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Article

Second Derivative Spectrophotometric Determination of Zinc(II) Using 2-Acetyl pyridine Thiosemicarbazone/Semicarbazone in Biological Samples

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ABSTRACT

2-Acetylpyridine thiosemicarbazone (APT) and 2-acetylpyridine semicarbazone (APS) have been used for the spectrophotometric determination of zinc(II) in aqueous medium. APT and APS react with zinc(II) in acidic medium. The color reactions between reagents with zinc(II) are instantaneous and the absorbance of complexes remains constant for over 24 h. The maximum absorbance (l_{max}), composition (M: L), molar absorptivity, and Sandells sensitivity of the Zn-APT and Zn-APS complexes, respectively, are 360, 355 nm, 1:2, 4.06×10^4, 8.12×10^3 L/mol/cm, and 0.0160, 0.080 µgcm^{-2} of Zn(II), respectively. The Zn-APT and Zn-APS systems obey Beer's law for 0.105–1.046, and 0.523–5.231 µg/ml of Zn(II), respectively. Large number of cations, anions, and complexing agents (e.g. triethanolamine, and thiourea) does not interfere in APT method. The method is successfully applied for the determination of zinc in biological samples.

Key words: Spectrophotometry, Zinc determination, 2-Acetylpyridine thiosemicarbazone, 2-Acetylpyridine semicarbazone, Biological samples.

1. INTRODUCTION

Zinc is an essential element in living organisms. It plays an important biological role. In human blood, 75–85% of zinc is distributed in erythrocytes in erythrocytes most as carbonic anhydrase, 12–22% 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 -globulins minor fractions complexed with glutamate, histidine and cysteine in carboxy peptidase, carbonic anhydrase, and liver alcohol dehydrogenases [1-3].

Zinc is associated with many enzymes and it is involved in the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). 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, causes ulcers, and scaling of the skin besides affecting the bones and joints. Children in under developed countries who are solely deficient of zinc fail to mature sexually. Less severe deficiency has been linked to a low sperm count and infertility. Zinc deficiency many produce serious defects and fetal loss [4].

Although a little zinc is vital to health, too much is harmful, a single 220 mg zinc sulfate capsule can cause nausea and vomiting. Toxic effects, which may also include abdominal pain, fever, and severe anemia can result from eating acidic foods or drinking liquids that have been stored in galvanized containers. Thus, zinc is an essential element and has significant importance, both biological and industrially. Hence, determination of zinc in biological samples is indispensable. A close literature survey [5-13] reveals that 2-acetylpyridine thiosemicarbazone (APT) and 2-acetylpyridine semicarbazone (APS) are not used for the spectrophotometric determination of zinc(II) in aqueous medium. In the light of above, the authors have investigated spectrophotometric determination of zinc(II) using APT and APS in biological samples.

2. EXPERIMENTAL

The reagents APT and APS were synthesized as described earlier [14] pKa values of reagents were determined spectrophotometrically using Phillips and Merrit method [15]. The values of deprotonation of APT and APS were 5.47 and 6.16 (pK_1); 8.56 and 8.30 (pK_2), as shown in Scheme 1.

The reagents (APT or APS) solution (0.01 M) was prepared by dissolving 50 mg of the compound in dimethylformamide in 25-ml standard flask. The reagent solution is stable for at least 12 h. Hydrochloric acid (1 M) – sodium acetate (1 M) (pH 0.5–3.5); 0.2 M NaOAc-0.2 M AcOH (pH 4–6), and 2 M NH_4Cl-2 M NH_4OH (pH 7–10) solutions were used. $ZnSO_4.7H_2O$ (0.2808 g) was dissolved in doubly distilled water containing a few drops of conc. H_2SO_4 in a 100 ml standard flask to get a 1×10^{-2} M solution, which was standardized gravimetrically using 8-hydroxyquinoline [16]. The working solutions were prepared by diluting the stock solution to an appropriate volume. All other chemicals used were of AR grade.

2.1. Procedures

2.1.1. Determination of zinc(II) (zero order)

An aliquot of the solutions containing zinc (II) in Beer's law validity range Table 1 and buffer solution pH 6.0 and 1 ml of 0.01 M reagent (APT or APS) were combined in a 25-ml volumetric flask and the resulting solution was diluted to the mark with distilled water and

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absorbencies were measured at 360 and 355 nm against corresponding reagent blank. The measured absorbance is used to compute the amount of zinc present in the real samples using predetermined calibration plot.

