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Study on Inclusion Complex of β-Cyclodextrin-Dithizone-Zinc (β-CD-H_2Dz-Zn) by Spectrophotometry and Its Analytical Application

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The non-covalent interaction of β-cyclodextrin and dithizone-zinc (H_2Dz-Zn) complex was studied by spectrophotometry. The results showed that β-cyclodextrin formed a complex with H_2Dz-Zn in a ratio of 1:1 with an apparent formation constant of 4.7×10^2 M^{-1}. Due to the enhancement of absorbance intensity of dithizone-zinc produced from complex formation, a spectrophotometry method was developed for determination of zinc in the presence of β-cyclodextrin. A linear relationship between absorbance and concentration of zinc was obtained in the range of 0.1-9.0 mg/L with correlation coefficient of 0.996. The detection limit obtained was 0.005 mg/L and the relative standard deviation (RSD) was 2.05 %. The developed method was applied in real samples and the recoveries obtained were from 85-95 %.

Key Words: β-Cyclodextrin, Dithizone, Zinc, Inclusion complex, UV- spectrophotometric.

INTRODUCTION

Cyclodextrin is a cyclic oligomer of α-D-glucose, which is connected through glycosidic α-1,4 bonds. β-Cyclodextrins, (Fig. 1) has seven glucose units which has polar exterior and non-polar at the interior cavity. Since the compound formed a doughnut-shaped molecule, it has the ability to form a non-covalent host-guest inclusion complex with many organic compounds that are capable of entering the cyclodextrin cavity and is referred as host[1]. The host-guest inclusion complex systems occurs through various interactions, such as hydrogen bonding, Van der Waals force, electrostatic or hydrophobic interactions[2]. Since β-cyclodextrin is low cost and commercially available, it is widely used to form an inclusion complex to increase the solubility and sensitivity in many analytical metal complexes[3,4].

One of the most important organic chelating reagents for metal complexes is diphenylthiocarbazone or commonly known as dithizone (Fig. 2) which was first introduced in 1925 by Hellmuth Fischer[5]. It forms a bright coloured product when it is complexed with metals but it has a very low solubility in water. It forms a complex with zinc and produced a pink colour solution. Zinc is found in many samples such as in the environment, biological and also in pharmaceutical samples[6-8]. Its determination is very important because either an excess or a deficiency of zinc can be harmful to humans. Zinc has been determined by atomic absorption spectrometry or inductively coupled plasma mass spectrometry (ICP-MS). These methods

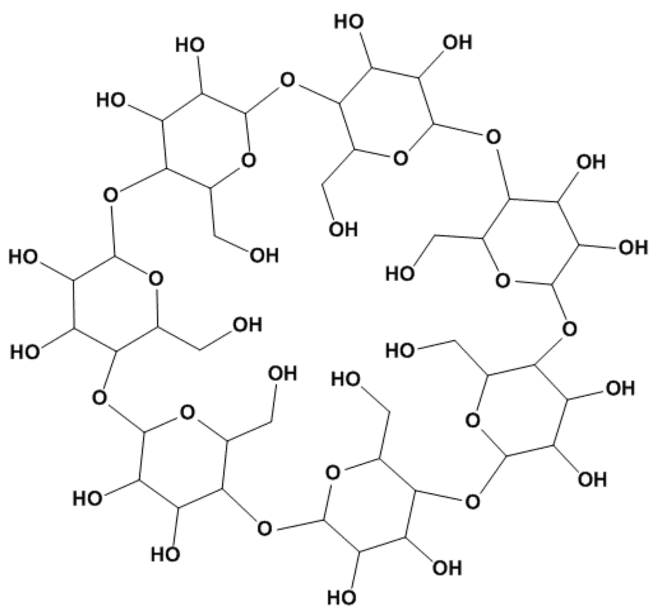

Fig. 1. β-Cyclodextrin

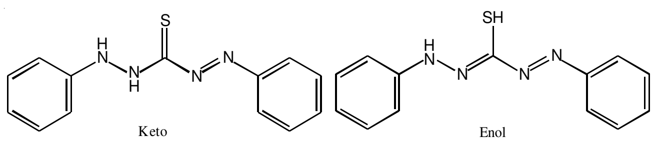

Fig. 2. Keto and enol form of dithizone

are highly sensitive, but the cost of the equipment is high and separation and/or preconcentration techniques are usually necessary. Therefore, these complicated and time consuming methods do not satisfy the requirements of routine analysis.

