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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF LEAD & ZINC IN BIOLOGICAL SAMPLES USING 2, 6-PYRIDINEDICARBOXALDEHYDE, PHENYLENEDIAMINE

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ABSTRACT

Analytical application of 2,6-Pyridinedicarboxaldehyde,phenylenediamine(PDAPDC) is described for the direct non-extractive spectrophotometric determination of lead (515nm),zinc (460nm), The synthesized and characterized using IR and NMR spectral data,. The reagents react with, lead (2.02 to 4.25 ppm) , zinc (66.26 to 28.52 ppm) in sodium acetate-acetic acid buffer solution to form light yellow colored 1:2 (M: L) complexes. The colour reactions are instantaneous and absorbance values remain constant for over 24 h. The molar absorptivity and Sandell's sensitivity of(PDAPDC)methods are found to be 2.15 X 10 ^{4} mol^{-1} cm^{-1} and 0.0052 µg cm^{-2} of Pb^{II} and 0.0047 µg cm^{-2} of Zn^{II}. The systems obey Beer's law in the range of 2.2 µg/ml of Pb^{II} and 1.9 µg /ml of Zn^{II} . Since (PDAPDC) method is more sensitive it was applied for the determination of, lead ,zinc in biological samples.

Keywords: Carboxaldehyde, Lead, Zinc

INTRODUCTION

Toxic metal is defined as that metal, which is neither essential nor has beneficial effect, on the contrary, it displays severe toxicological symptoms at low levels. With increasing industrialization, more and more metals are entering into the environment. These metals stay permanently because they cannot be degraded from the environment. They pass into the food and from food they ultimately make their passage into the tissue (Baykov *et al.,* 1996)[1].

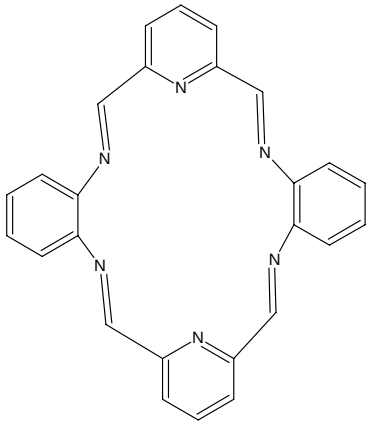
The lead, zinc are among the main toxic metals. They accumulate in food chains and have a cumulative effect (Cunningham & Saigo, 1997)[2]. Heavy metals often have direct physiologically toxic effects and are stored or incorporated in living tissues, sometimes permanently (Bokori e*t al.,* 1996)[3]. The contents of lead, zinc were detected in several tissues of goats. The results showed that the levels of these toxic metals were found to be very high generally above the permissible level (John & Jeanne, 1994) [4].

Similarly, the distribution and localization of some heavy metals in the tissues of some calf organs were detected. The most affected organs, which showed higher levels of trace metals, were livers, kidneys and small intestines (Horky *et al.,* 1998) [5].

Lead is a metabolic poison and a Neurotoxin that binds to essential enzymes and several other cellular components and inactivates them (Cunningham & Saigo, 1997)[6]. Toxic effects of lead are seen on hemopoietic, nervous, gastrointestinal and renal systems (Baykov *et al.,)*[7] metal fumes or suspended particulates from fuel combustion or smelting and disposal of wastes however, most of the lead poisoning is from leaded gasoline. Zinc concentrations were found to be highest in meat, liver, fish and eggs (Janet & Carl, 1994)[8].

2,6-Pyridinedicarboxaldehyde,phenylenediamine (PDAPDC) is new important reagent used For the spectrophotometric determination of lead1996). Lead is released into the air in the form of, zinc metal ions. with increasing industrialization, more and more industrial waste get accumulated in various regions and make their passage through soil into animal body, especially, in their liver, kidney and lean meat[9]. The present study was planned to determine the prevalence of selected Trace elements in lean and organ meat of beef, mutton which are the items of every day, consumption in.

This paper describes synthesis, characterization and analytical properties of new reagents viz.–2,6-Phyridinedicarboxaldehyde, phenylenediamine The spectrophotometric determination of lead, zinc using (PDAPDC). is included in this paper. This method is far more sensitive, non-sensitive, simple and rapid than all of the existing spectrophotometric methods[10-14]. It was used for the determination of lead, zinc, in various biological samples.



PDAPDC

RESULTS AND DISCUSSION

The reagent PDAPDC may be easily prepared. The reagent solutions (0.01M) are found to be stable for 24 h. The absorption bands are

515nm, 460nm indicates that in solution on increasing the pH, The colour reactions of some important metal ions with PDAPDC are summarized in Table1. In basic medium (above pH 8-9) coordinates the pentavalent metal ion as mono anion to give neutral complexes[15].

