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Simple and sensitive spectrophotometric determination of Zn(II) in Biological and Pharmaceutical samples with 5-methylfuran-2-carbaxaldehyde thiosemicarbazone(5-MFAT)

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method was developed for the determination of Zn(II) using 5-methylfuran-2-carbaxaldehyde thiosemicarbazone (5-MFAT) as an analytical reagent. The metal ion in aqueous medium forms yellow colored complex with BPT at pH 6.0 showing maximum absorbance at 430 nm. At 430 nm the reagent itself does not show considerable absorbance. Hence, analytical studies were further carried out at 430 nm. Beer's law was obeyed in the range of 0.26-2.61 µg/ml of Zn(II). The molar absorptivity and sandall's sensitivity of the method were 2.5 x 10^4 L mol^{-1} cm^{-1} and 0.0035 µg/cm^2 respectively. The detection limit of the method was achieved to be 0.064 µg/ml. The percentage of relative standard deviation was calculated to be less than 2% by replicate determinations of different concentrations. The composition of the Zn(II) complex with 5-MFAT was studied by the method of Job's continuous variation, and molar ratio method. It has been satisfactorily applied for the determination of Zn(II), when present alone or in presence of diverse ions. The proposed methods were applied for the determination of Zn(II) in real samples such as 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 spectrophotometry.

Keywords: Flame atomic absorption spectrophotometry, 5-methylfuran-2-carbaxaldehyde, thiosemicarbazone Determination of Zn(II)**,** Pharmaceutical samples, and Biological samples.

INTRODUCTION

Zinc an essential element for all animals including human beings plays an important physiological role in human blood 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 reminder 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 such as with the synthesis of deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid (RRNA). 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. 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 may include abdominal pain, fever and also severe anemia resulting from eating acidic foods or drinking liquids that have been stored in galvanized containers. It is clear that zinc is an essential element and has significant importance, both biologically and industrially.

The review of literature indicates only a few thiosemicarbazones[5-7] have been exploited for the spectrophotometric determinations of zinc(II). Not much attention has been paid for the spectrophotometric determination of Zn(II) with thiosemicarbazones. This has prompted the researcher to make a systematic investigation for utilizing benzoylpyridine thiosemicarbazone (BPT) first time for the spectrophotometric determination of zinc(II) in microgram quantities. Later, the established method was successfully applied for the determination of zinc(II) in pharmaceutical and biological samples. The proposed method when compared with other reported spectrophotometric method [8-14] was found to be more sensitive and selective Table (1). It also offers advantages such reliability and reproducibility in addition to its simplicity instant color development and less interference.

MATERIALS AND METHODS

Experimental

A Perkin-Elmer (lamda-25) UV-VIS spectrophotometer with 1.0 cm quartz cell was used for the absorbance studies. An Elico LI-120 digital pH meter was used for pH adjustment. A Perkin-Elmer 2380 atomic absorption spectrophotometer was used for the comparison of results. The experimental condition of AAS for determinations of zinc was: wavelength 213.9 nm, current 30 mA, band width 0.7 nm and gas, air-acetylene.

REAGENTS

Preparation of 5-methylfuran-2-carbaxaldehyde thiosemicarbazone (5-MFAT)

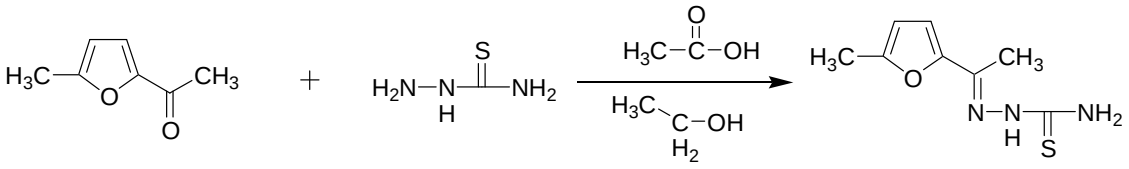
All reagents used were of analytical reagent grade unless otherwise started. 5-methylfuran-2-carbaxaldehyde thiosemicarbazone (5-MFAT) was prepared as per the procedure reported.[15] 5 ml of ethanolic solution containing 5-methylfuran-2-carbaxaldehyde (2g, 0.008 mol) and thiosemicarbazide (0.713 g, 0.008 mol) dissolved in 10 ml of hot water were taken in 250-ml round bottom flask. Suitable quantity (~ 2ml) of glacial acetic acid was added to the reaction mixture and refluxed for 3 hours. On cooling the reaction mixture, light yellow colored product

was separated out. It was collected by filtration and washed several times with hot water and 50 cold methanol. This compound was recrystallised from ethanol and dried in *vacuum.* Percentage of Yield is 91; m.p. 165-167 ^{0}C.

