SPECTROPHOTOMETRIC DETERMINATION OF ARSENIC, LEAD IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES USING 2,5-THIOPHENE DICARBOXYALDEHYDE, THIOSEMICARBAZONE

MD. FAKRUDDIN ALI AHMED, Y. LINGAPPA, A. GLORY

ABSTRACT

Analytical application of 2,5-thiophene dicarboxaldehyde, thiosemicarbazone (TDATSC) is described for the direct non-extractive spectrophotometric determination of AsIII and Pbi in aqueous environmental and biological samples. The reagents react with arsenic, in acidic medium (pH 6.0, sodium acetate-acetic acid buffer) to form light yellow colored, 2:1 (M: L) complexes. The reagents react with lead in basic medium (pH 9.0, buffer) to form light blue colored, 2:1 (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 TDATSC methods are found to be 1.45 X 10^4 L mol^-1 cm^-1 and 0.0014 µg cm^-2 of AsIII, of. 1.89 X 10^4 L mol^-1 cm^-1 and 0.0019 µg cm^-2 of Pbi respectively. The systems obey Beer’s law in the range of 0.123-2.537 µg/ml of AsIII, 2.0-2.4 µg/ml of Pbi. Since TDATSC method is more sensitive it was applied for the determination of Arsenic, lead in environmental and biological samples.

Key words: Spectrophotometry, Arsenic, Lead, 2,5-thiophene dicarboxaldehyde, thiosemicarbazone in environmental and biological samples.

INTRODUCTION

Thiosemicarbazones are important reagent widely used for the spectrophotometric determination of metal ions2a,10,17. 2, 5-thiophene dicarboxaldehyde, thiosemicarbazone is new important reagent used for the spectrophotometric determination of metal ions arsenic, lead. Arsenic compound widely used and have long been recognized as toxicants5,15,22. Trace concentration of arsenic can also affect the physical and mechanical properties of metal and alloys23. In environmental arsenic is widely distributed in the nature. It occurs as inorganic and organic compounds in many environmental matrices such as natural water and soil as trivalent and tetravalent19,20,21. Animals vary in their arsenic accumulation depending upon the type of food they consume (John & Jeanne, 1994). Acute arsenic exposure can give symptoms with rapid onset of headache, nausea and severe gastrointestinal irritation (Allan et al., 1995). Similarly, increased levels of copper cause liver, kidney and brain damage, which may follow hemolytic crisis (Judith, 1994). 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 meat22.

Lead is a cumulative poison that enters the body from lead water pipes, lead-based paints and leaded petrol (Renner, R. 1995)24. Presence of even traces of Pb(II) in environmental samples leads to environmental pollution and many fatal diseases including dysfunction of renal blood and neurological systems. Pb(II) easily deposits in blood, kidney, reproductive system, nervous system and brain, and acute lead poisoning can result in colic shock, severe anaemia and irreversible brain damage. Lead compounds as ant-knocking agents in automobile fuels cause air pollution.

The determination of trace amounts of lead is very important in the context of environmental monitoring however a large number of spectrometric methods for determination of lead are reported to face interference due to the presence of several metal ions (Du, B. et al. 20023, Fargussion, J. 19905, Ferreira, S. L. C. et.al 19916, Dutta, S. & Das, A. K. 20027, Dutta, S. & Das, A. K. 20074, Kiehei, U. et. al 200011, Mondal14, B. C., Das, A. K. 20022, Dutta, S. & Das, A. K. 200719. The present study was planned to determine the prevalence of selected Trace elements in lean and organ pig kidney and liver, and in water samples.