Lead (II) reacts with PDAPDC in basic pH s to give water insoluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition of metal ion, reagent (PDAPDC), and buffer has no effect on the absorbance of complexes. Analytical characteristics of the complexes are summarized in Table 1. The stiochiometry of the complexes (M:L = 1:2) was determined by job's continuous variation and molar ratio methods. Sodium acetate (0.2M)-acetic acid (0.2M) buffer solution (pH 6.0-9.0 and T=300 K) and equimolar (2.15X10^{4}M) solutions of Pb^{II} , (1.80X10^{4}M) solutions of Zn^{II} and PDAPDC were used in the calculation of stability constants of the complexes.

The effect of various cations and anions which are generally associated with the metal ion in the determination lead, zinc was studied by measuring the absorbance of lead and zinc complexes containing 2.1µg/ml of lead (II),and 1.3µg/ml of zinc in solution. The colour reaction is developed as described in the standard procedure. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions

and cations in PDAPDC methods respectively are as follows: citrate (1152,1152); tarrate (888,888); ascorbate (752,752); iodate (761,761); iodide(612,761), thiocyanate (507,507); phosphate(465,465); urea (384,384); bromide (317,317); sulphate (252,384); thiosulphate (246,246), nitrate (212,244); oxalate (281,281); fluoride (75,75); Ba^{2+} (675,800); Mn^{2+} (275, 325); Mg^{2+} (125,150); Sr^{2+} (100,125); W^{6+} (110,110); Sn^{2+} (47,47); Mo^{6+} (19,23); Tl^{3+} (14,14); Fe^{2+} (12,10); Cr^{6+} (10,12); ,Pt^{4+} (8,8); Fe^{3+}(5,4); Au (4,4); Ag^{+} (5,5); Cd^{2+} (4,4); Pb^{2+} (3,4); Ni^{2+} (1,1.2); Cu^{2+} 1,1).

Higher amounts of Fe^{3+} (13,17) do not interfere in the presence of 70ppm of fluoride. Larger amounts of As^{3+} (42,52) do not interfere in the presence of 600ppm of iodide.

Table 1: Physico-chemical and analytical properties of Pb ^{II}, Zn ^{II} complexes with PDAPDC

S.No.	Characteristics	Pb -PDAPDC	Zn-PDAPDC
1.	λmax (nm)	515	460
2.	pH range (optimum)	8.0-9.0	6.0-7.0
3.	Mole of reagent required per mole of metal ion for full colour development	10-fold	10-fold
4.	Time stability of the complex (in hours)	24	24
5.	Beer's law validity range (µg/ml)	2.2	1.2
6.	Molar absorptivity (L $mol^{-1}cm^{-1}$)	2.15 X 10^{4}	1.80X 10^{4}
7.	Specific absorptivity (ml g^{-1} cm^{-1})	0.212	0.25
8.	Sandell's sensitivity (µg of Hg ^{II} cm-2)	0.0052	0.0047
9.	Composition of the complex as obtained in Job's and molar ration methods (M:L)	1 : 2	1 : 2
10.	Stability constant of the complex	3.40 X 10^{13}	9.12 X 10^{13}
11.	Standard deviation	0.0079	0.0039
12.	Relative standard deviation (RSD)	0.56%	0.30%

The present method (PDAPDC) was applied for the determination of lead, zinc when present alone and present in biological samples (Table 2). The lead, zinc concentration as determined in lean and organ meat of beef, mutton has been summarized in the Tables II,. Highest lead concentration was found in the liver of mutton (4.25 ppm) and lowest (2.02ppm) in the in the kidney of beef. The results showed that the lead concentration in the liver and kidney of all species was higher than the permissible limit of 1 ppm (ANZFA)[16]. Similarly, in the lean meat of beef, mutton and poultry the lead concentration was observed to be higher than the permissible limit. A higher concentration of lead than the permissible limit in the liver and kidney of animals has been reported by Aranha (1994) and Danev *et al.* (1996) and showed that 86% samples of liver and 100% samples of kidney were contaminated above the limits set by the country regulations. Similarly, Maldonado *et al.* (1996 The zinc concentration in the lean and organ meat of beef, mutton has been summarized in Tables II, Highest zinc concentration (66.26 ppm) was found in the lean meat of beef and lowest concentration (28.52 ppm) in the lean meat of poultry. All the values in the study samples were below the permissible limit (150 ppm) set by (ANZFA)[16]. Jozef *et al.* (1997) [17] reported the zinc and copper intoxication by industrial emission in the livers,kidneys, spleen, musculature and in the ovaries and uterus of some experimental sheep. Results showed that the highest concentration of zinc in the experimental animals, died of zinc intoxication, was in the liver and kidneys.The low concentration of zinc may be attributed to zinc deficient soils, consequently the fodder/cereals available to poultry and cattle are deficient of zinc. Perhaps, this is one of the reasons for low tissue content of zinc.