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

Reagent	λmax (nm)	pH	Molar absorptivity (∈*) (L mol^{-1} cm^{-1})	Liner range (µg/ml)	M; L	Ref
Benzildithiosemicarbazone	395	9.5	0.42	1.0-18.0	1:1	8
Glyoxaldithiosemicarbazone	433	9.0-11.0	1.3	N.R	1:1	9
1,3-cyclohexanedionedithio semicarbazones	570	6.3	1.42	N.R	N.R	10
Xylenol orange and cetlpyridine chloride	580	5.0-6.0	1.1	1.0-20.0	1: 2: 4	11
Methylglyoxal bis(4-phenyl-3-thiosemicarbazone)	445	6.0-8.5	0.21	0.2-0.4	1:1	12
1,2- cyclohexanedionedithio semicarbazone	415	1.1-6.6	0.73	N.R	1:2	13
7-(4-naitrophenylazo)-8-hydroxy quinoline-5-Sulphonic acid	520	9.2	3.75	0.05-1.0	1:2	14
5-methylfuran-2-carboxaldehyde thiosemicarbazone	430	5.0-7.0	2.5	0.26-2.6	1:1	P.M

M; L Metal: Ligand; N.R Not Reported; P. M Present method. ***∈*:*** *10^4 L mol^{-1} cm^{-1}*



5-methylfuran-2-carbaxaldehyde Thiosemicarbazide 5-methylfuran-2-carbaxaldehyde thiosemicarbazone

A sample of 2.0847 g of zinc chloride was taken in a liter standard flask. This was then dissolved and made up to 1 Liter with double distilled water. The exact content of zinc was determined, gravimetrically by 8-hydroxy quinoline.[16] the working solutions were obtained by diluting the stock solution to the requisite concentrations with double distilled water.

Buffer solutions

The buffer solutions were prepared by mixing 1M hydrochloric acid and 1M sodium acetate (pH 1.0-3.0) and 0.2M acetic acid and 0.2 M sodium acetate (pH 3.5-7.0). The pH of these solutions was prepared in doubly distilled water. Suitable portions of these solutions were mixed to get the desired pH.

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 300^0C. The residue obtained was dissolved in nitric acid (1:1) and then slowly heated 2 hours to produce dry mass. Finally the residue obtained was dissolved in a minimum amount of double distilled water. The same solutions were quantitatively transferred into 50 ml volumetric flasks and than made up to the mark with double distilled water.

Biological samples

10 grams of the powdered palm leaves, chilli samples or 10 ml of milk sample was taken in a silica crucible, heated to oxidize 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 (whatman No-41) and than 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 collected into double distilled water separately and made up to 50 ml in volumetric flasks.

General procedure

Direct spectrophotometry

In each set of different 25 ml of volumetric flasks, 10 ml of buffer solution (pH 6.0), 1 ml of 5-MFAT (1 x 10^{-2}M) and various volumes of 1 x 10^{-4} M zinc(II) solution were made up to the mark with double distilled water. The absorbance was measured at 430 nm against the reagent blank. The calibration plot was prepared by plotting the absorbance against the amount of zinc(II).

RESULTS AND DISCUTION

Zinc(II) reacts with 5-methylfuran-2-carbaxaldehyde thiosemicarbazone (5-MFAT) and forms yellow colored complex in acidic medium at pH = 6.0.

Absorption spectra of the reagent and Zn(II)-5-MFAT complex

The absorption spectra of the solution containing zinc(II) complex against the reagent blank and that of the reagent solution against the corresponding buffer blank were recorded in the wavelength region 300-500 nm. Typical spectra are presented in the (Fig 1) showing absorption maximum of Zn(II) complex at 430 nm. Whereas the reagent itself does not show any considerable absorbance at this wavelength. Hence, 430 nm was chosen for further studies.

The study of the effect of pH on the color intensity of the reaction mixture showed that the maximum color was obtained in the wide range of pH 5.0-7.0. Analytical studies were therefore, carried out at pH 6.0. A 10 fold molar excess of 5-MFAT was necessary for complex and

constant color development. Excess of the reagent has no effect on the absorbance of the complex. The absorbance of the complex solution was found independent of the order of the addition of the reactants. The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex it was observed that the color development is instantaneous and remains constant for more than 71 hours.