In the present work, a spectrophotometric study of the supramolecular interaction between H_2Dz-Zn complex and β-cyclodextrin is carried out. Formation of an inclusion complex with β-cyclodextrin has an advantage where the addition of β-cyclodextrin can enhance the sensitivity of the absorption spectra of dithizone-zinc complex in a spectrophotometric determination. Based on the results obtained, a spectrophotometric method for the determination of zinc was successfully developed.

EXPERIMENTAL

All the absorbance measurements were carried out on a Shimadzu UV-VIS spectrophotometer (UV-1650 PC) with matched 1 cm quartz cell. All pH measurements were made by a Thermoline pH meter.

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

Effect of pH: The pH of the inclusion complex of β-cyclodextrin-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 8-14. The absorbance was detected at 522 nm by using a UV-visible spectrophotometer.

Phase solubility study: The phase solubility studies were successfully done for the inclusion complex of β-cyclodextrin-H_2Dz according to the Higuchi and Connors method[9]. For these studies, an excess amount of dithizone was added into a series of aqueous solution of β-cyclodextrin with increasing concentration from 0 to 0.02 M.

The solutions were then sealed and shaken at room temperature for 24 h and were carefully filtered through 0.45 μm Whatmann filter paper. The solutions were analyzed to get their absorption through a UV spectrophotometer (426 nm).

Formation constant: The dithizone and zinc concentration was held constant at 1.5×10^{-5} M and 1 mg/L respectively while varied amounts of β-cyclodextrin were added sequentially in a 10 mL volumetric flask. Acetone)15 %) was added to the mixture. The mixed solution were then diluted to the calibration mark and mixed well. The absorbances were measured at 522 nm for the inclusion complex of β-cyclodextrin-H_2Dz-Zn. The stoichiometry ratio of the inclusion complex was obtained through the double reciprocal plot.

Determination of zinc in 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 was followed to analyze zinc.

1 mL of tap water was taken and used without any pretreatment. 0.5 mg/L of zinc was spiked into the solution and the standard procedure was followed to analyze zinc at 522 nm by a UV-visible spectrophotometer.

RESULTS AND DISCUSSION

Effect of solvents: Due to low solubility of dithizone in many solvents, only five best solvents were chosen in this study. The absorbance of the inclusion complex of β-cyclodextrin-H_2Dz-Zn was measured at 522 nm. The result was shown in Table-1. From the result, it was found that acetone gives 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-cyclodextrin[10]. The absorbance for the effect of solvents decreases as the polarity of solvents increases. Compared with all these solvents, dithizone easily dissolved in acetone. Hence, when the solubility of dithizone increased, it will also increase the absorption sensitivity in UV-spectrophotometer. Since acetone has the highest absorbance compared to other solvents, thus, acetone was chosen as the solvent throughout the experiment.

TABLE-1
EFFECT OF SOLVENTS AT 522 nm (CONCENTRATION Of β-CYCLODEXTRIN = 0.06 M, CONCENTRATION OF DITHIZONE = 1.5×10^{-5} M, CONCENTRATION of Zn = 1 mg/L)

Solvents	Absorbance
Acetone	0.103
Acetonitrile	0.076
Dimethyl formamide	0.078
Ethanol	0.069
Methanol	0.066

Absorption spectra: The absorption spectra of β-cyclodextrin, dithizone (H_2Dz), complex of dithizone-zinc (H_2Dz-Zn), inclusion complex of β-cyclodextrin-H_2Dz and inclusion complex of β-cyclodextrin-H_2Dz-Zn was recorded and shown in Fig. 3. It could be seen that there was no absorption spectra observed for β-cyclodextrin in the range of 350-700 nm. While for H_2Dz, there was 2 maximum absorption points at 450 and 620 nm, which indicates the enol and keto form respectively. As for inclusion complex of β-cyclodextrin-H_2Dz, there was only 1 absorption peak at 426 nm that shows the stable condition of the complex formed. For H_2Dz-Zn complex, the absorption peak has shifted to 511 nm due to the formation of a metal-ligand complex. Formation of the inclusion complex of β-cyclodextrin-H_2Dz-Zn shifts the absorption spectra to 522 nm and increased the sensitivity of the absorbance. This phenomena indicates that there is a formation of an inclusion complex.