2.1.2. Determination of zinc(II) using second derivative spectrophotometry

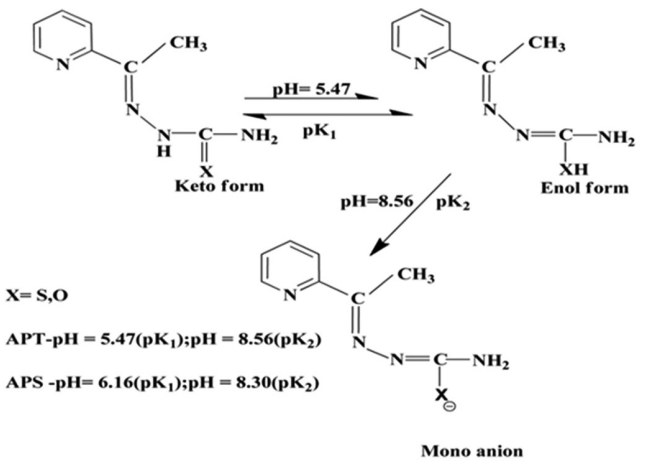
Selective second-order derivative spectrophotometric methods were developed for the determination of zinc(II). For the Zn-APT and Zn-APS second derivative spectra, the derivative amplitudes at 465 nm, 434 nm (peak) and at 522 nm, 414 and 474 nm (valley), respectively, were proportional to the concentration of Zn(II). The derivative amplitudes were measured for different concentration of Zn(II) and plotted against the amount of Zn(II). The plots were linear and obeyed Beer's law in the range 0.76–0.002, 0.0116–0.0005 µg/mlat 465 nm, and 434 nm and 0.523–0.006, 0.005–0.0007 µg/mlat 498 nm, and 440 nm in the second-order derivative method using APT and APS, respectively.

2.1.3. Apparatus

Shimadzu 160A ultraviolet-visible spectrophotometer equipped with 1.0 cm quartz cell and an ELICO model LI - 610 pH meter were used in the present study.

3. RESULTS AND DISCUSSION

The reagent solutions (0.01 M) are stable for 12 h. The bathochromic shift of absorption band from 290 to 300 nm indicates that in solution



Scheme 1: Different species of 2-acetylpyridine thiosemicarbazone and 2-acetylpyridine semicarbazone are solution at different pH values.

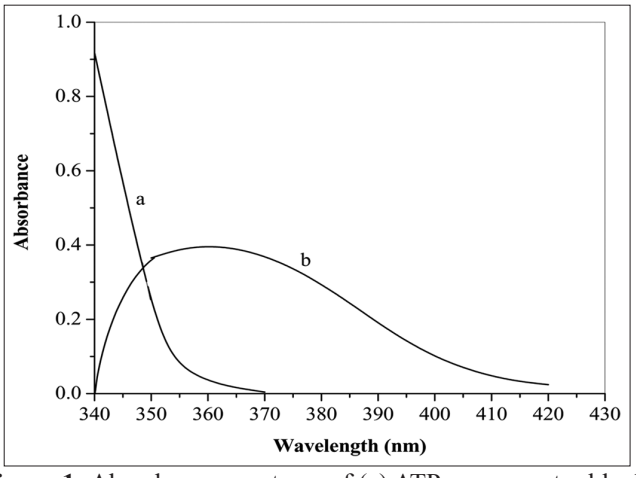


Figure 1: Absorbance spectrum of (a) ATP versus water blank, (b) Zn^{II}-APT complex.

on increasing the pH, the acid is neutralized, and the >C=S groups is enolized and dissociated [17]. The color reactions of some important metal ions with APT and APS are summarized in Table 2. In basic medium (above pH 8.56), the ligands presumably exists in enolic form and coordinates the divalent metal ion as mono anion to give neutral complexes.