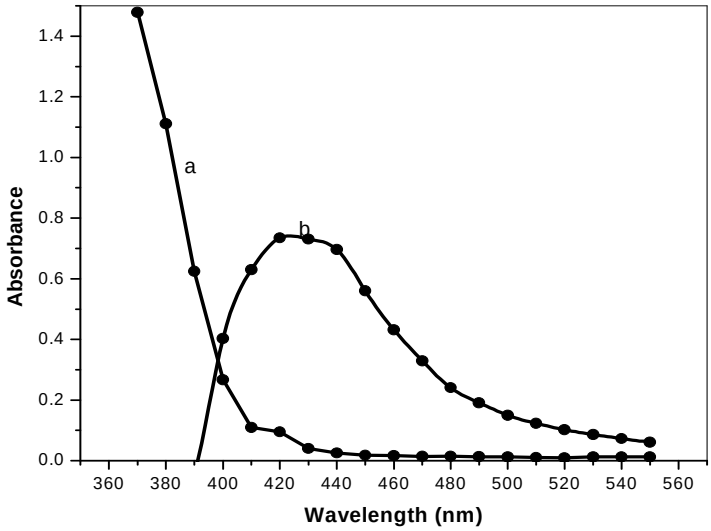


Fig. 1. Absorption spectra of (a) 5-MFAT Vs Buffer blank, (b) [Zn(II)-5-MFAT] Vs reagent Blank [Zn(II)] = 4 x 10^{-5} M; [5-MFAT] = 4 x 10^{-4} M; pH = 6.0.

For the possible determination of Zn(II) at micro levels, the absorbance of the solution containing different amounts of the metal ion was measured. The liner plot between the absorbance and the amount of zinc(II) ion is drawn and the straight line obeyed the equation A_{430} = 0.2779 C - 0.0116. Further Beer's law was obeyed in the range of 0.26-2.61 µg/ml. The molar absorptivity and sandall's sensitivity were 2.5 x 10^{4} L mol^{-1} cm^{-1} and 0.0035 µg/cm^{2} respectively. The standard deviation of the method for ten determinations of 1.18 µg/ml is ±0.0069.

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 2%. It representing the accuracy of the method. The lower detection limit of Zn(II) was found to be 0.064 µg/ml.

Effect of foreign ions

The effect of various foreign ions that are generally associated with zinc(II) on its determination under optimum conditions developed was studied and the results are presented in the Table 2.Among the various ions studied, all the anions and the cations Pb(II), Cd(II), Te(IV), U(VI), Na(I), K(I), Li(I), Th(IV), W(VI), Ce(VI), Ti(IV), Al(III) do not interfere even when present in more than 100 fold excess. Cr(III), Zr(IV), Mn(II), Ru(III), Pd(II), Mo(VI) anf Pt(IV) are tolerable when present between 50 fold excess. Cu(II) and Fe(II) interfere when present more

than 10 fold excess, Ni(II) and V(V) interfere when present is more than 5 fold excess. However in the presence of 1860 µg/ml of EDTA, Cu(II) and Ni(II) do not interfere even in 100-fold excess. Fe(II) is tolerable up to 90-fold excess in presence 1270 µg/ml of iodide. In presence of 950 µg/ml of phosphate, V(V) is tolerable up to 100 fold excess.

Table. (2). Tolerance limit of foreign ions amount of Zn(II) in 1.3 µg/ml

Diverse ion	Tolerance limit (µg/ml)	Diverse ion	Tolerence limit (µg/ml)
EDTA	1860	Pb(II)	1025
Ascorbaic acid	1760	Cd(II)	880
Iodide	1270	Te(IV)	750
Citrate	1215	U(VI)	480
Phasphate	950	Na(I)	460
Tartarate	900	K(I)	390
Oxalate	880	Li(I)	320
Bromide	800	Th(IV)	280
Urea	760	W(VI)	250
Nitrate	720	Ce(IV)	210
Sulphate	680	Ti(IV)	200
Bromate	640	Cu(II)	12,130[a]
Acetate	600	Al(III)	110
Thiosulphate	560	Ni(II)	6,107[a]
Thiourea	380	Fe(II)	11,112[b]
Chloride	355	Cr(III)	88
Flouride	200	Zr(IV)	80
		Mn(II)	74
		Ru(III)	65
		Pd(II)	60
		Mo(VI)	59
		Pt(IV)	55

In the presence of a) 1860µg of EDTA; b) 1270 µg of iodide;