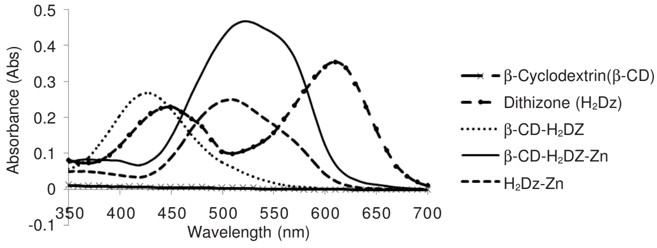

Fig. 3. Absorption spectra of β-cyclodextrin, H_2Dz, complex of H_2Dz-Zn, inclusion complexes of β-cyclodextrin-H_2Dz and β-cyclodextrin-H_2Dz-Zn

Effect of pH: The inclusion complex of β-cyclodextrin-H_2Dz-Zn is only formed in a basic medium. Therefore, the effect of pH was conducted in the range of pH 8-13. pH 8 has the highest absorbance intensity and therefore it was chosen throughout the study.

Phase solubility study: The phase solubility study was done according to the Higuchi and Connors method[9]. Phase solubility diagram was shown in Fig. 4 and it is an Ap-type. It can be seen that the solubility of dithizone increased with increasing concentration of β-cyclodextrin. This confirms that 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.

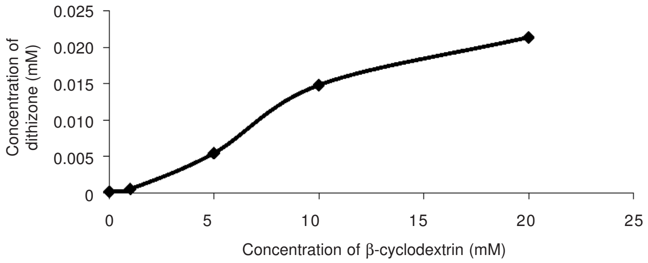

Fig. 4. Phase solubility diagram of β-cyclodextrin-H_2Dz system in water

Job's method: Job's method also called the method of continuous variation is used to determine the stoichiometry of a complex. There were two results obtained in Fig. 5a and b. Fig. 5a shows Job's method for the metal-ligand complex of dithizone-zinc, while Fig. 5b shows results for the inclusion complex of β-cyclodextrin-H_2Dz-Zn. A series of solutions was prepared with a same total number of moles but different ratios. The results show both of the complexes adopts the same 1:1 complex ratio.

Formation constant: Formation constant (K) is a very important parameter to be investigated. In this study, the concentration of dithizone and zinc was held constant at 1.5×10^{-5} M and 1 mg/L respectively, while concentration of β-cyclodextrin was varied from 0 to 3.2×10^{-3} M. The absorption spectrum of inclusion complex of β-cyclodextrin-H_2Dz-Zn was shown in Fig. 6 and it is measured at 522 nm.

When the concentration of β-cyclodextrin was increased, an increasing absorption intensity of the complex was observed. This suggests that there is an inclusion complex formation.

The apparent formation constant (K) value can be determined from the changes in absorbance. The apparent formation constant (K) value for the inclusion complex with a ratio of 1:1 or 2:1 can be calculated from the Benesi-Hildebrand equation[11]:

$$\frac{1}{A} = \frac{1}{\varepsilon[G]_o K[CD]} + \frac{1}{\varepsilon[G]_o} \quad (1)$$

$$\frac{1}{A} = \frac{1}{\varepsilon[G]_o K[CD]^2} + \frac{1}{\varepsilon[G]_o} \quad (2)$$

