

Studies of Zinc(II) in Pharmaceutical and Biological Samples by Extractive Spectrophotometry: Using Pyridoxal-4-phenyl-3-thiosemicarbazone as Chelating Reagent

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Pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) é proposto como um novo reagente sensível para a determinação espectrofotométrica de zinco(II). PPT reage com zinco(II) no intervalo de pH entre 5,0 e 6,0, para formar um complexo colorido amarelo, o qual foi adequadamente extraído em *n*-butanol. A absorvância do complexo Zn(II)-PPT foi medida em diferentes intervalos de tempo, a 430 nm, para averiguar a estabilidade do complexo. Foi observado que o desenvolvimento da cor foi instantâneo e estável por mais de 48 h. O sistema obedece a lei de Beer até 6,0 µg mL⁻¹ de zinco(II), com uma excelente linearidade em termos do valor do coeficiente de correlação, de 0,999. A absorvância molar e sensibilidade de Sandell da espécie extraída é 1,6 × 10⁴ L mol⁻¹ cm⁻¹ e 4,085 × 10⁻³ µg cm⁻², respectivamente, a 430 nm. O limite de detecção do método é 0,04 µg mL⁻¹. Para avaliar a precisão do método, determinações foram efetuadas em diferentes concentrações; o desvio padrão relativo não excedeu 3,1%. A composição do complexo de zinco(II) com PPT foi estudada pelos métodos de variação contínua de Job, da razão molar, método de Asmus e da razão da inclinação. PPT tem sido empregado satisfatoriamente para a determinação de zinco(II) isoladamente, ou na presença de outros íons, normalmente associados ao zinco(II) em amostras farmacêuticas e biológicas. Vários materiais de referência (NIST 1573, NBS 1572 e NIST SRM 8435) têm sido testados para a determinação de zinco, com o propósito de avaliar a exatidão do presente método. Os resultados do método proposto são concordantes com os obtidos por espectrometria de absorção atômica com chama.

Pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) is proposed as a new sensitive reagent for the sensitive extractive spectrophotometric determination of zinc(II). PPT reacts with zinc(II) in the pH range 5.0-6.0 to form a yellow colored complex, which was well extracted into *n*-butanol. The absorbance value of Zn(II)-PPT complex was measured at different intervals of time at 430 nm, to ascertain the stability of the complex. It was observed that the color development was instantaneous and stable for more than 48 h. The system obeyed Beer's law up to 6.0 µg mL⁻¹ of zinc(II), with an excellent linearity in terms of correlation coefficient value of 0.999. The molar absorptivity and Sandell's sensitivity of the extracted species is 1.6 × 10⁴ L mol⁻¹ cm⁻¹ and 4.085 × 10⁻³ µg cm⁻² at 430 nm. The detection limit of the method is 0.04 µg mL⁻¹. To assess precision of the method, determinations were carried out at different concentrations; the relative standard deviation does not exceed 3.1%. The composition of the zinc(II) complex with PPT was studied by the method of Job's continuous variation, molar ratio method, Asmus' method and slope ratio method. It has been satisfactorily applied for the determination of zinc(II), when present alone or in presence of diverse ions, which are usually associated with zinc(II) in pharmaceutical and biological samples. Various certified reference materials (NIST 1573, NBS 1572 and NIST SRM 8435) have been tested for the determination of zinc for evaluating the accuracy of the developed method. The results of the proposed method are in agreement with flame atomic absorption spectrometry.

Keywords: pyridoxal-4-phenyl-3-thiosemicarbazone, zinc(II), pharmaceutical samples, biological samples

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Introduction

Zinc is an essential element for all animals including human beings and it plays an important physiological role. In human blood, zinc is distributed 75- 85% in erythrocytes (mostly as carbonic anhydrase), 12 to 22% in plasma, and 3% in leukocytes. One third of zinc in plasma is loosely bound to serum albumins, the remainder being more firmly attached to α -globulins, with minor fractions complexed in histidine and cysteine.¹⁻³

Zinc is associated with many enzyme systems, both as metallo-enzyme and enzyme-activator, as well as filling a structural role. In addition, it plays a number of important biological roles. Its most vital function may be concerned with the synthesis of deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid. Zinc deficiency leads to impaired DNA synthesis, delayed wound healing and decrease in collagen synthesis. Deficiency of zinc leads to retarded growth, lower feed efficiency, inhibits the general well-being, causes ulcers, scaling of the skin, besides affecting the bones and joints. Children in underdeveloped countries who are solely deficient in zinc, fail to mature sexually. Less severe zinc deficiency has been linked to a low sperm count and infertility. Zinc deficiency during pregnancy may produce serious defects and foetal loss.⁴

Although a little zinc is vital to health, too much is harmful, a single 220 mg zinc sulphate capsule can cause nausea and vomiting. Toxic effects, which may also include abdominal pain, fever and severe anaemia can result from eating acidic foods or drinking liquids that have been stored in galvanised containers.

It is clear that zinc is an essential element and has significant importance, both biologically and industrially. When the quantity is more than what is required, zinc produces toxic effects. Hence, separation and determination of zinc(II) from its associated metal ions is indispensable.

