CHAPTER V
SPECTROPHOTOMETRIC DETERMINATION OF MERCURY AND SOME SULPHUR CONTAINING COMPOUNDS

A: MERCURY:

Mercury with electronic configuration 5d\textsuperscript{10} 6s\textsuperscript{2}, can exist in two different oxidation states i.e. 1+ and 2+. Mercurous ion (oxidation state 1+) exists as a dimer Hg\textsubscript{2}\textsuperscript{2+}. The Hg(II) ion being a soft acid can form highly stable complex with soft bases. (eg. HgI\textsubscript{4}\textsuperscript{2−}). In its 2+ oxidation state, mercury forms four co-ordinated (tetrahedral) compounds and exhibits a high tendency to form complexes with ligands containing S or N donor atoms. Mercury(II) also forms two co-ordinated linear complexes.

Mercury is a virulent poison. The element itself and its compounds are readily absorbed through the respiratory tract, the gastrointestinal tract or even the unbroken skin. The toxic limit of mercury vapour in air is of the order of 100 γ/m\textsuperscript{3} but continuous exposure to levels as low as 1 γ/m\textsuperscript{3} may lead to symptoms of chronic mercury poisoning within a few weeks. Hence, mercury compounds must be handled with utmost care.

Spectrophotometric reagents for mercury:

Dithizone is the most widely used reagent for the determination of mercury in different types of materials like biological (1,2), soil (3), coal (4) etc. Other reagents which have been used for the purpose are: di-2-naphthylthiocarbazone (5), oxamide bis(phenylhydrazone) (6), nitrofurazone (7,8), sulpharsazen (9),
thiothenoyltrifluoroacetone (10,11), crystal violet (12), rhodamine B (13), brilliant green (14), xylenol orange (15), methylene blue (16), pyrazolene dyes (17). ruhemann's purple (18), antipyrinyl dithioformic acid (19), 1,3-bis(4-nitrophenyl)triazine (20), bendschedler's green (21). jenus green (22), solid reagent prepared from the reaction of brilliant green and NaBPh₄, mixed in 1:1 ratio (23) thiomaltol(2-methyl-3-hydroxy-4-thiopyrone (24), Phloxine B-1,10-phenanthroline (25), 1(5)-(2-chloro-3-pyridinyl)-3-(4-methoxyphenyl)-5-(1)-(3-chloro-2-quinoxaliny1) formazan (26), nitroso-R-salt (27), phenyl-α-pyridyltriazone and p-nitro phenyl-α-pyridyltriazone (28), dithizone-S (29), phthalazinylformazans (30), N-substituted-4,6-diphenylpyridine-2-thiones (31), glycine cresol red and methylthymol blue (32), hydrazinecarbothioamide-2-[(4-hydroxy-3-methoxyphenyl)methylene] (33), xylenol orange (34) mono and dithiosemicarbazones (35), safranine T (36), rhodamine 6-G (37), copper diethyldithiocarbamate (38), copper thiuramate (39), 4,4-dinitrodiazamino benzene (40), EDTA (41), ferric tris-(4-methylpyridine-2-thione-1-oxide (42), ferrocyanide (43), 2,2'-bipyridyl (44), neutral red (45), methyl violet (46), nitrofurazone (47), 5-nitro-2-furaldehydesemicarbazone (48), thiamine (49), titan yellow (50), variamine blue B (51), caprolactam (52).

Among the heterocyclic azo dyes used for the purpose, mention may be made of 4-(2-pyridylazo)resorcinol (53), 1-(2-pyridyl-
azo)resorcinol (53), 1-(2-pyridylazo)phenanthrene-9-ol and 2-(2-pyridylazo)acenaphthylene-8-ol (54), 2-(2-pyridylazo)-p-cresol and its halo derivatives (56), 5-diethylamino-2-(2-pyridylazo)phenol and its halo derivatives (57), 2-(2-thiazolylazo)-4-methoxyphenol (58), 4-(4,5-dimethyl-2-thiazolylazo)-2-methyl resorcinol (59), azoxine ash [4-(8-hydroxy-7-quinolylazo)-5-hydroxy-2,7-naphthalene disulphonic acid], and azoxine Ts [di-k_{3}{(8-hydroxy-7-quinolylazo)-1,5-naphthalenedisulphonate}] (60), 2-(2-pyridylazo)-1-naphthol, 1-(4-methyl-2-thiazolylazo)naphthol and 1-(2-thiazolylazo)-2-naphthol (61), 5-(2-thiazolylazo)-2-monoethylamino-p-cresol, 2-(2-thiazolylazo)-5-diethylamino-m-phenol and 5-(2-quinolylazo)-2-monoethylamino-p-cresol (62) and 2-(4-antipyrylazo)-5-diethylaminophenol (63).