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

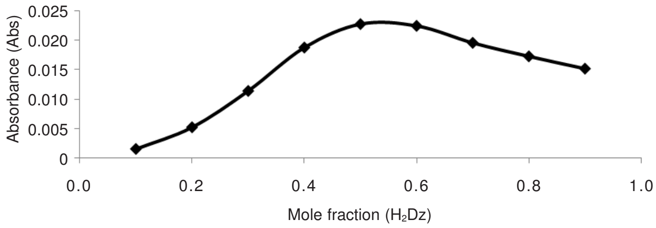

Fig. 5a. Job's plot for complex of H_2Dz-Zn at 511 nm

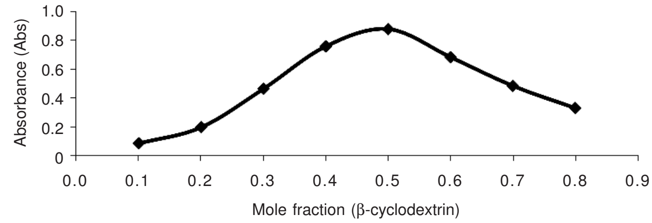

Fig. 5b. Job's plot for inclusion complex of β-cyclodextrin-H_2Dz-Zn at 522 nm

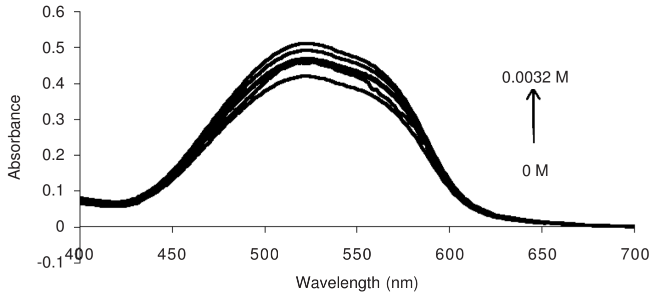

Fig. 6. Absorption spectrum of inclusion complex of β-cyclodextrin-H_2Dz-Zn with varied concentration of β-cyclodextrin from 0 M to 0.0032 M at pH 8 at 522 nm

Based on the absorbance detected, two double reciprocal plots was plotted to confirm the stoichiometric mole ratio of the inclusion complex. The double reciprocal plot for inclusion complex of β-cyclodextrin-H_2Dz-Zn is shown in Fig. 7 (a and b).

It could be seen that for inclusion complex of β-cyclodextrin-H_2Dz-Zn, a good linear relationship was obtained when 1/Abs was plotted against 1/[CD] in Fig. 7a compared to a plot of 1/Abs against $1/[CD]^2$ in Fig. 7b. This results confirmed that inclusion complex of β-cyclodextrin-H_2Dz-Zn was in a ratio of 1:1, which support the result obtained in Job's method. The calculated formation constant (K) was 4.7×10^2 M^{-1}.

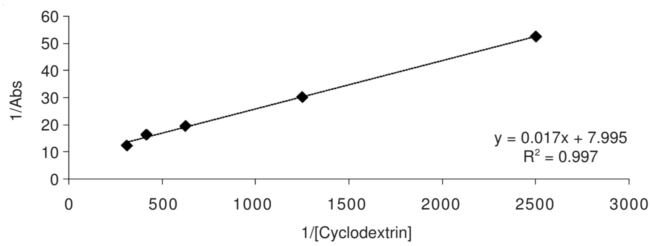

Fig. 7a. Double reciprocal plot for inclusion complex of β-cyclodextrin-H_2Dz-Zn for 1/Abs *vs.* 1/[CD]

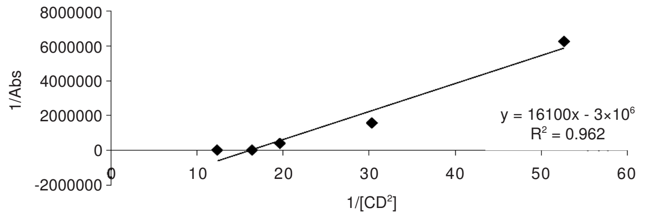

Fig. 7b. Double reciprocal plot for inclusion complex of β-cyclodextrin-H_2Dz-Zn for 1/Abs *vs.* $1/[CD]^2$

Effect of amount of dithizone: The effect of the amount of dithizone on the absorbance of the inclusion complex was studied. The results obtained (Fig. 8) indicate that the absorbance of the 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.