This paper describes synthesis, characterization and analytical properties of new reagents 2,5-thiophenedicarboxaldehyde, thiosemicarbazone TDATSC. The spectrophotometric determination of arsenic, lead using TDATSC is included in this paper. Since the latter reagent is more sensitive, it was used for the determination of lead, Arsenic in various biological and water samples.
Experimental

Preparation of TDATSC

The reaction mixture containing 2,5-thiophene dicarboxaldehyde
(1g, 0.00708 mol in 20ml of methanol ) thiosemicarbazone (0.64g,
0.00708 mol in 20ml of methanol dissolved in hot condition) was
taken in 250-ml round bottom flask and refluxed for 4h. On cooling
the reaction mixture, dark yellow coloured product was formed. It
was collected by filtration and washed with hot water and 50
percent cold methanol. This compound recrystallised from ethanol
and dried in vacuo, Yield 1.4. g; m. p. 86°C.

Characterisation of TDATSC

The reagents have been characterized by IR and 1H NMR spectral
data. Infrared spectrum of TDATSC shows bands at [ 3 3 4 0 ( s ];
3251(m, brj); 3178(m), 3140(m);1609(m); 1552(s),1435(s);
1362(s); 1248(m) 1174(m), 1297(m); 842(8), 733(6); cm⁻¹
respectively corresponding to NH asymmetric, NH asymmetric,
(C-H) aromatic stretch, υ(C=O) stretching, υ(C≡N) symmetric, υ(C=C)
aromatic ring, υ(C=O) of Thiophene ring, (TDATSC) and υ (C=O)‐COOP
( aromatic ) and υ (C‐C)‐COOP aromatic ring vibrations. 1H
NMR spectra of TDATSC (CDCl₃ + DMSO‐d₆) showed signals at
3.4(1H; s); 7.24-7.5 7.81-7.88, (4H,m). The δ values are respectively
N=NH(hydrazine) proton groups of thiosemicarbazone.

pKₐ values of reagents

The pKₐ values were determined by recording the UV-Visible spectra
of 4 X 10⁻⁵ mol solutions of the reagents 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 TDATSC were 6.91 (pKₐ); 8.94(pKₐ).

The reagent (TDATSC) 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
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 NaHc₂H₅O (pH 7-10)
solutions were used. A stock solution (1 mg l⁻¹) was prepared by
dissolving 312.01 mg of Na₂HAsO₄.7H₂O (E.Merck preanalysis) in
1000-ml de-ionized water. Dilute standard solutions were
prepared from this stock solutions as and when required.

1000 ppm stock solution of Lead was prepared by
dissolving 1.83 g of lead acetate in one litre of distilled water.
(E.Merck preanalysis) in 1000-ml de-ionized water. Dilute standard
solutions were prepared from this stock solutions as 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 TDATSC 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
calculate 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-610H meter were used in the present study.

Dried pig 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 13,12,2. 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.

Determination of arsenic, lead in natural and polluted water

Water samples from a river receiving in polyethylene bottles and
filtered through whatman 41 filter paper. Pb(II)|As(V) if any is
reduced to As(III) by the process described. Arsenic and lead
content was determined directly according to the recommended
method and also by the reported method15.

RESULTS AND DISCUSSION

The reagents TDATSC may be easily prepared. The reagent solutions
(0.01M) are found to be stable for 24 h. The absorption band from
279 to 295 nm indicates that in solution on increasing the pH, the
colour reactions of some important metal ions with TDATSC are
summarized in Table1. In basic medium (above pH 9.0) coordinates
the tetravalent metal ion as mono anion to give neutral complexes13.