Some of the recently introduced reagents are Michler's thioketone (64), 3-hydroxypicolinaldehydeazine (65), 4-(4'-dimethylamino)phenyl-1-phenylthiosemicarbazide (66), 5-(benzothiazol-2-yl)-3-(2-hydroxyphenyl)-1-phenyl formazan (67), ammonium (2'-amino-3'-hydroxyphenyl-4'-azo)benzene-4-arsonate (68), 3-methyl-5-oxopyrazoline-4-carbodithioic acid (69), 5',6'-dibromo-2',3',4'-trihydroxyacetophenone (70), N-p-chlorophenyl benzoxyhydroxamic acid (71) and ammonium(2',3'-dithydroxyphenyl-4-azo)benzene-4-arsonate (72), 1-(2-lepidylazo)-2-acenaphthylenol (73), tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine
trisodium salt (74), a critical account for the various spectrophotometric reagents used for mercury has also been given by S. Chilov (75).

**EXPERIMENTAL**

**Reagents:**

**Stock solution of mercury(II):**

A stock solution of mercury(II) was prepared by dissolving appropriate amount of mercuric chloride (Analytical grade) in acidulated doubly distilled water. The solution was standardized gravimetrically (76).

**Buffer solutions** (77)

- **Borate buffer, pH 10:** This buffer was prepared by diluting 250 ml of a solution containing 12.369 g boric acid and 24.911 g of potassium chloride per litre and 220 ml of 0.2 M sodium hydroxide solution to 1 l with distilled water.

- **Acetate buffer, pH 5.5:** This buffer was prepared by mixing 0.2 M acetic acid solution and 0.2 M sodium acetate solution in the ratio 1:9, in a total volume of 1 l.
A.1 Spectrophotometric determination of mercury with PBA

1. Preliminary studies:

A series of solutions containing mercury(II) and excess of reagent (PBA) was maintained at different pH levels in a total volume of 10ml. The complex was precipitated when ethanolic concentration was less than 40% (v/v); however, precipitates solublised when ethanolic concentration was more than 60% (v/v). The precipitates were also soluble in water-immiscible solvents. It was found that colour intensity of the complex after extraction in chloroform was found to maximum. The complex was stable for about 4h.

However there was no colour reaction of mercury(II) with reagent PBP.

2. Spectral behaviour of the complex and effect of pH:

A series of solutions containing 1ml of 2x10^{-4}M mercury(II) and 4ml of reagent (5x10^{-4}M) were prepared and pH's were adjusted at different levels. The colour of the complex was extracted in 10ml of chloroform. The spectra of the solutions were recorded against the corresponding reagent blank. Fig V.1 shows the spectra of the mercury(II)-PBA complex which absorbs maximum at 520nm. A plot of pH vs. absorbance at \( \lambda_{\text{max}} \), showed that the constant and maximum absorbance was exhibited in the pH range 8.5-10.5 (Fig.V.2). Numerous buffers were tried in this pH range, however, borate buffer of pH 10 was found to be best suited.
Fig X.2. EFFECT OF pH ON Hg(II)-PBA COMPLEX.

Fig XI. ABSORPTION SPECTRA OF Hg (II)-PBA COMPLEX AT DIFFERENT pH.

Fig X.1. ABSORPTION SPECTRA OF Hg (II)-PBA COMPLEX AT DIFFERENT pH.

Fig X.2. EFFECT OF pH ON Hg(II)-PBA COMPLEX.
for this purpose. 1-5ml of this buffer had constant and maximum absorbance and therefore, in subsequent studies 2ml of this buffer was used.

3. **Effect of reagent concentration:**

For this study a series of solutions containing 1ml of $2 \times 10^{-4}$M mercury(II) and varying amounts of reagent and 2.0ml of borate buffer was prepared. The complex was extracted in 10ml of chloroform and absorbance measured against corresponding reagent blank at 520nm. It was found that 3 times molar excess of PBA was required for maximum complexation. (Fig V.3) However, in further studies 5 times molar excess of the reagent was maintained.