3.1. Determination of Zinc(II)

Zinc(II) react with APT in acidic pHs, respectively, to give colored complexes in Table 2. The color reactions are instantaneous at room temperature. The change in the order of addition of metal ion, reagent (APT or APS), buffer has no adverse effect on the absorbance of complexes. Absorption spectra of Zn-APT and Zn-APS complexes are given in Figures 1 and 2. Various physicochemical and analytical characteristics of the complexes are summarized in Table 3. Second derivative spectra of Zn-APT and Zn-APS systems are given in Figures 3 and 4, respectively. The derivative amplitudes are proportional to concentration of Zn(II).

The stoichiometry of the complexes was determined by well-known Job's continuous variation, molar ratio methods (Figures 5-8). Sodium acetate (0.2 M)-acetic acid (0.2 M) (pH 6.0) buffer solution and equimolar (3×10^{-4} and 4×10^{-4}M) solution of Zn(II) and APT or APS were used in these methods. The data obtained in Job's method were used in the calculation of stability constants of the complexes. The structure of the complexes $Zn(APT)_2$ and $Zn(APS)_2$ complexes is given in Scheme 2. $Zn(APT)_2$ complex is more stable than $Zn(APS)_2$ complex. Higher stability of $Zn(APT)_2$ complex is due to higher affinity of zinc to bind with sulfur containing ligand (APT).

Table 1: Chromogenic characteristics of APS

Metal ion	λ_{max}	$\varepsilon\times10^4$ L/mol/cm	pH range	Color of the complex
Cu^{II}	355	0.98	5.0–70	Yellow
Ni^{II}	350	2.80	8.0–10.0	Pale yellow
Co^{II}	360	1.45	5.0–8.0	Orange yellow
Zn^{II}	355	0.81	5.0–7.0	Greenish yellow
Fe^{II}	365	0.14	4.0–7.0	Deep yellow with red tinge
Fe^{III}	370	0.14	4.0–7.0	Deep yellow with red tinge
V^{V}	370	0.27	4.0–7.0	Yellow colored

APS: 2-acetylpyridine semicarbazone

Table 2: Chromogenic characteristics of APT.

Metal ion	λ_{max}	$\varepsilon\times10^4$*	pH range	Color of the complex
Cu^{II}	376	0.26	5.0–70	Pale greenish yellow
Ni^{II}	375	1.6	5.0–7.0	Yellow greenish
Co^{II}	355	1.25	5.0–8.0	Orange yellow
Zn^{II}	360	4.06	5.0–7.0	Pale greenish yellow
Fe^{II}	370	0.17	4.0–7.0	Deep yellow with red tinge
Fe^{III}	375	0.14	4.0–7.0	Deep yellow with red tinge
Hg^{II}	351	5.4	5.0–7.0	Yellow colored
V^{V}	360	0.37	3.0–7.0	Yellow colored

APT: 2-Acetylpyridine thiosemicarbazone

Table 3: Physicochemical and analytical characteristics of Zn^{II} complexes with APT and APS.

S. no.	Characteristics	Results	
		$Zn(APT)_2$	$Zn(APS)_2$
1.	λ_{max} (nm)	360	355
2.	pH range (optimum)	5.0–7.0	5.0–7.0
3.	Mole of reagent required per mole of metal ion for full color development	10 fold	10fold
4.	Time stability of the complex (in minutes)	24 h	12
5.	Beer's law validity range (µg/ml)	0.10–1.04	0.52–5.23
6.	Molar absorptivity (L/mol/cm)	4.06×10^4	8.12×10^3
7.	Specific absorptivity (ml/g/cm)	0.62	0.124
8.	Sandell's sensitivity (µg of Zn (II) cm^{-2})	0.0160	0.080
9.	Composition of the complex as obtained in Job's and molar ratio methods (M: L)	1: 2	1:2
10.	Stability constant of the complex	1.3×10^{12}	1.71×10^{11}
11.	Standard deviation	0.019	0.016
12.	Relative Standard deviation (RSD), (coefficient of variation)	1.2%	0.65%