The present ligands containing heterocyclic ring are found to be potential and cost effective for the determination of lead(II),zinc(II) without the need for extraction using the toxic solvents. Further, the reagents are easy to synthesize using commercially available precursors. Moreover, the present method is simple, rapid and very sensitive for non-extractive spectrophotometric determination of lead(II) ,zinc(II) in aqueous medium.

Table 2: Determination of lead, zinc in liver & Kidney samples

Sample	Lead (µg/g)[a]	Recovery	Added	Found	±S.D.%
Beef liver	0	2.18	100	102.12	98.3 ± 0.38
			500	504.30	100.2 ± 0.5
Sheep liver	0	4.25	100	104.50	100.0 ± 0.8
			500	504.10	100.5±0.19
Beef kidney	0	2.02	100	102.02	98.8 ± 0.44
			500	504.10	100.5 ± 0.72
Sheep kidney	0	5.1	100	105.03	97.2 ± 0.12
			500	509.10	100.9 ± 0.31

Sample	Zinc (µg/g)[a]	Recovery	Added	Found	±S.D.%
Beef liver	0	5.8	100	105.02	96.9 ± 0.71
			500	505.10	107. ± 0.14
Sheep liver	0	5.6	100	106.80	99.3 ± 0.67
			500	505.10	100.5±0.
Beef kidney	0	4.61	100	104.02	96.9 ± 0.10
			500	508.10	100.5 ± 0.22
Sheep kidney	0	5.1	100	105.03	100.0 ± 0.12
			500	509.10	100.9 ± 0.51

[a]Average of five determinations.

MATERIALS AND METHODS

Preparation of PDAPDC:

The reaction mixture containing 2,6-Pyridinedicarboxaldehyde, (2g,0.01183mol in 20ml of methanol) phenylenediamine (1.2781,.0.01183 mol in 20ml of methanol dissolved in hot condition) was taken in 250-ml round bottom flask and refluxed for 10h. On cooling the reaction mixture, light browen coloured product was formed. It was collected by filtration and washed with hot water and 50 percent cold methanol. This compound was recrystallised from ethanol and dried in vacuo, yield 2.6 g;m.p. 172°C.

Characterization of PDAPDC:

The reagents have been characterized by IR and ^{1}H NMR spectral data. Infrared spectrum of PDAPDC shows bands at [3437(s); 3258(m,br)];3358(m),3080(s),1680(m);1623(s),1583(s),1509(br);1418(s),1384(br),1246(m)1162(br),891 (δ), 756(δ),722(δ),696(δ); 649(δ) cm-1 respectively corresponding to υNH-symmetric, υ (C-H) aromatic stretch, stretching υ (C=N) aromatic ring, δ(C-H) of pyridine ring, δ(C-H) -oop(aromatic) and δ (C-C)-oop bend aromatic ring vibrations. ^{1}H NMR spectra of HTT ($CDCl_3$ + DMSO-d6) showed signals at 2.27, (1H,s); 8.15-8.32(1H),7.10,-7.86(4H,s) 3.25(1Hs)due toC=N(C_5H_4N),NH.

pK_a values of reagents:

The pK_a values were determined by recording the UV-Visible spectra of 2 X 10^5 M solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wave lengths determined spectrophotometrically using Phillips and Merrit method. The values of deprotonation of PDAPDC were 6.61 (pK_1); 8.30(pK_2).

The reagent PDAPDC solution (0.01 M) was prepared by dissolving 50 mg of the compound in dimethylformamide (DMF) 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 NH_4OH (pH 7-10) solutions were used.

1000 ppm stock solution of Lead was prepared by dissolving 1.83 g of lead acetate in one litre of distilled water. One gram of zinc metal was dissolved in one ml of HCl and volume was made up to one litre with distilled water to make 1000 ppm stock solution of zinc.

Recommended procedure:

An aliquot of the solutions containing 2.1, 1.3 mg/ml(or ppm) of Lead(II) ,Zinc(II)10 ml of NH_4Cl-2 NH_4OH , NaOAc, AcOH buffer solution (pH 8.0-9.0)(pH 6.0-7.0) and 1.0 ml of 0.01 M PDAPDC were mixed in a 25-ml volumetric flask and resulting solution was diluted to the mark with distilled water. The absorbance of this solution was measured at 515, 460 nm against respective reagent blank. The measured absorbance is used to compute the amount of Lead, Zinc present in the samples using predetermined calibration plot.

Schimadzu 160A UV-Visible spectrophotometer equipped with 10. cm quartz cell and an ELICO model LI-610pH meter were used in the present study.

Dried Beef and sheep liver and kidney samples (2-5 g) were taken in a 250 ml beaker. A 6 ml of concentrated nitric acid was added and gently heated for half an hour. After the disappearance of the froth, 6 ml of 1:1 nitric acid and perchloric acid were added[18-19]. The contents were digested for one hour and repeatedly treated with 6 ml portions of nitric acid and perchloric acid mixture until the solution becomes colourless. The acidic 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 following the recommended procedure.

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