Arsenic (III), Lead(II) reacts with TDATSC in acyclic, basic 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 (TDATSC), and buffer has no effect on the absorbance of
complexes. Analytical characteristics of the complexes are
summarized in Table 1. The stoichiometry of the complexes (M:L =
2:1) was determined by job's continuous variation and molar ratio
methods. Sodium acetate (0.2M)-acetic acid (0.2M) buffer solution
(pH 6.0, pH 9.0, and T=300 K) and equimolar (1.45 X 10⁻⁴ M),(1.89 X
10⁻⁴ M) solutions of As (III), Pb (II) and TDATSC were used in the
use of 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 Arsenic (III),
Pb(II) was studied by measuring the absorbance of Arsenic the a
complexes containing 1.42 mg/ml of Arsenic (III), 1.22 mg/ml Pb(II) in
solution. The colour reaction is developed as described in the
standard procedure. An error of ±2% in the absorption reading was
considered tolerable. The tolerance limit (TL) values in ppm for
various anions and cations in TDATSC methods respectively are as
follows: citrate (1152,1152); tannate (888,888); ascorbate
(752,752); iodate (761,761); iodide(612,761), thioanate
(507,507); phosphate(465,465); urac (384,384); bromide
(317,317); sulphate (252,384); thiosulphate (246,264), nitrate
(212,244); oxalate (281,281); fluoride (75,75), Ba²⁺ (675,800); Mn²⁺
(275,325); Mg²⁺ (125,150); Sr²⁺ (1 00,125); W²⁺ (110,110); Sn²⁺
(47,47); Cd²⁺ (32,63); Mo⁶⁺ (9,193); Tl³⁺ (14,14); Fe³⁺ (12,10); Cr³⁺
(10,12); Zn (10,10), Pt⁺⁺ (88); Fe⁺⁺(5,4); Au (4,4); Ag⁺⁺ (2,5); Ni²⁺
(1,12); Cu²⁺(1,1). Higher amounts of Fe³⁺ (13,17) do not interfere in
the presence of 70ppm of fluorid. Larger amounts of Hg²⁺ (40,48)
do not interfere in the presence of 500 ppm of iodide.

The present method (TDATSC) 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),lead(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. A non-extractive spectrophotometric
determination of Arsenic (III),lead(II) in aqueous medium.
Table 1: Physico-chemical and analytical properties of As⁵⁺, Pb⁰ complexes with TDATSC

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characteristics</th>
<th>As-TDATSC</th>
<th>Pb-TDATSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λmax (nm)</td>
<td>280</td>
<td>442</td>
</tr>
<tr>
<td>2</td>
<td>pH range (optimum)</td>
<td>6.0</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>Mole of reagent required per mole of metal ion for full colour development</td>
<td>10-fold</td>
<td>10-fold</td>
</tr>
<tr>
<td>4</td>
<td>Time stability of the complex (in hours)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Beer’s law validity range (µg/ml)</td>
<td>0.236-2.357</td>
<td>2.0-2.4</td>
</tr>
<tr>
<td>6</td>
<td>Molar absorptivity (L mol⁻¹ cm⁻¹)</td>
<td>1.4 X 10⁴</td>
<td>1.89 X 10⁴</td>
</tr>
<tr>
<td>7</td>
<td>Specific absorptivity (ml g⁻¹ cm⁻¹)</td>
<td>0.25</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>Sandell’s sensitivity (µg of As⁵⁺ cm⁻³)</td>
<td>0.0014</td>
<td>0.0019</td>
</tr>
<tr>
<td>9</td>
<td>Composition of the complex as obtained in Job’s and molar ration methods (M:L)</td>
<td>1:2</td>
<td>2:1</td>
</tr>
<tr>
<td>10</td>
<td>Stability constant of the complex</td>
<td>4.40 X 10¹⁰</td>
<td>8.40 X 10¹⁰</td>
</tr>
<tr>
<td>11</td>
<td>Standard deviation</td>
<td>0.0049</td>
<td>0.0042</td>
</tr>
<tr>
<td>12</td>
<td>Relative standard deviation (RSD)</td>
<td>0.46%</td>
<td>0.36%</td>
</tr>
</tbody>
</table>

