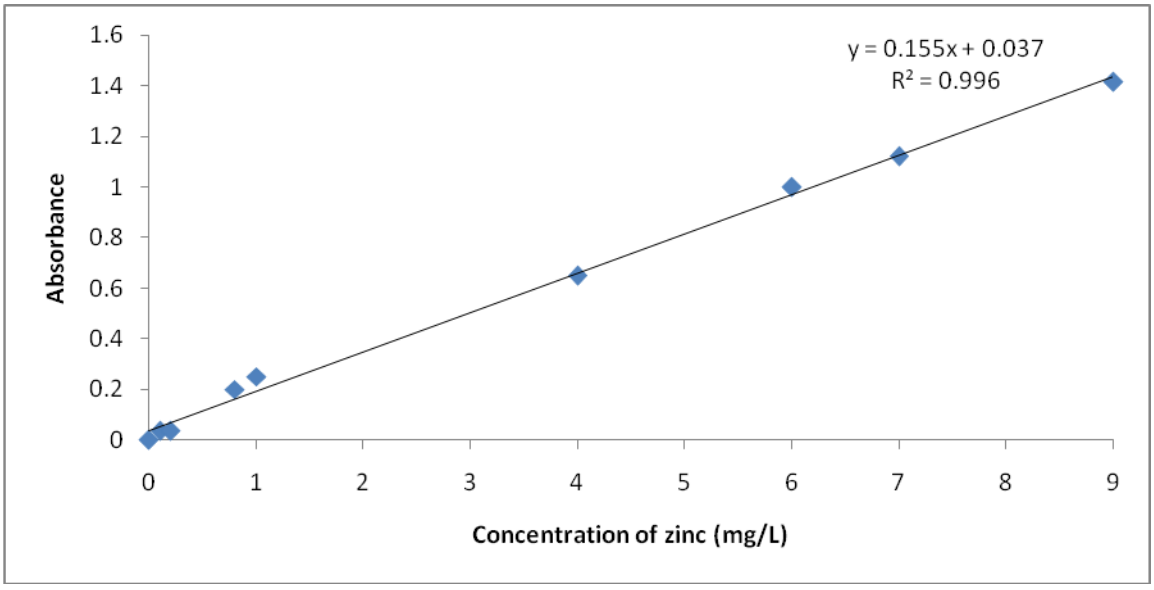

Figure 5.5: Calibration curve of inclusion complex of β-CD-H_2Dz-Zn at 522 nm, pH 8.

Based on the graph in Figure 5.5, the specific absorptivity, molar absorptivity and Sandell sensitivity of the inclusion complex of β-CD-H_2Dz-Zn were calculated. The specific absorptivity, a, for inclusion complex of β-CD-H_2Dz-Zn is

15.5 x 10^{-2} ml g^{-1} cm^{-1}, while the molar absorptivity, ε, is 1.02 x 10^{4} L mol^{-1} cm^{-1} and Sandell sensitivity, S, is 6.45 x 10^{-2} μg cm^{-2}.

5.7 Limit of Detection

Limit of detection is the lowest concentration of mass of analyte that can be detected (Connors, 1973). The detection limit is given by (Long *et al*., 1983),

$$C_L = X_L - M_B / m = 3S_B / m \qquad \text{Equation 5.3}$$

where C_L is the detection limit, X_L is the smallest discernable analytical signal, M_B the mean blank response value, S_B the standard deviation of the blank response and m is the mean value of the slope of the calibration curve. The calibration curve for the detection limit of zinc with addition of β-CD is also shown in Figure 5.5. Data for the limit of detection calculation can be reviewed in Table A-1 in Appendix A. From the calibration curve obtained, the detection limit of zinc was calculated and the value is 0.005 mg/L.

5.8 Reproducibility of the method

The absorbance of inclusion complex of β-CD-H_2Dz-Zn was measured on ten replicates (Figure 5.6) to determine the precision of the method under the optimum experimental condition by using standard solution. The calculated relative standard deviation (R.S.D) was 2.05% for 1 mg/L of zinc (Zn). This indicates that this method is highly precise and reproducible.