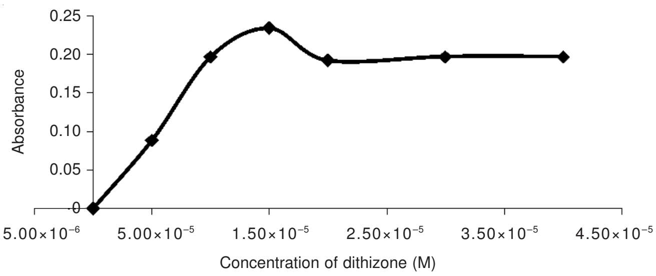

Fig. 8. Effect of the amount of dithizone (H_2Dz) at 522 nm, pH 8

Effect of foreign ions: Effect of foreign ions on the determination of 1 mg/L of zinc was carried out and the result was tabulated in Table-2. If interference occurred, the ratio was reduced gradually until the interference halted. The criterion for interference was fixed at ± 5.0 % variation of the average absorbance intensity calculated for the established level of zinc. From the result that was shown in Table-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 metal-ligand complexes with these metal ions[12]. These results show that the proposed method had good selectivity towards foreign ions.

Calibration graph of zinc: A spectrophotometric determination of zinc in aqueous was developed based on the enhancement of the absorbance intensity produced from complex formation. Under the optimum conditions, a linear relationship between absorbance and concentration of zinc in the range of 0.1 to 9.0 mg/L was obtained with a correlation coefficient of 0.996 and molar absorptivity of 1.02×10^4 L mol^{-1} cm^{-1}.

TABLE-2
EFFECT OF FOREIGN IONS AT 522 nm AT pH 8

Tolerence limit (mg/L)	Foreign ions
3000	Ca^{2+}, K^+, Ba^{2+}, Cl^-, CO_3^{2-}, F^-, SO_4^{2-}, acetate, citric acid
2000	Na^+, Br^-, NO_3^{2-},oxalic acid
1000	NH_4^+, oxalate
750	Mg^{2+}
5	Mn^{2+}, Cu^{2+}
3	Al^{3+}, Cd^{2+}
2	Ni^{2+}, Co^{2+}

Reproducibility and detection limit: Ten replicates of the sample prepared by standard procedure were determined at 522 nm with a RSD of 2.05 % for 1 mg/L of zinc. This shows that the method was highly precise and reproducible. The detection limit was determined by analyzing ten blank samples without addition of zinc and the value is 0.005 mg/L. These results were compared with previously studied methods in Table-3 and show a good sensitivity towards determination of zinc.

TABLE-3
COMPARISON OF PRESENT METHOD WITH OTHER REPORTED METHODS ON SPECTROPHOTOMETRIC DETERMINATION OF ZINC

Reagent	Molar absorptivity (L mol^{-1} cm^{-1} $\times 10^4$)	LOD (mg/L)	Remarks	Ref.
Pyridoxal-4-phenyl-3-Thiosemicarbazone	1.60	0.04	Less sensitive	13
N-ethyl-3-carbazolecar-boxaldehyde-3-thiosemicar-bazone	1.55	-	Less sensitive	14
β-CD-H_2Dz-Zn	2.20	0.005	Highly sensitive	Present study

Application to real samples: The proposed method was applied in two different matrixes which are 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 quantitative and the results are shown in Table-4. Therefore, it can be concluded that this method is effective for the determination of zinc in environmental samples and also in pharmaceutical samples.

TABLE-4
RECOVERY OF ZINC FROM SAMPLES

Sample	Actual conc. (mg/L)	Conc. found (mg/L)	Recovery (%)
Tap water	0.0747 ± 0.003	0.5497 ± 0.005	95
Chinese herbal medicine (%)	1.2115 ± 0.004	1.6365 ± 0.001	85

Conclusion

The results presented in this paper clearly demonstrate that the inclusion complex of β-cyclodextrin-H_2Dz-Zn was successfully formed in a ratio of 1:1. The solubility of dithizone in water was also successfully increased by the formation of the inclusion complex hence reducing the usage of organic solvents. At the optimum experimental conditions, there were not much interference from foreign ions and there was a linear relationship between the absorption intensity and concentration of zinc in the range of 0.1 to 9.0 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 (RSD) was 2.05 %. This method was successfully applied in environmental and also pharmaceutical samples to determine the concentration of zinc by a UV-visible spectrophotometer as the recoveries are 95 % and 85 % respectively.

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