The review of literature indicates only a few phenylthiosemicarbazones have been exploited for the spectrophotometric determination of zinc(II). Not much attention has been paid for the extractive spectrophotometric determination of zinc(II) with phenylthiosemicarbazones. This has prompted the researcher to make a systematic investigation for utilizing pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) first time for the extractive spectrophotometric determination of zinc(II) in microgram quantities. Later, the established method is successfully applied for the determination of zinc(II) in pharmaceutical and biological samples. The proposed method when compared with other reported spectrophotometric methods⁵⁻¹¹ was found to be more sensitive and selective (Table 1). It also offers advantages like reliability and reproducibility in addition to its simplicity instant color development and less interference.

Experimental

Apparatus

A Shimadzu 240 UV-VIS spectrophotometer with 1.0 cm quartz cell was used for absorbance studies. An Elico LI-120 digital pH meter was used for pH adjustment. A Perkin-

Table 1. Comparison of present method with other reported spectrophotometric methods

Reagent	λ_{\max} (nm)	pH	Molar absorptivity ($L \text{ mol}^{-1} \text{ cm}^{-1}$) $\times 10^4$	Linear range (mg L^{-1})	M:L	Remarks	Reference
Benzildithiosemicarbazone	395	9.5	0.42	1.0-18.0	1:1	Cu(II), Ni(II), Co(II), Pb(II), Mn(II), Ag(I) are interferents	5
Glyoxaldithiosemicarbazone	433	9.0-11.0	1.3	N.R	1:1	Less sensitive	6
1,3-Cyclohexanedithiosemicarbazone	570	6.3	1.42	N.R	N.R	Less sensitive	7
Xylenol orange and cetlpyridium chloride	580	5.0-6.0	1.1	1.0-20.0	1:2:4	Less sensitive	8
Methylglyoxal bis(4-phenl-3-thiosemicarbazone)	445	6.0-8.5	0.21	0.2-0.4	1:1	Less sensitive	9
1,2-Cyclohexanedithiosemicarbazone	415	1.1-6.6	0.73	N.R	1:2	Hg(II), Cu(II), Cd(II), Fe(II), Ni(II), Co(II), V(V) are interferents	10
7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid	520	9.2	3.75	0.05-1.0	1:2	Cu(II), Ni(II), Co(II), Cd(II), Fe(III) and Fe(II) are interferents	11
Pyridoxal-4-phenyl-3-thiosemicarbazone	430	5.5	1.60	0.5-6.0	1:1	Highly sensitive and selective	P.M

M:L = Metal:Ligand; P.M= Present Method; N.R = Not Reported.

Elmer 2380 atomic absorption spectrometer was used for the comparison of results. The experimental conditions of AAS for determination of zinc was: wavelength 213.9 nm, current 30 mA, band width 0.7 nm and gas, air-acetylene.

Reagents

All reagents used were of analytical reagent grade unless otherwise stated. Pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) was prepared as per the procedure reported.¹² 1.0 g of pyridoxal hydrochloride was dissolved in 15 mL of demineralised double distilled water and mixed in a flask with 50 mL of ethanol containing 0.8 g of 4-phenyl-3-thiosemicarbazide. The resulting solution was neutralized with sodium acetate and refluxed under heating for 30 min. It was allowed to stand at room temperature until yellow crystals were formed. These were separated and recrystallized from ethanol (Scheme 1).

A sample of 2.0847 g of zinc chloride was taken in a litre standard flask. This was then dissolved and made up to 1 L with double distilled water. The exact content of zinc was determined, gravimetrically by 8-hydroxyquinoline.¹³ The working solutions were obtained by diluting the stock solution to the requisite concentrations with double distilled water.

1.0 mol L⁻¹ sodium acetate and 1.0 mol L⁻¹ acetic acid solutions were prepared in double distilled water. Suitable portions of these solutions were mixed to get the desired pH.

General procedure

To an aliquot of a working standard solution containing 12.5 - 150 µg zinc(II), were added pH 5.5 buffer (3 mL), 0.5% reagent solution (2 mL) and a salting-out agent, 0.1 mol L⁻¹ magnesium sulphate (1 mL). The mixture was shaken two times with 10 mL portions of *n*-butanol each time for 1 min and allowed to stand for a few minutes. The two organic phases were collected into a 25 mL volumetric flask and made up

to the mark with *n*-butanol. The absorbances of all the organic phases were measured at 430 nm against the reagent blank.

Analytical procedures for various samples

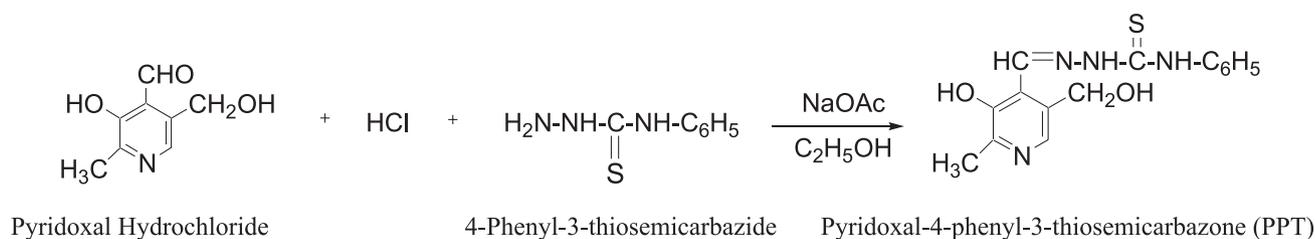
Pharmaceutical samples. The samples were treated separately with concentrated nitric acid on a hot-plate, at a low temperature, to avoid violent spurting. The temperature of the hot-plate was increased to 300 °C. The residue obtained was dissolved in nitric acid (1:1) and then slowly heated 2 h to produce a dry mass. Finally the residue was dissolved in a minimum amount of double distilled water. The same solution was quantitatively transferred into a 50 mL volumetric flask and then made up to the mark with double distilled water.