APT: 2-Acetylpyridine thiosemicarbazone, APS: 2-Acetylpyridine semicarbazone

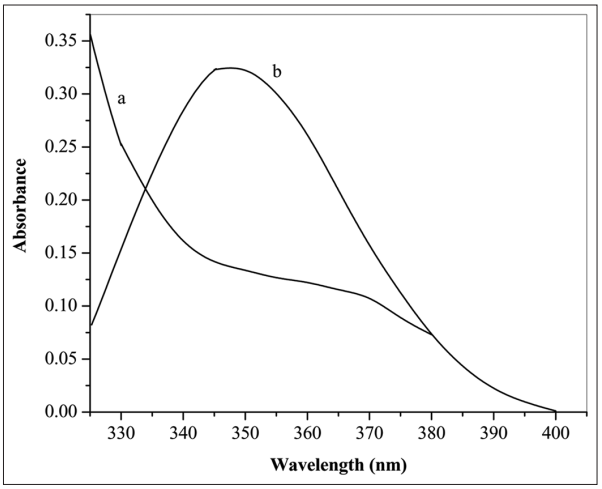


Figure 2: Absorbance spectrum of (a) APS versus water blank, (b) Zn^{II}-APS complex.

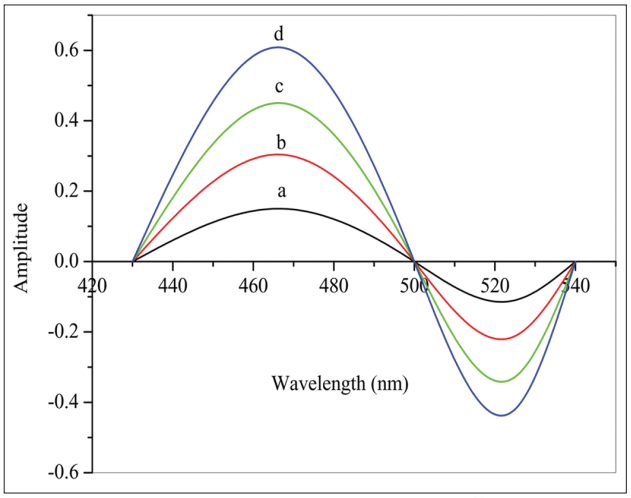


Figure 3: Second derivative spectra of Zn^{II} – APT versus reagent blank Zn^{II}, µg/ml; a. 0.2092; b. 0.4185; c. 0.6277; d. 0.8370 [APT]=6×10^{-4}M; pH=6.0

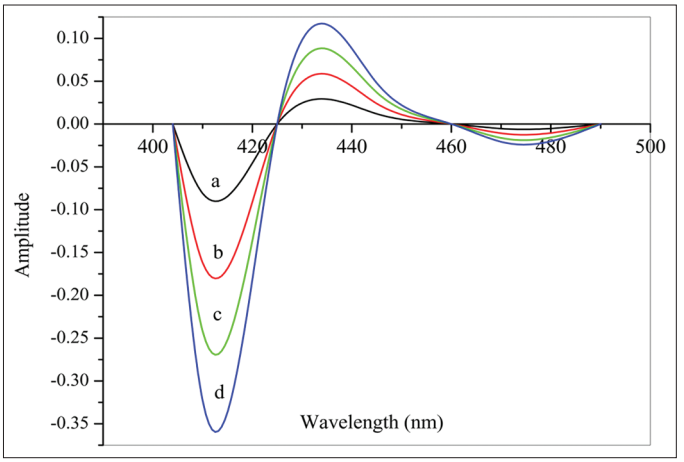


Figure 4: Second derivative spectra of Zn^{II} – APS *Vs* reagent blank Zn^{II}, µg/ml; a. 1.0462; b. 2.0925; c. 3.1387; d. 4.1850 [APS]=6×10^{-4}M; pH=6.0.