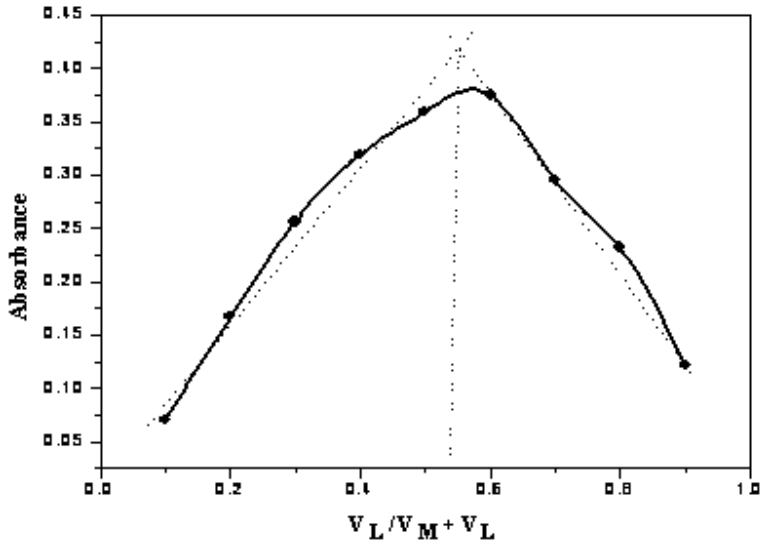


Fig. 2. Job's curve [Zn(II)-5-MFAT] = 2 x 10^{-4} M; Wave length = 430 nm; pH = 6.0

Composition and stability constant of the complex

The composition of the complex by determined by Job's method and molar ratio method and the stichiometry was found to be 1:1 (Metal: Ligand). The stability constant of the complex was determined by Job's method as 2.4 x 10^6**.** Fig.(2)

Applications

The proposed method was applied for the determination of zinc(II) in pharmaceutical biological samples and certified reference materials.

Table 3. determination of Zn(II) in biological samples using 5-MFAT

Name of sample	composition certified value mg/tablet	Amount of Zn(II)[a] Found mg/tablet		Present method	
		FAAS method	Present method	S.D	% RSD
Antoxid	Zinc sulphate monohydrate, 27.45 mg (equivalent to elemental Zinc, 9.99 mg); Selenium oxide, 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 panttothenate, 2.5 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) 4mg.	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%) 133.54 mg, Vit.A 5000 IU, C 150 mg; E IU 25mg, Zinc sulphate monohydrate 27.45 mg; (equivalent to Elemental Zinc, 9.99 mg);	9.99	9.97	0.1010	1.01
Maxmin-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

[a] Avarage five determinations; [b]Masked with fluoride; [c]Masked with oxalate

Determination of Zn(II) in pharmaceutical samples

Pharmaceutical samples like antoxid, benzoic, magnical, polyzee, ridage, and maxamin forte were analyzed for zinc(II). The results are presented in compared with other reported spectrophotometric method (FAAS) Table 3.

Determination of Zn(II) in biological samples

Biological samples like palm leaves, chilli, and milk samples were analyzed for zinc(II) using the proposed method. The leaves of sago plam *(Metroxylon sagu Rottb.)* and oil plam *(Elaeis guineensin Jacq.)* plants, the chilli samples were collected in and around Anantapur, 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 spectrophotometry Table .4.

Determination of Zn(II) in certified reference materials

The presented 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 (5).

CONCLUSION

Table.4 determination of Zn(II) in biological samples by using 5-MFAT

Name of the biological sample	Amount of Zn(II)[a] Found mg/tablet		Present Method	
	FAAS method	Present method	S.D	(%) RSD
Leafy samples[b]				
Sago plam leaves *(Metroxylonsagu Rotb)*[b]	28.2	28.1	0.2200	0.78
Oil plam leaves (Elaeis guineensim Jacq.) [b]	46.7	46.6	0.1800	0.39
Chilli samples [b] ***(sample location)***				
Rayachoty	20.4	20.2	0.0920	0.45
Anantapur	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
Thiruchanoor	26.0	25.3	0.0985	0.38
Akuthotapalli	27.0	27.0	0.1256	0.46
Rennigunta	22.0	21.8	0.1458	0.66
Milk samples[c] ***(Animal origin)***				
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

[a] *Avarage four determinations;* [b]*Concentration in µg/g;* [c] *Concentration µg/mL*$^{-1}$

A through literature survey revealed that many thiosemicarbazones were used for the determination of zinc(II). Studies upon the use of benzoylpyridine thiosemicarbazone (BPT) as an analytical reagent are limited. Hence, the present investigations were carried out with a view to test the potentiality of BPT as complexing agent for Zn(II) and subsequent determination spectrophotometrically. The method has good sensitivity, compared with other spectrophotometric determination methods. The selectivity of this method is enhanced by using masking agents for Cu(II), Ni(II), Fe(II) and V(V). Finally, the developed method can be considerably declared for the determination of Zn(II) in pharmaceutical and biological samples.

Table 5. determination of Zn (II) in certified reference materials using 5-MFAT

Certified reference materials	Amount of Zn(II)[a] Found mg/tablet		Present method	
	Certified value	Present method	S.D	(%)RSD
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

[a] *Avarage four determinationss*

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