Biological samples. Ten grams of the powdered leafy/chilli or 10 mL of milk sample was taken in a silica crucible, heated to oxidise organic matter, and ashed at 550 °C, in a muffle furnace for 4-5 h. The ash was then dissolved by heating with 10 mL of 2 mol L⁻¹ hydrochloric acid, filtered through an acid, washed filter paper (Whatmann No. 41) and then washed with hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally, made up to the mark with double distilled water.

Certified Reference Materials. About 0.1 g of each sample was dissolved in 10 mL of aqua-regia. They were heated to near dryness and the nitrate was expelled from the residue, using 5 mL of concentrated hydrochloric acid. Each residue was extracted into double distilled water separately and made up to 50 mL in volumetric flasks. The concentration of zinc was determined by following the procedure described in general procedure.

Results and Discussion

Zinc(II) reacts with pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) and forms a yellow colored complex



Scheme 1.

which is extracted into *n*-butanol from sodium acetate-acetic acid buffer of pH 5.5. The complex has a maximum absorbance at 430 nm. The extraction of the complex into the solvent is instantaneous and is stable for more than 48 h. Hence, a detailed study of the extraction of zinc(II) with PPT has been undertaken with a view to develop a rapid and sensitive extractive spectrophotometric method for the determination of zinc(II), when present alone or in presence of diverse ions, which are usually associated with zinc(II) in pharmaceutical and biological samples.

Absorption spectra of the reagent and Zn(II)-PPT complex

1.0 mL of zinc(II) solution containing about 87.5 μg of metal ion ($2.68 \times 10^{-3} \text{ mol L}^{-1}$) was transferred into a 25 mL standard flask and to it, 3.0 mL of buffer (pH 5.5), 2.0 mL of $7.65 \times 10^{-3} \text{ mol L}^{-1}$ PPT solutions were added and the volume of the aqueous phase was brought up to 10.0 mL with double distilled water. The solution was shaken with 10.0 mL of *n*-butanol for 2 min and then allowed to form two separate layers. The organic phase was collected in a 25 mL standard flask and made up to the mark with fresh *n*-butanol. The organic solution was transferred into the quartz cell of the spectrophotometer and the spectrum was recorded, using the reagent solution as a blank, which was prepared by using the same solution without zinc(II). Similarly, the absorption spectrum of the reagent was also recorded, using the solvent as a blank. The absorption spectra of both the reagent and the complex are depicted in Figure 1.

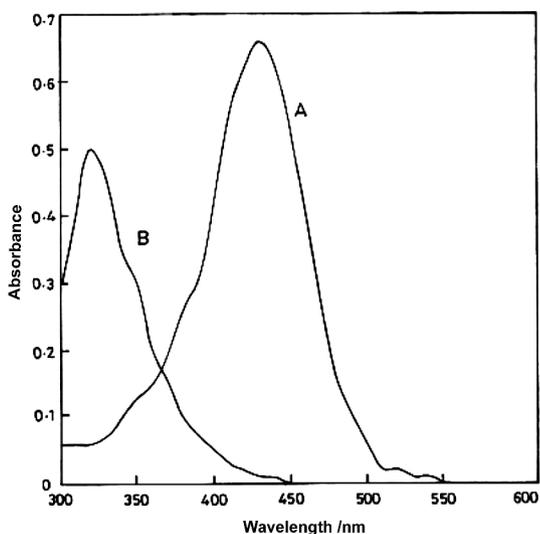


Figure 1. (A) Absorption spectra of Zn(II)-PPT complex vs PPT blank, (B) Absorption spectra of PPT vs *n*-butanol blank. Zn(II): 1 mL of $2.7 \times 10^{-3} \text{ mol L}^{-1}$; pH: 3.0 mL of 5.5; PPT: 2.0 mL of $7.65 \times 10^{-3} \text{ mol L}^{-1}$.

From the two spectra it is clear, that the Zn(II)-PPT complex and the reagent have maximum absorbances, at 430 nm and 320 nm, respectively. The reagent has a minimum absorbance at the maximum absorbance of the complex and does not interfere in the determination of zinc(II). Hence, all further absorbance measurements of the complex are made at 430 nm.

Effect of pH on the extraction of Zn(II)-PPT complex

The effect of pH on the formation of Zn(II)-PPT complex was studied to find out the optimum pH for zinc(II) determination. The pH studies were carried out using hydrochloric acid-potassium chloride (pH 1.0-2.6), sodium acetate-acetic acid (pH 3.4-6.5) and ammonium chloride-ammonium hydroxide (pH 7.0 - 11.0) buffers.