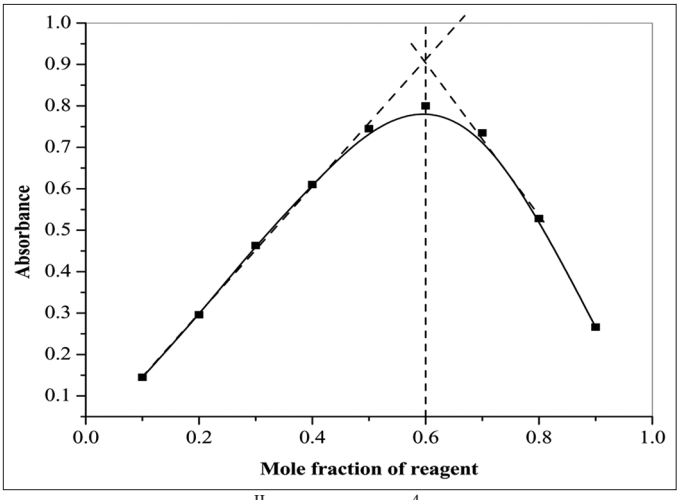
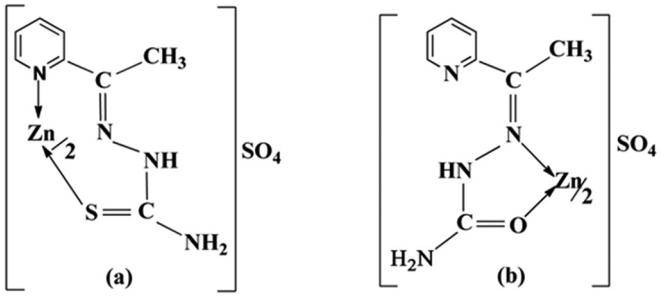


Figure 5: Job's curve Zn^{II}=APT=3×10^{-4}M, wavelength=360 nm, pH=6.0.

Table 4: Tolerance limit of foreign ions in the determination of 0.52 (µg/ml) APT and 2.61(µg/ml) APS of zinc.

Tolerance limit (ppm)					
Ions	**Zero-order**		**Ions**	**Zero-order**	
	APT	**APS**		**APT**	**APS**
EDTA	1581	2972	Sn(II)	47	4.7
Urea	847	847	Ba(II)	44	0.7
Cyanate	641	641	Pb(II)	41[a]	4.1[a]
Ascarbate	624	624	W(VI)	37	32
Tartrate	592	592	Al(III)	26	26
Iodate	507	507	Cd(II)	22	2.2
Thiosulfate	394	394	Mo(VI)	19.2	0.19
Citrate	384	384	Ag(I)	12	12
Sulfate	384	384	Mn(II)	11	2.1[b]
Oxalate[b]	352	352	Au(II)	10.25	10.25
Thiourea	307	309	Pt(II)	10.25	10.25
Iodide	304	612	Cr(VI)	10	2.6
Nitrate	248	248	Ca(II)	8.0	9.6
Bicarbonate	244	246	Sr(II)	5.0	21
Acetate[a]	236	236	Ti(III)	5.0	10
Thiocyanate	232	232	Pd(II)	5.0	10
Fluoride[c]	78	78	Mg(II)	4.9	0.046
Phosphate	39	38	Zr(IV)	3.8	3.6
Bromide	31	31	Hg(II)	2.4	0.24
Chloride	14	14	Ni(II)	1.4	0.11
			Zn(II)	0.842	1.5
			Fe(III)	0.67[c]	0.675
			Co(II)	0.2	0.07
			Fe(II)	0.067	0.67
			V(V)	0.06[b]	0.6

[a]Masked with 230 µg/ml of acetate, [b]Masked with 300 µg/ml of oxalate, [c]Masked with 70 µg/ml fluoride. APT: 2-Acetylpyridine thiosemicarbazone, APS: 2-Acetylpyridine semicarbazone



Scheme 2: Tentative structure of (a) $[Zn(APT)_2]^{2+}$, (b) $[Zn(APS)_2]^{2+}$ complexes.

3.1.1. Interferences

The effect of various cations and anions which are generally associated with the metal ion on the determination of zinc(II) is studied by measuring the absorbance of the zinc complex in the presence of foreign ions. The color reaction is developed as described in the standard procedure. An error of ±2% in the absorbance or amplitude reading in the case of derivative methods considered tolerable. The results are given in Table 4. The dada obtained in

