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Research Article

SPE SPECTROPHOTOMETRIC DETERMINATION OF MERCURY IN BIOLOGICAL SAMPLES USING HYDANTOIN 5-AMINO-1,3,4-THIADIAZOLE-2-THIOL

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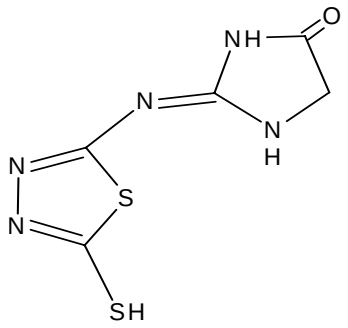
ABSTRACT

Analytical application of Hydentoin,5-amino1,3,4-thiadizole-2-thiol (HTT) is described for the direct non-extractive spectrophotometric determination of Mercury(490nm) The synthesized and characterized using IR and NMR spectral data,. The reagents react with Mercury II (31.47 to 78.96 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 HTT methods are found to be 6.45 X 10^4 L mol^{-1} cm^{-1} reagents have been. The systems obey Beer's law in the range of 2.2 µg/ml of Hg^{II}. Since HTT method is more sensitive it was applied for the determination of Mercury (II) in biological samples.

Keywords: Spectrophotometry, Mercury, Hydentoin,5-amino1, 3, 4-thiadizole-2-thiol, biological samples.

INTRODUCTION

The analysis and monitoring of mercury in environmental ,biological, industrial and food samples is extremely important because of the high toxicity of this metal both in its inorganic and organic compound [1] Mercury can exist in the environment as metal, as monovalent, divalent salts, methyl mercury and dimethyl mercury. The major sources of mercury are geothermal steam used for power production, paper industry, chemical industry, paint industry, pesticides and fungicides. Mercury escapes into the air and soil and from there get accumulated into the plants. The major effects of mercury poisoning are neurological and renal distrbances[2]. Damaging, particularly irritability, paralysis, insanity or blindness, chromosome breaking and birth defects; liver and brain damage[3].one example of acute mercury poisoning is "Minamata disease" Which causes metal disturbance; a loss of balance .speech, sight and hearing difficulty; in swallowing; and finally coma and death[4].



Hydentoin,5-amino-1, 3, 4-thiadizole-2-thiol is new important reagent used For the spectrophotometric determination of metal ions mercury. 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[5]. 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. –Hydentoin-5-amino1,3,4-thiadizole-2-thiol (HTT). The spectrophotometric determination of mercury using HTT is included in this paper. This method is far more sensitive, non-sensitive, simple and rapid than all of the existing spectrophotometric methods[6-10]. It was used for the determination of mercury in various biological samples.

RESULTS AND DISCUSSION

The reagents HTT may be easily prepared. The reagent solutions (0.01M) are found to be stable for 24 h. The absorption band from 485 to 505 nm indicates that in solution on increasing the pH, The colour reactions of some important metal ions with HTT are summarized in Table1. In basic medium (above pH 8.56) coordinates the tetravalent metal ion as mono anion to give neutral complexes[11].

Mercury (II) reacts with HTT in acidic pH s to give water soluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition of metal ion, reagent (HTT), 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 and T=300 K) and equimolar (6.45X10^4M) solutions of Hg^{II} and HTT 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 Mercury (II) was studied by measuring the absorbance of Mercury the a complexes containing 2.2µg/ml of Mercury (II) 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 HTT 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); Zn and Pd^{2+} (10,10), 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.

The present method (HTT) was applied for the determination of Mercury when present alone and present in biological samples (Table 2).

The mercury concentration as determined in lean and organ meat of beef, mutton has been summarized in the Tables II,. Highest mercury concentration was found in the liver of mutton (77.76 ppm) and lowest (31.47 ppm) in the liver of beef. All the study samples

showed mercury concentration much higher (31.4 to 78.96 ppm) than the permissible limit of 0.03 ppm(ANZFA)[14].

The present ligands containing heterocyclic ring are found to be potential and cost effective for the determination of mercury(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 mercury (II) in aqueous medium.