The studies were carried out keeping the 1.0 mL of $1.34 \times 10^{-3} \text{ mol L}^{-1}$ zinc(II) solution and 2.0 mL of $3.35 \times 10^{-3} \text{ mol L}^{-1}$ PPT solution constant and varying the pH values of aqueous phases from 1.0 to 7.5 using suitable buffer solutions. The volume of each aqueous phase was adjusted to 10.0 mL with double distilled water. Each of the solution was shaken with 10.0 mL of *n*-butanol separately, for 2 min. The organic phase was collected in a 5 mL standard flask and made up to the mark with fresh *n*-butanol. The optical densities of the complex in the organic layers collected were measured at 430 nm, using their corresponding reagent blanks. The plot between pH and its absorbance is shown in Figure 2. From the graph, it is observed that the extraction of the metal ion into the organic phase increases with increase in pH from 1.0 to 5.0. There is no noticeable difference in the absorbance values between pH 5.0 to 6.0. Hence, sodium acetate-

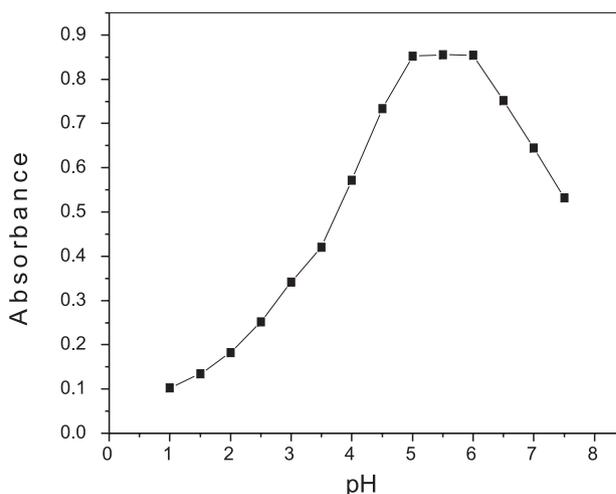


Figure 2. Effect of pH on Zn(II)-PPT complex. Zn(II): 1 mL of $1.34 \times 10^{-3} \text{ mol L}^{-1}$; PPT: 2.0 mL of $3.35 \times 10^{-3} \text{ mol L}^{-1}$; λ_{max} : 430 nm.

acetic acid buffer is used for further studies, keeping 5.5 as the optimum pH.

Effect of solvents on the extraction of Zn(II)-PPT complex

The *n*-amyl alcohol, isoamylalcohol, *n*-butanol, benzene, carbon tetrachloride, chloroform, chlorobenzene, cyclohexane, methylisobutylketone, nitrobenzene and propyl acetate solvents were employed to study the extraction of Zn(II)-PPT complex. Of the various solvents studied, *n*-butanol was found to extract the complex effectively. The physical properties of solvents such as polarity, polarisability, molar volume and also the chemical properties such as acid-base strength and hydrogen bonding ability are responsible for the behavior of a solvent *i.e.*, they influence the interactions with other organic liquids with non-polar solutes with water and electrolytes. The above solvents, which were used in present investigation shows poor extraction compared to *n*-butanol may be due to physical and chemical properties of solvents. Hence, *n*-butanol is chosen for all further investigations.

Effect of reagent concentration on the absorbance of Zn(II)-PPT complex

The effect of the reagent concentration has been studied by using different solutions containing 1.0 mL of 1.34×10^{-3} mol L⁻¹ (87.5 µg) zinc(II) solution and 3.0 mL of pH 5.5 buffer solution. To these solutions, 1.0 mL of the reagent solution containing varying concentrations from 1.34×10^{-3} mol L⁻¹ to 13.4×10^{-3} mol L⁻¹ was added to get maximum color formation. The total volume of the aqueous phases were brought to 10.0 mL with double distilled water. The complex solutions were extracted into 10.0 mL of *n*-butanol, in each case and the organic phases were collected in 25 mL standard flasks. The volume of the organic phases were made up to the mark of the flasks with *n*-butanol and their absorbances were measured at 430 nm, against their corresponding reagent blanks. The above data reveal that a five-fold molar excess of the reagent to that of metal ion.

Effect of salting-out agents on the extraction of Zn(II)-PPT complex

Various salting-out agents such as lithium nitrate, lithium sulphate, ammonium sulphate, magnesium sulphate and ammonium chloride were used to enhance the metal complex extraction into organic phase, in a single step. It was noticed that the presence of 1.0 mL

of 0.1 mol L⁻¹ magnesium sulphate facilitated a maximum absorbance. Hence, with 1.0 mL of 0.1 mol L⁻¹ magnesium sulphate solution as a salting-out agent and five-fold molar excess of the reagent, zinc(II) is quantitatively extracted into the organic phase. The aqueous phase is tested for zinc(II) with 8-hydroxyquinolinate method, which discloses its complete extraction into the organic phase.

Adherence of the Zn(II)-PPT complex system to Beer's law

Known aliquots of various solutions (10.0 mL), each containing constant volumes of 3.0 mL of sodium acetate-acetic acid buffer (pH 5.5), 2.0 mL of 6.6912×10^{-3} mol L⁻¹ reagent, 1.0 mL of 0.1 mol L⁻¹ magnesium sulphate and varying volumes of zinc(II) (12.5-175.0 µg) were prepared. Each solution was shaken with two 10.0 mL portions of *n*-butanol and the organic phases were taken in a 25 mL standard flask. The solution was made up to the mark with *n*-butanol. The absorbances of all the organic phases were measured at 430 nm. A graph plotted between the amount of Zn(II) and its absorbance is shown in Figure 3. It is observed from the graph, that a linear plot passing through the origin obeys the Beer's law in the range 0.5-6.0 mg L⁻¹ of zinc(II).