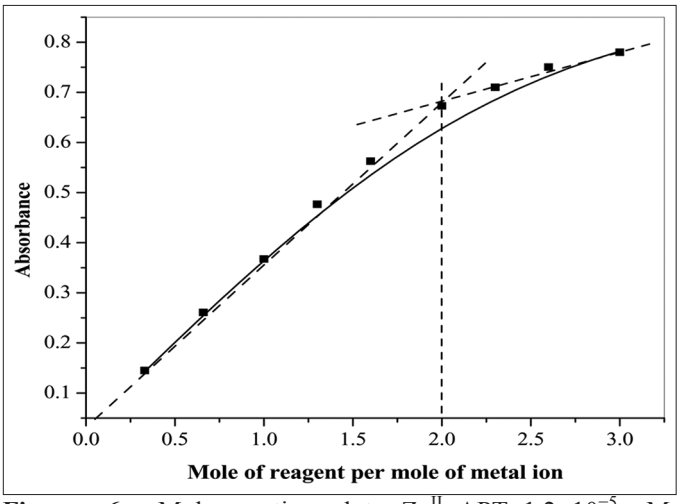


Figure 6: Molar ratio plot Zn^{II}=APT=1.2×10^{-5} M, wavelength=360 nm, pH=6.0.

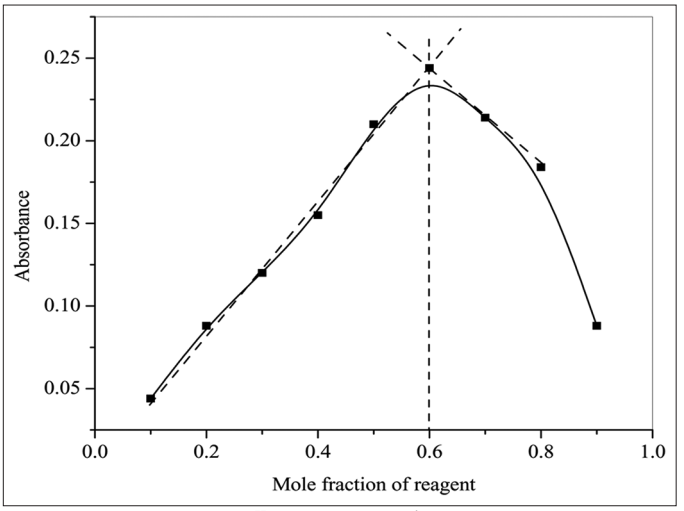


Figure 7: Job's curve Zn^{II}=APS=4×10^{-4} M, wavelength=355 nm, pH=6.0.

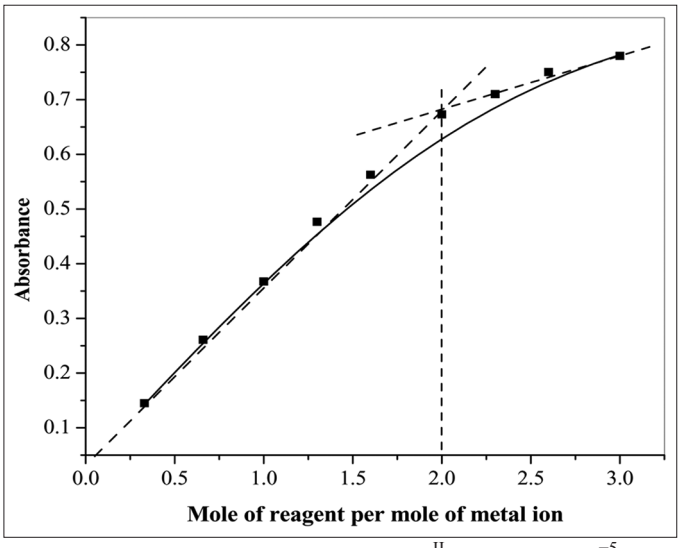


Figure 8: Molar ratio plot Zn^{II}=APS=1.6×10^{-5} M, wavelength=355 nm, pH=6.0.

second derivative method are also incorporated. The data suggest that several associated anions and cations do not interfere when they are present in large excess. The tolerance limit values for many anions and cations are high in derivative methods. The interference of associated metal ions such as Fe(III) and Al(III) is decreased with triethanolamine. Larger amounts of copper(II) do not interfere in the presence of thiourea.

3.2. Applications

The present method was successfully applied to the determination of zinc when present alone. The method was also extended to the determination of zinc in biological samples. Data are given in Tables 5 and 6.

Table 5: Analysis of fish and sheep liver sample/

Sample	Zinc[a] (µg/g)		Recovery (%)
	Added	Found	
Sheep liver	0	0.172	-
	100	101.02	100.80
	500	500.92	100.15
Fish liver	0	1.10	-
	100	101.21	100.10
	500	502.10	101.20

[a]Average of five determinations

Table 6: Determination of zinc in blood samples.