Table 1: **Physico-chemical and analytical properties of Hg^{II} complexes with ATT**

S.No.	Characteristics	Hg-HTT
1.	λmax (nm)	490
2.	pH range (optimum)	6.0-8.0
3.	Mole of reagent required per mole of metal ion for full colour development	10-fold
4.	Time stability of the complex (in hours)	24
5.	Beer's law validity range (µg/ml)	2.2
6.	Molar absorptivity (L $mol^{-1}cm^{-1}$)	6.45 X 10^4
7.	Specific absorptivity (ml g^{-1} cm^{-1})	0.25
8.	Sandell's sensitivity (µg of Hg^{II} cm-2)	0.0064
9.	Composition of the complex as obtained in Job's and molar ration methods (M:L)	1 : 2
10.	Stability constant of the complex	9.54 X 10^{13}
11.	Standard deviation	0.0059
12.	Relative standard deviation (RSD)	0.36%

Table 2: **Determination of Mercury(II) in liver & Kidney samples**

Sample	Mercury (µg/g)[a]		Recovery
	Added	Found	±S.D.%
Beef liver	0	3.14	
	100	103.02	98.9 ± 0.25
	500	505.10	100.2 ± 0.5
Sheep liver	0	7.7	
	100	107. 80	100.0 ± 0.3
	500	505.10	100.5±0.19
Beef kidney	0	4.60	
	100	104.02	96.9 ± 0.42
	500	509.10	100.5 ± 0.72
Sheep kidney	0	6.3	
	100	106.03	100.0 ± 0.1
	500	509.10	100.9 ± 0.51

[a]Average of five determinations

Experimental

Preparation of HTT

The reaction mixture containing Hydentoin(3g,0.02976mol in 10ml of methanol) 5-amino1,3,4-thiadizole-2-thiol(3.964g,.0297mol in 20ml of methanol dissolved in hot condition) was taken in 250-ml round bottom flask and refluxed for 8h. On cooling the reaction mixture, light yellow 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 7.2. g;m.p. 190°C.

Characterisation of HTT

The reagents have been characterized by IR and ^{1}H NMR spectral data. Infrared spectrum of HTT shows bands at [3256(s); 3252(m,br)]; 3143(s),3135(m);3060(s),1670(m);1610(s),1431(s); 1362(s);1290(s)1202(s),1075(m),:756(δ),722(δ),

689(δ); cm-1 respectively corresponding to υNH-symmetric, υC=N symmetric, υ (C-H) aromatic stretch, υ (C=S) stretching υ (C=N) aromatic ring, δ(C-H) of Thiadiazole ring, (HTT and δ (C-H)-oop, bend (aromatic) and δ (C-C)-oop bend aromatic ring vibrations. ^{1}H NMR spectra of HTT ($CDCl_3$ + DMSO-d6) showed signals at 3.34, (2H,s); 7.70, (3H,m); 7.56 (1H,s) ,4.86(1H,s),3.25(1Hs)due$C_3H_2N_2O$(Hydentoin to CH),due to(1H)-$C_2H_3N_3S_2$(Thiadiazole),) C=N and =C-SH,-C-NH(hydrazine) proton groups.

pK_a values of reagents

The pK_a values were determined by recording the UV-Visible spectra of 6.45 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 HTT were 6.61 (pK_1); 8.30(pK_2).

The reagent (HTT) 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.

A stock solution (1 mg L-1) was prepared by dissolving 271.50 mg of $HgCl_2$ (E.Merck preanalysis) in 1000-ml de-ionized water. . Dilute standard solutions were prepared from this stock solutions as and when required.

Recommended procedure

An aliquot of the solutions containing 0.24-2.36 mg/ml(or ppm) of mercury(II) ,10 ml of NaOAc-AcOH buffer solution (pH 6.0) and 1.0 ml of 0.01 M HTT 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 490 nm against

respective reagent blank. The measured absorbance is used to compute the amount of mercury 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[12-13]. 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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