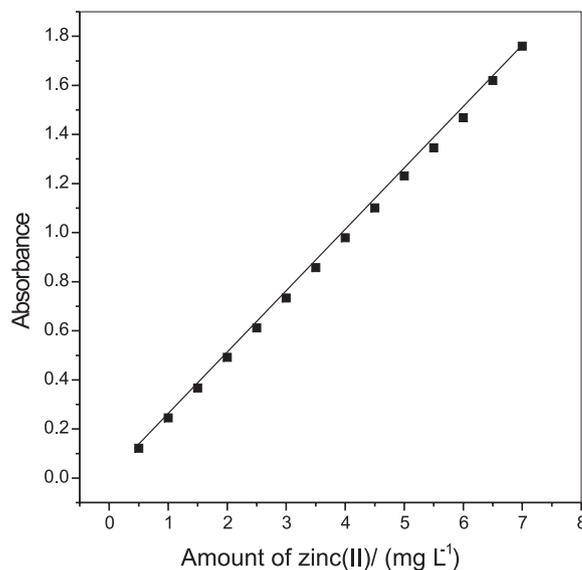


Figure 3. Applicability of Beer's law on Zn(II)-PPT complex. Zn(II): 12.5 – 175 µg; PPT: 2.0 mL of 6.7×10^{-3} mol L⁻¹; pH: 5.5; λ_{max} : 430 nm.

Sensitivity, molar absorptivity and correlation coefficient of the Zn(II)-PPT complex

The molar absorptivity of the complex is calculated and noted as 1.6×10^4 L mol⁻¹ cm⁻¹ and the Sandell's sensitivity of the complex is 4.085×10^{-3} µg cm⁻². The regression line

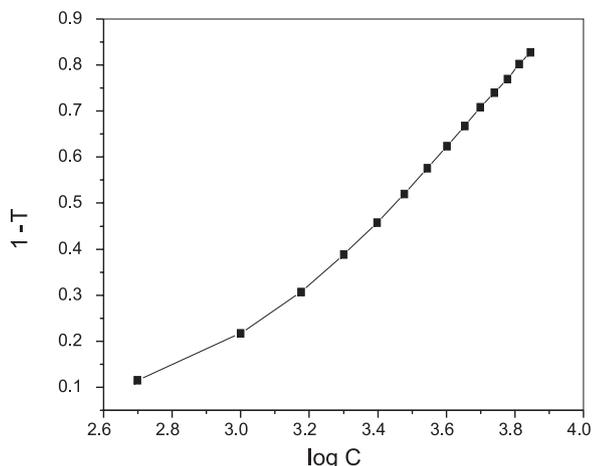


Figure 4. Ringbom plot of Zn(II)-PPT complex. Zn(II): 500-7000 $\mu\text{g L}^{-1}$; PPT: 2.0 mL of $6.7 \times 10^{-3} \text{ mol L}^{-1}$; pH: 5.5; λ_{max} : 430 nm.

is given by Absorbance = 0.24497 X + 0.00005. The correlation coefficient value of Zn(II)-PPT system, with independent variable as concentration in $\mu\text{g mL}^{-1}$ and dependent variable as absorbance, was found to be 0.999. This indicates excellent linearity between the two variables.

Ringbom plot for Zn(II)-PPT complex

Ringbom plot was drawn between log C of Zn(II) and (1-T), where 'T' is transmittance. The plot has a sigmoid shape with a linear segment, at intermediate absorbance values 0.26-1.23 and concentration values 1.5-5.0 mg L^{-1} of zinc(II). The slope of the Ringbom plot from Figure 4 is 0.768. Based on this value, the ratio between the relative error in concentration and photometric error is 2.646. For a photometric error of one percent, $\Delta P=0.01$. Hence, the relative error in concentration is 0.02646.

Precision, accuracy and detection limit of the method

To assess the precision and accuracy of the method, determinations were carried out with different concentrations of zinc(II), under optimum conditions. The standard deviation is found to be not more than 0.007 and the relative standard deviation is less than 3.1%. It is evident from these results that, the method is precise, besides being accurate. The detection limit, C_{min} , was determined as the amount of zinc(II) corresponding to three times the standard deviation of the blank values and a value of $0.04 \mu\text{g mL}^{-1}$ was obtained.

Calculation of instability constant of Zn(II)-PPT complex

The instability constant of the complex was calculated by using Edmonds and Birnbaum's Method.¹⁴ The

absorbance values of the extracts obtained after shaking the solutions containing fixed volumes of zinc(II) (1.0 mL of $1.34 \times 10^{-3} \text{ mol L}^{-1}$), buffer (pH 5.5) and 0.1 mol L^{-1} magnesium sulphate with different known volumes of $1.34 \times 10^{-3} \text{ mol L}^{-1}$ PPT with *n*-butanol were recorded at 430 nm. The average value is noted as $2.3911 \times 10^{-3} \text{ mol L}^{-1}$ at room temperature.

Determination of the composition of Zn(II)-PPT complex

Spectrophotometric investigation of the metal complex was made to obtain the composition of the complex. Job's method of continuous variation, molar ratio, Asmus' and slope ratio methods were employed to elucidate the composition of the complex.

Job's method of continuous variation. Equimolar solutions of zinc(II) and the PPT ($4.015 \times 10^{-3} \text{ mol L}^{-1}$) were prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume constant at 12.0 mL. To each solutions, 3.0 mL of sodium acetate-acetic acid buffer (pH =5.5) solution and 1.0 mL of 0.1 mol L^{-1} magnesium sulphate solution as salting-out reagent were added and the volume of the aqueous phase brought to 10.0 mL of *n*-butanol individually and the organic phases were collected in 25 mL standard flasks. They were made up to the mark with *n*-butanol. The absorbances of these organic phases were recorded at 430 nm, against their corresponding reagent blanks. The plot corresponding to its absorbance versus mole fraction of the metal ion is shown in Figure 5. From the Figure 5, it is observed that one mole of zinc(II) reacts with one mole of the reagent showing the composition of the complex.

