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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF ARSENIC IN BIOLOGICAL SAMPLES USING 2-ACETYL-5-CHLORO THIOPHENE 5-AMINO-1,3,4-THIADIAZOLE-2-THIOL

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ABSTRACT

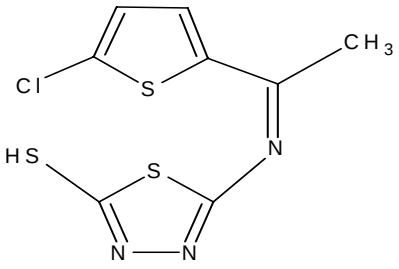
Analytical application of 2-acetyl-5-chlorothiophene5-amino1,3,4-thiadizole-2-thiol (ATT) is described for the direct non-extractive spectrophotometric determination of Arsenic. The reagents react with arsenic, in acidic medium (pH 6.0., sodium acetate-acetic acid buffer) to form light yellow colored1:2 (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 ATT methods are found to be 1.45 X 10^{4} L mol^{-1} cm^{-1} and 0.0040 µg cm-2 of As^{III} respectively. The systems obey Beer's law in the range of 0.123-2.537 µg/ml of As^{III}. Since ATT method is more sensitive it was applied for the determination of Arsenic in biological samples.

Keywords: Spectrophotometry, Arsenic, 2-Acetyl-5-chlorothiophene5-amino1, 3, 4-thiadizole-2-thiol, Biological samples.

INTRODUCTION

2-acetyl-5-chlorothiophene[1-3] 5-amino amino1, 3, 4-thiadizole-2-thiol is new important reagent used for the spectrophotometric determination of metal ions Arsenic. Arsenic compound widely used and have long been recognized as toxicants[4-6]. Arsenic is widely distributed in the nature. It occurs as inorganic and organic compounds as trivalent [7-8].Animals vary in their arsenic accumulation depending upon the type of food they consume (John & Jeanne, 1994)[9].Acute arsenic exposure can give symptoms with rapid onset of headache, nausea and severe gastrointestinal irritation (Allan *et al.,* 1995)[10]. Similarly, increased levels of copper cause liver, kidney and brain damage, which may follow hemolytic crisis (Judith, 1994)[11]. 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[12]. 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. -acetyl-5-chlorothiophene5-amino1,3,4-thiadizole-2-thiol (ATT). The spectrophotometric determination of Arsenic using ATT is included in this paper. Since the latter reagent is more sensitive, it was used for the determination of Arsenic in various biological samples.



ATT

MATERIALS AND METHODS

Preparation of ATT

The reaction mixture containing 2-acetyl-5-chloro-thiophene(3g,0.0186mol in 10ml of methanol)5-amino1,3,4-thiadizole-2-thiol (2.48g, 0.044mol 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 3.2. g;m.p. 212°C.

Characterisation of ATT

The reagents have been characterized by IR and ^{1}H NMR spectral data. Infrared spectrum of ATT shows bands at [3340(s); 3251(m,br)]; 3178(m), 31340(m);1609(m); 1552(s),1435(s); 1362(s); 1174(m), 1297(m); 760(δ), 720(δ); cm-1 respectively corresponding to υNH-symmetric, υC=N symmetric, υ (C-H) aromatic stretch, υ (C=S) stretching υ (C-C) aromatic ring, δ(C-H) of Thiophene ring, (ATT and δ (C-H)-oop bend (aromatic) and δ (C-C)-oop bend aromatic ring vibrations. ^{1}H NMR spectra of ATT ($CDCl_3$ + DMSO-d6) showed signals at 2.54, (3H,s); 7.15-7.69, (4H,m); 7.69 (1H,s) due to CH_3, C_4H_2S(Thiophene),$C_2H_2N_2S$(Thiadiazole) C=N and =C-NH(hydrazine) proton groups of pK_a values of reagents:

The pK_a values were determined by recording the UV-Visible spectra of 4 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 ATT were 6.6 (pK_1); 8.3 (pK_2).

The reagent (ATT) 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 NH4cl-2 NH_4OH (pH 7-10) solutions were used.

A stock solution (1 mg L-1) was prepared by dissolving 312.01 mg of $Na_2HAsO_4.7H_2O$ (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 Arsenic(III) ,10 ml of NaOAc-AcOH buffer solution (pH 6.0) and 1.0 ml of 0.01 M ATT 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 280 nm against respective reagent blank. The measured absorbance is used to compute the amount of Arsenic 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[14-15]. 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.

RESULTS AND DISCUSSION

The reagents ATT may be easily prepared. The reagent solutions (0.01M) are found to be stable for 24 h. The absorption band from 270 to 295 nm indicates that in solution on increasing the pH, The colour reactions of some important metal ions with ATT are summarized in Table1. In basic medium (above pH 8.56) coordinates the tetravalent metal ion as mono anion to give neutral complexes[13].

Arsenic (III) reacts with ATT in acidic pHs to give water soluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition of metal ion, reagent (ATT), 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 ($4X10^{-5}$M) solutions of As (III) and ATT were used in the calculation of stability constants of the complexes.

Table 1: Physico-chemical and analytical properties of As^{III} complexes with ATT

S.No.	Characteristics	As-ATT
1	λmax (nm)	280
2	pH range (optimum)	5.0-8.0
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)	0.236-2.357
6	Molar absorptivity (L $mol^{-1}cm^{-1}$)	$1.45 X 10^4$
7	Specific absorptivity (ml g^{-1} cm^{-1})	0.25
8	Sandell's sensitivity (µg of As^{III} cm-2)	0.0047
9	Composition of the complex as obtained in Job's and molar ration methods (M:L)	1:2
10	Stability constant of the complex	$4.40 X 10^{10}$
11	Standard deviation	0.0049
12	Relative standard deviation (RSD)	0.46

The effect of various cations and anions which are generally associated with the metal ion in the determination Arsenic (III) was studied by measuring the absorbance of Arsenic the a complexes containing 1.4µg/ml of Arsenic (III) 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 ATT 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); Pb^{2+} (41,47); Cd^{2+} (23,26); 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^{+} (2,5); 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 Hg^{2+} (40,48) do not interfere in the presence of 600ppm of iodide.

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

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

Table 2: Determination of Arsenic in liver & kidney samples

Sample	Arsenic (µG/G)[a] Added	Found	Recovery ±S.d.%
Beef Liver	0	5.23	
	100	105.02	95.9 ± 0.55
	500	506.2	100.6 ± 0.48
Sheep Liver	0	3.80	
	100	103.80	100.0 ± 0.3
	500	505.10	100.5±0.19
Beef Kidney	0	4.60	
	100	104.02	96.9 ± 0.42
	500	506.10	100.4 ± 0.2
Sheep Kidney	0	4.0	
	100	104.03	100.0 ± 0.1
	500	506.10	100.5 ± 0.51

[a] Average of five determinations

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