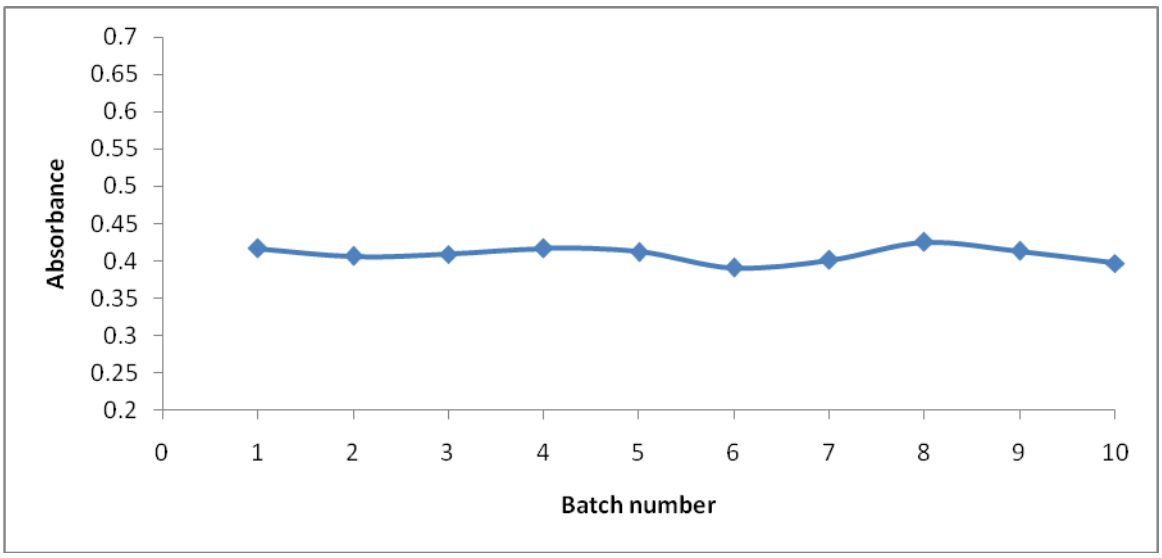

Figure 5.6: Reproducibility study on inclusion complex of β-CD-H_2Dz-Zn

These results are compared with previously studied method in Table 5.3 and it shows a good sensitivity towards determination of zinc.

Table 5.3: Comparison of present method with other reported methods on spectrophotometric determination of zinc.

REAGENT	MOLAR ABSORPTIVITY (L mol^{-1} cm^{-1} x 10^4)	LOD (mg/L)	REMARKS	REFERENCES
Pyridoxal-4-phenyl-3-thiosemicarbazone	1.60	0.04	Less sensitive	Reddy *et al.*, 2006
N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone	1.55	N.R	Less sensitive	Reddy *et al.*, 2007
β-CD-H_2Dz-Zn	2.20	0.005	Highly sensitive	P.M

N.R: Not reported; P.M: Proposed method

5.9 Application: Analysis of zinc in spiked water samples

The proposed method was applied in two different matrixes which were tap water and digested herbal medicine. 0.5 mg/L of zinc was spiked into both of the samples and the recovery of zinc was found to be in the range of accepted value and the results are

shown in Table 5.4. Therefore, it can be concluded that this method is effective for the determination of zinc in environmental samples such as tap water and also in pharmaceutical samples (herbal medicine)

Table 5.4: Recovery of zinc from real samples

SAMPLE	ACTUAL CONC. (mg/L)	CONC. FOUND (mg/L)	RECOVERY
Tap water	0.0747 ± 0.003	0.0550 ± 0.005	95 %
Chinese herbal medicine	1.2115 ± 0.004	1.6365 ± 0.001	85 %

CHAPTER 6: CONCLUSION

The inclusion behavior of β-CD with H_2Dz and H_2Dz-Zn has been investigated. The results from Fourier Transform Infra Red (FTIR), Solid State ^{13}C CP/MAS NMR Spectroscopy, Thermal Gravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and powder X-ray Diffraction (XRD) confirmed the formation of inclusion complex between β-CD-H_2Dz and β-CD-H_2Dz-Zn. The ratio was confirmed by Job's method and formation constant study through the double reciprocal plot. Inclusion complex of β-CD-H_2Dz forms at 2:1 ratio while inclusion complex of β-CD-H_2Dz-Zn forms at 1:1 host-guest system complex. The solubility of dithizone in aqueous was also successfully increased by the formation of inclusion complex hence reducing the usage of organic solvents. The apparent formation constant (K) for inclusion complex of β-CD-H_2Dz was found to be 0.8441 M^{-1} while for inclusion complex of β-CD-H_2Dz-Zn, the formation constant increased to 434.78 M^{-1}. It is shown that the presence of zinc influence the stability of the inclusion complex. The inclusion complex was applied for determination of zinc using spectrophotometric technique. At the optimum experimental conditions, there were no interferences from foreign ions and there was a linear relationship between the absorption intensity and concentration of zinc in the range of 0.1 to 9 mg/L with a correlation coefficient of 0.996. The limit of detection was determined to be 0.005 mg/L and the relative standard deviation (R.S.D.) was 2.05%. The proposed method can be used as an alternative method for the determination of zinc in environmental and also pharmaceutical samples as the recoveries are 95% and 85% respectively.

APPENDIX A

Calculation of the limit of detection

Table A-1: Blank signal for β-CD-H_2Dz-Zn system at 522 nm.

Number of measurements	Absorbance
1	0.0002
2	0.0010
3	0.0007
4	0.0002
5	0.0001
6	0.0001
7	0.0001
8	0.0001
9	0.0001
10	0.0001

From Eq. 5.3:

$C_L = X_L - M_B / m = 3S_B / m$

Mean of the blank (X_L) = 0.0003

Standard deviation of the blank (S_B) = 0.000253

Slope (m) = 0.1553 (From Figure 5.5)

Detection limit = 0.005 mg/L