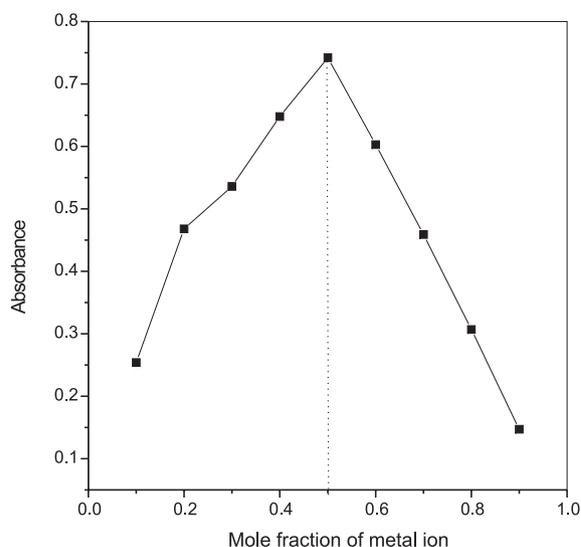


Figure 5. Job's method of continuous variation of Zn(II)-PPT complex. Zn(II) or PPT: $4.05 \times 10^{-3} \text{ mol L}^{-1}$; pH: 5.5; λ_{max} : 430 nm.

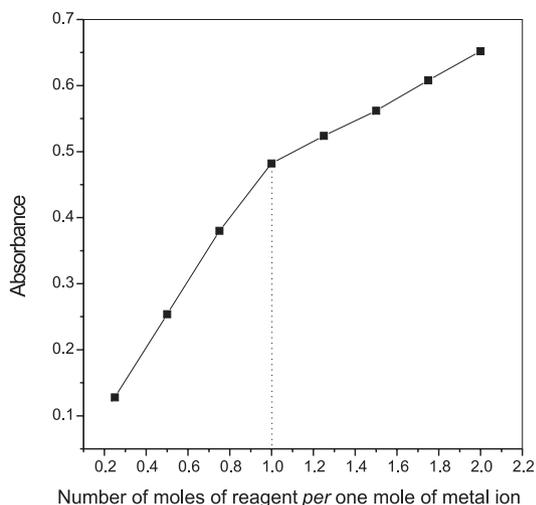


Figure 6. Molar ratio method of Zn(II)-PPT complex. Zn(II): 1.0 mL of 1.34×10^{-3} mol L⁻¹; PPT: 1.0 mL of $0.335 - 2.677 \times 10^{-3}$ mol L⁻¹; pH: 5.5; λ_{\max} : 430 nm.

Molar ratio method. The amount of metal ion (1.0 mL of 1.34×10^{-3} mol L⁻¹) and volume of buffer taken into the separatory funnel were maintained constant and the reagent concentration varied ($0.335 - 2.676 \times 10^{-3}$ mol L⁻¹), with a proportional increase from 0.25 to 2.0 ratio of PPT to that of the metal ion. The complex present in the solutions was extracted with 10.0 mL of *n*-butanol and the extracts were collected in 25 mL standard flask. These organic solutions were made up to mark with fresh *n*-butanol. The absorbances of these organic phases were recorded at 430 nm, against their corresponding reagent blanks and a plot is drawn between the absorbance with mole proportion of the metal ion (Figure 6). From the graph, it is observed that one mole of the reagent and one mole of the metal ion participate in the complex formation, which is in good agreement with the results of Job's method of continuous variation.

Asmus' method. In Asmus' method, the data obtained from the molar ratio method was used. $1/m$ values, where m = extinction modulus, were calculated by dividing the optical density with the cell width, along with $1/V$, $1/V^2$ and $1/V^3$ values and the plots between $1/m$ and $1/V$, $1/V^2$, $1/V^3$, (Figure 7) shows linear plot between $1/m$ and $1/V$ only, indicating the composition of the complex as 1:1(M:L).

Slope ratio method. Two series of mixtures were prepared using 2.67×10^{-3} mol L⁻¹ solutions of the reagent and zinc(II): (i) Excess of metal ion. In one series, the content of zinc(II) (1.0 mL of 2.67×10^{-3} mol L⁻¹) was kept constant and to them varying volumes (0.1 - 1.0 mL of 2.67×10^{-3} mol L⁻¹) of the reagent solutions were added.

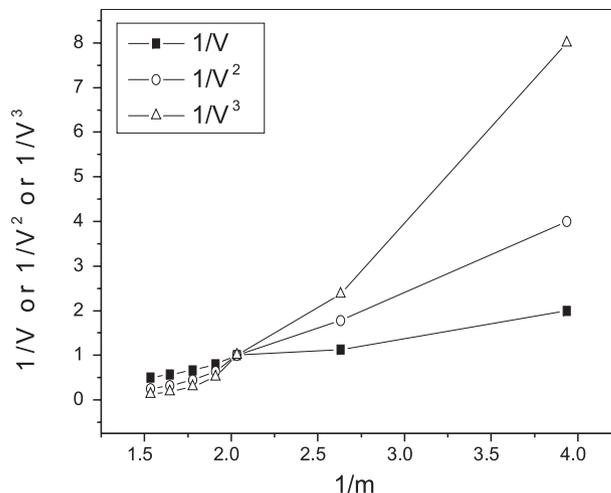


Figure 7. Asmus' method of Zn(II)-PPT complex. Zn(II): 1.0 mL of 1.34×10^{-3} mol L⁻¹; PPT: 0.5-2.0 mL of 1.34×10^{-3} mol L⁻¹; pH: 5.5; λ_{\max} : 430 nm.