Table 2: Determination of Arsenic, lead in liver & kidney

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arsenic (µg/g)</th>
<th>Recovery</th>
<th>Sample</th>
<th>Lead (µg/g)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADDED</td>
<td>FOUND</td>
<td></td>
<td>ADDED</td>
<td>FOUND</td>
</tr>
<tr>
<td></td>
<td>±S.D.%</td>
<td>±S.D.%</td>
<td></td>
<td>±S.D.%</td>
<td>±S.D.%</td>
</tr>
<tr>
<td>Pig liver</td>
<td>0</td>
<td>0.517</td>
<td>Pig liver</td>
<td>0</td>
<td>0.317</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100.52</td>
<td>98.9 ± 0.53</td>
<td>100</td>
<td>103.02</td>
</tr>
<tr>
<td>500</td>
<td>501.02</td>
<td>100.6 ± 0.48</td>
<td>100.6 ± 0.48</td>
<td>500</td>
<td>505.10</td>
</tr>
<tr>
<td>Pig kidney</td>
<td>0</td>
<td>0.28</td>
<td>Pig kidney</td>
<td>0</td>
<td>0.243</td>
</tr>
<tr>
<td>100</td>
<td>100.30</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100</td>
<td>102.02</td>
</tr>
<tr>
<td>500</td>
<td>503.10</td>
<td>100.3 ± 0.19</td>
<td>100.3 ± 0.19</td>
<td>500</td>
<td>505.10</td>
</tr>
</tbody>
</table>

Average of five determinations

Table 3: Determination of arsenic in water sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>proposed µg/ml±S</th>
<th>Reference² µg/ml±S</th>
<th>t-test² method</th>
<th>F-test² Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polluted water sample</td>
<td>1.02 ± 0.02</td>
<td>1.01 ± 0.03</td>
<td>0.62</td>
<td>2.25</td>
</tr>
<tr>
<td>(d) ground water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polluted water sample</td>
<td>2.02 ± 0.05</td>
<td>1.99 ± 0.07</td>
<td>0.52</td>
<td>1.62</td>
</tr>
<tr>
<td>(e) tap water</td>
<td>1.19 ± 0.05</td>
<td>17 ± 0.05</td>
<td>0.7</td>
<td>1.56</td>
</tr>
<tr>
<td>Polluted water sample</td>
<td>1.30 ± 0.02</td>
<td>1.34 ± 0.03</td>
<td>1.62</td>
<td>2.25</td>
</tr>
<tr>
<td>(f)</td>
<td>1.52 ± 0.05</td>
<td>1.59 ± 0.06</td>
<td>0.57</td>
<td>1.62</td>
</tr>
<tr>
<td>(g)</td>
<td>1.40 ± 0.04</td>
<td>1.37 ± 0.03</td>
<td>0.45</td>
<td>1.76</td>
</tr>
<tr>
<td>Polluted water sample</td>
<td>0.49 ± 1.44</td>
<td>0.501 ± 1.60</td>
<td>2.35</td>
<td>2.25</td>
</tr>
<tr>
<td>(h)</td>
<td>1.49 ± 0.37</td>
<td>1.50 ± 0.07</td>
<td>2.63</td>
<td>2.78</td>
</tr>
<tr>
<td>(i)</td>
<td>2.49 ± 0.20</td>
<td>3.02 ± 0.39</td>
<td>2.73</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Mean ± Relative Standard Deviation (n = 5)

Table 4: Determination of lead in water sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>proposed µg/ml±S</th>
<th>Reference² µg/ml±S</th>
<th>t-test² method</th>
<th>F-test² Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polluted water sample</td>
<td>0.49 ± 1.44</td>
<td>0.501 ± 1.60</td>
<td>2.35</td>
<td>2.25</td>
</tr>
<tr>
<td>(d)</td>
<td>1.49 ± 0.37</td>
<td>1.50 ± 0.07</td>
<td>2.63</td>
<td>2.78</td>
</tr>
<tr>
<td>(e)</td>
<td>2.49 ± 0.20</td>
<td>3.02 ± 0.39</td>
<td>2.73</td>
<td>2.26</td>
</tr>
</tbody>
</table>

REFERENCES