Sample	Zinc µg/g				Sample sources[a]
	Present method *n*=5		AAS method *n*=5		
	Found	RSD %	Found	RSD%	
Blood	95.78	0.88	99.62	1.5	Kidny damage patient(M)
Blood	228.11	1.3	230.37	1.9	Paralysis patient(M)
Blood	9.62	1.2	7.72	1.5	Normal adult(M)

[a]Sample was government of hospital Anantapur.

3.2.1. Biological samples

A 2–5 g of dried fish, sheep liver samples were taken in a 250 ml beaker. A 6 ml of concentrated nitric acid was added and gently heated for ½ h. After the disappearance of the forth, 6 ml of 1:1 nitric acid and perchloric acid were added [18]. The contents were digested for 1 h and repeatedly treated with 6 ml portions of nitric acid and perchloric acid mixture until the solution becomes colorless. The acid solution was evaporated to dryness and the resulting white residue was dissolved in minimum volume of 1 M nitric acid and made up to the volume in a 50-ml volumetric flask. Aliquots of this solution were taken for analysis by following recommended procedure. The results are given in Table 5.

3.2.2. Human blood serum samples

Human blood samples were collected from four patients with a 10 ml polypropylene syringe equipped with stainless steel needle. These samples were transferred into glass tube and centrifuged at 3000 rpm for 20 min. The supernatants were collected as the blood serum samples. Freeze-dried human serum reference material (GHPA No. 4) issued by the government hospital patients Anantapuramu was also used as a test sample in the preliminary study.

3.2.3. Digestion procedure for human blood serum

Blood serum samples were digested as follows. The blood serum (4 ml) was placed in a Teflon beaker (100 ml) and after adding 1 ml of concentrated HNO_3 the serum sample was heated almost to dryness on a hot plate at 110°C. Then, 1 ml of concentrated HNO_3 was again added to the residue and the solution was heated at 150°C for 2 h. A 1 ml of concentrated HNO_3 and 1 ml of 60% $HClO_4$ were added and the solution was heated at until white fumes appeared. This procedure was repeated twice. Finally, 0.35 ml of concentrated HNO_3 and 1 ml of pure water were added to dissolve the residue with heating at 110°C for 1 h, and solution was diluted to 50 ml with distilled water. The amount of zinc was estimated using recommended procedure. The results are given in Table 6.

4. CONCLUSION

The present methods for the determination of zinc (II) are compared with other spectrophotomeric methods. The present APT method seems to rank among highly sensitive methods for the determination of zinc(II). The results obtained in the analysis of different samples using present method are comparable to the data obtained (Table 7). The present ligands containing heterocyclic ring are found to be potential and cost effective for the determination of zinc(II) without the need for extraction using the toxic solvents. Further, the reagents are easy

Table 7: Comparison of spectrophotometric method for the determination of zinc(II).

Name of the reagent	λ_{max}	pH	Det. µg/g	$\varepsilon \times 10^4$ L/mol/cm	M:L	Ref.
Benzildithiosemicarbazone (BT)	395	9.5	1.0–18.0	0.42	1:1	8
Glyoxaldithiosemicarbazone (GT)	433	9.0–11.0	-	1.3	1:1	9
1,3-cyclohexanedionedithiosemicarbazone	570	3.6	-	1.42	-	10
Xylenol orange and cetylpyridinium chloride	580	5.0–6.0	1.0–20.0	1.1	1:2	11
Methylglyoxal bis(4-phenyl-1,3-thiosemicarbazone	445	6.0–8.5	0.2–0.4	0.21	1:1	12
1,2-cyclohexanedionedithiosemicarbazone	415	1.1–6.6	-	0.73	1:2	13
7(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid	520	9.2	0.05–1.0	3.75	1:2	14
Benzoylpyridine thiosemicarbazone	375	5.0–7.0	0.26–2.6	1.87	1:1	15
2-Acetylpyridinethiosemicarbazone	360	5.0–10.0	0.15–1.04	4.06	1:2	Present work

to synthesize using commercially available precursors. Moreover, the present methods are simple, rapid, and very sensitive for the spectrophotometric determination of zinc(II) in aqueous medium.

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