To each solution, 3.0 mL of pH 5.5 buffer and 1.0 mL of 0.1 mol L⁻¹ magnesium sulphate solutions were added. These solutions were brought to 10.0 mL with double distilled water. The aqueous phases were individually shaken with 10.0 mL of *n*-butanol for 2 min. The organic phases were then collected in 25 mL standard flasks and made up to the mark with *n*-butanol. The absorbance values of these were noted at 430 nm, against their corresponding reagent blanks and graphically represented in Figure 8. (ii) Excess of reagent. In another series, the concentration of the reagent was kept constant (1.0 mL of 2.67×10^{-3} mol L⁻¹) and to them varying volumes (0.1-1.0 mL of 2.67×10^{-3} mol L⁻¹) of zinc(II) solutions were added and the rest of the procedure is the

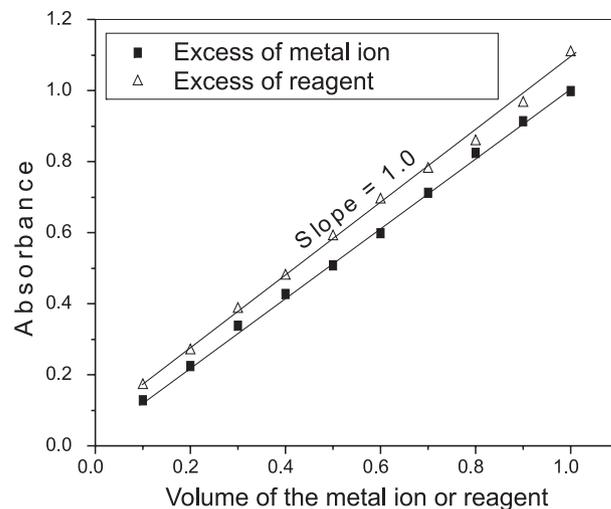


Figure 8. Slope ratio method of Zn(II)-PPT complex. Excess of reagent: Zn(II): 0.1-1.0 mL of 2.67×10^{-3} mol L⁻¹; PPT: 1.0 mL of 2.67×10^{-3} mol L⁻¹; pH: 5.5; λ_{\max} : 430 nm; Excess of metal ion: Zn(II): 1.0 mL of 2.67×10^{-3} mol L⁻¹; PPT: 0.1-1.0 mL of 2.67×10^{-3} mol L⁻¹; pH: 5.5; λ_{\max} : 430 nm.

same as described in excess of metal ion. The absorbance values of the organic phases were recorded at 430 nm, against their corresponding reagent blanks and graphically represented in Figure 8. The above two plots indicate the formation of 1:1 complex, metal and ligand, under experimental conditions.

From all the four methods mentioned above, it is evident that the composition of zinc(II) and PPT in complex is 1:1 (M:L) .

Effect of foreign ions on the extraction of Zn(II)-PPT complex

In order to assess the possible analytical applications of this color reaction, the effect of some foreign ions was examined, by carrying out determinations of 87.5 μg of zinc(II) with a known amount of foreign ion in question, using the recommended general procedure. The criterion for interference is an absorbance varying more than $\pm 2\%$ from the expected value for zinc(II) alone.

The results indicated that Al(III), Mn(II), Mo(VI), Mg(II), Pb(II), V(V) and W(VI) can be tolerated up to 5000 μg . Bi(II), Sb(II), Ca(II), Cr(III), Hg(II) and Ag(I) when present up to 2500 μg can be tolerated. Ni(II), Co(II), Cu(II), Fe(II), Fe(III), Zn(II), Cd(II) and Pd(II) interfere severely, even when present in trace amounts.

Anions like tartrate, oxalate, fluoride and thiocyanate do not interfere in the determination even when present up to 5000 μg or more. Chloride, bromide, citrate, thiosulphate, iodide, nitrate and sulphate do not have any effect on the extraction of zinc(II), even when present up

to 2500 μg . Thiourea, thiosulphate, phosphate and EDTA interfere severely during the extraction of zinc (II).

The interference of Co(II), Cu(II) and Pd(II) was suppressed by using 1.0 mL of 0.3% (m/v) oxalate solution as a masking agent. The interference of Fe(II) and Fe(III) was avoided by masking them with 1.0 mL of 0.5% (m/v) sodium fluoride solution and interference due to the presence of Ni(II) can be avoided by using 1.0 mL of 0.5% (m/v) thiocyanate, masking agents.

Applications of the developed method

The proposed method was applied for the determination of zinc(II) in pharmaceutical, biological samples and Certified Reference Materials.

Determination of zinc(II) in pharmaceutical samples.

Pharmaceutical samples like antoxid, becozinc, magnical, polyzee, ridage and maxamin forte were analysed for zinc(II). The results are presented in Table 2.

Determination of zinc(II) in biological samples.

Biological samples like leafy, chilli and milk samples were analysed for zinc(II) using the proposed method. The leaves of sago palm (*Metroxylon sago Rottb.*) and oil palm (*Elaeis guineensis Jacq.*) plants, the chilli samples are collected in and around Tirupati, A.P., India and the milk samples of different animal origin was collected. The content of the zinc(II) present in the organic solution was determined by using a calibrated plot and the results obtained were confirmed by direct flame atomic absorption spectrometry (Table 3).

Table 2. Determination of zinc(II) in pharmaceutical samples

Name of the sample	Composition, Certified value mg/tablet	Amount of Zn(II) ^a found (mg/tablet)		Present method	
		FAAS method	Present method	S.D	R.S.D (%)
Antoxid	Zinc sulphate monohydrate, 27.45 mg (equivalent to elemental zinc, 9.99 mg); Selenium dioxide, 70 μg .	9.99	9.98	0.1020	1.02
Becozinc	Zinc sulphate monohydrate, 54.93 mg (equivalent to elemental zinc, 19.99 mg); Niacinamide, 50 mg; Calcium pantothenate, 12.5 mg; Folic acid, 1 mg.	19.99	19.97	0.2800	1.40
Magnical	Calcium carbonate, 500 mg; Dicalcium phosphate dehydrate, 100 mg; Magnesium hydroxide, 90 mg; Zinc (as zinc sulphate) 4 mg.	4.00	3.98	0.0360	0.90
Polzee	Vit.B-1, 10 mg; B-6, 3 mg; Nicotinamide, 50 mg; Calcium penta	22.50	22.49	0.3320	1.47
Ridage	Beta-carotene(7.5%) 133.34 mcg, Vit.A, 5000 IU, C 150 mg; E IU 25 mg, Zinc sulphate monohydrate, 27.45 mg (equivalent to elemental zinc, 9.99 mg);	9.99	9.97	0.1010	1.01
Maxamin forte	Folic acid, 1.5 mg; Dried ferrous sulphate ^b , 6.32 mg; Manganese sulphate, 4.06 mg; Copper sulphate ^c , 3.93 mg; Zinc sulphate monohydrate, 50 mg (equivalent to elemental zinc, 18.2 mg);	18.20	18.18	0.2420	1.33

^aAverage of four determinations; ^bMasked with fluoride; ^cMasked with oxalate.

Table 3. Determination of zinc(II) in biological samples

Biological samples	Amount of Zn(II) ^a found		Present method	
	FAAS method	Present method	S.D	R.S.D (%)
<i>Leafy samples^b</i>				
<i>Name of the sample</i>				
Sago palm leaves (<i>Metroxylonsagu Rotb.</i>)	28.2	28.1	0.2200	0.78
Oil palm leaves (<i>Elaeis guineensis Jacq.</i>)	46.7	46.6	0.1800	0.39
<i>Chilli samples^b</i>				
<i>Sample location</i>				
Rangampet	20.4	20.2	0.0920	0.45
Ithepalli	19.5	19.0	0.1980	1.04
Chandragiri	19.0	18.8	0.1230	0.65
Muthyalareddypalli	30.0	29.6	0.1623	0.54
Alipiri	17.5	17.1	0.0986	0.57
Tiruchanoor	26.0	25.3	0.0985	0.38
Pudi	27.0	27.0	0.1256	0.46
Renigunta	22.0	21.8	0.1458	0.66
Akkarampalli	20.0	19.3	0.1658	0.85
Gajulamandyam	22.0	21.2	0.1354	0.63
<i>Milk samples^c</i>				
<i>Animal origin</i>				
Buffalo	3.00	2.96	0.0495	1.67
Cow	3.90	3.86	0.0385	0.99
Goat	4.20	4.16	0.0256	0.62
Sheep	2.40	2.38	0.0223	0.94
Dairy	3.70	3.56	0.0359	1.00

^aAverage of four determinations; ^bConcentration in $\mu\text{g g}^{-1}$; ^cConcentration in $\mu\text{g mL}^{-1}$.

Table 4. Determination of zinc(II) in Certified Reference Materials

Certified Reference Material	Zinc ^a (mg kg ⁻¹)		Present method	
	Certified value	Present method	S.D	R.S.D (%)
Tomato leaves (NIST 1573)	62.0	61.55	0.2980	0.48
Citrus leaves (NBS 1572)	29.0	28.42	0.1980	0.69
Whole milk powder (NIST SRM 8435)	28.8	27.90	0.0985	0.35

^aAverage of four determinations.

Determination of zinc(II) in certified reference materials.

The present method is applied for the determination of zinc(II) in certified reference materials such as Tomato leaves (NIST 1573), Citrus leaves (NBS 1572) and Whole milk powder (NIST SRM 8435) (Table 4).

Conclusions

A thorough literature survey revealed that many thiosemicarbazones were used for the determination of zinc(II). Studies upon the use of pyridoxal-4-phenyl-3-thiosemicarbazone (PPT) as an analytical reagent are limited. Hence, the present investigations were carried out with a view to test the potentiality of PPT as a complexing agent for Zn(II) and its subsequent determination by extractive spectrophotometry. The method has good sensitivity, compared with other existing extractive spectrophotometric determination methods. The selectivity of this method is enhanced by using masking agents for Co(II), Ni(II), Cu(II), Cd(II), Pd(II) and Fe(III). Finally, the developed method can

be conclusively declared apt for the determination of Zn(II) in pharmaceutical and biological samples.

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