4. **Physico-chemical characteristics of the complex. Beer's law validity and ringbom plots:**

Linearity between the absorbance of the complex and mercury(II) concentration was examined by varying the concentration of mercury(II) in solution, containing a fixed amount of ligand (sufficient excess), maintaining the required pH with buffer and extracting the complex in 10ml of chloroform. Absorbance was measured at $\lambda_{\text{max}}$ against corresponding reagent blank. Results obtained for validity of Beer's law, optimum range of concentration as calculated from Ringbom's plot (Fig. V.4). Sensitivity and molar extinction coefficient are summarised in Table V.1.
Fig. 3. EFFECT OF REAGENT CONCENTRATION ON Hg (II) - PBA COMPLEX.

Fig. 4. RINGBOM PLOT FOR Hg(II) - PBA COMPLEX.

Hg(II) = \(2 \times 10^{-5}\) M
Table V.1: Physico-chemical constants of the mercury(II)-PBA complex.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>520</td>
</tr>
<tr>
<td>Beer's law validity range, ppm</td>
<td>0.0-7.0</td>
</tr>
<tr>
<td>Optimum concentration range, ppm</td>
<td>1.5-5.0</td>
</tr>
<tr>
<td>Sandell's sensitivity (ug.Hg(II).cm$^{-2}$)</td>
<td>0.005</td>
</tr>
<tr>
<td>Molar extinction coefficient ($\epsilon$) (l.mol$^{-1}$.cm$^{-1}$)</td>
<td>4.0x10$^4$</td>
</tr>
</tbody>
</table>

5. Molar composition of the complex:

The stoichiometry of the complex was established by Job's method of continuous variations. Mercury(II) and reagent solutions of equal molarities were mixed in different ratios followed by buffer to adjust pH and then extracting in chloroform. The curve obtained by plotting absorbance vs. mole fraction of the metal ion is shown in (Fig. V.5). It is clear from the curve that metal to ligand ratio is 1:2 in mercury(II)-PBA complex.

6. Recommended procedure:

To an aliquot containing 15-50 ug of mercury(II), add sufficient excess of reagent (4ml of 5x10$^{-4}$ M PBA) and 2ml of borate buffer pH10. Extract the complex in 10ml of chloroform and measure the absorbance at 520nm, against corresponding reagent blank. Knowing the absorbance, the amount of mercury in the unknown solution is deduced from the calibration curve drawn under similar conditions.
Fig. 5. COMPOSITION OF Hg(II)-PBA COMPLEX BY JOB'S METHOD.
7. **Absorbance deviations and accuracy of the method:**

A series of ten solutions were prepared containing 1ml of 2x10^{-4}M of mercury(II), sufficient excess of the reagent and 2.0ml of buffer. The complex was extracted in 10ml of chloroform and absorbance was found to be 0.80 with a standard deviation of 0.0052.

To check the accuracy of the method a series of solutions containing different amounts of mercury(II) and a fixed amount of the reagent were prepared. Mercury(II) contents were determined in these solutions, following the recommended procedure. Table V.2 shows that the accuracy of method lies within permissible range, accepted for spectrophotometric determinations.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Amount of Hg(II)/10ml taken(ppm)</th>
<th>Hg(II) found (ppm)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15.2</td>
<td>15.4</td>
<td>+1.3%</td>
</tr>
<tr>
<td>2.</td>
<td>22.8</td>
<td>22.5</td>
<td>-1.3%</td>
</tr>
<tr>
<td>3.</td>
<td>30.4</td>
<td>30.6</td>
<td>+0.66%</td>
</tr>
<tr>
<td>4.</td>
<td>38.0</td>
<td>38.0</td>
<td>0.0%</td>
</tr>
<tr>
<td>5.</td>
<td>45.6</td>
<td>45.0</td>
<td>-1.31%</td>
</tr>
</tbody>
</table>
8. Effect of diverse ions:

In the determination of mercury(II) the effect of diverse ions has been studied by preparing a synthetic solution containing 4.0 µg/ml of mercury(II) and different amounts of diverse ions. The mercury content in these mixtures were determined following the recommended procedure. Fluoride, chloride, bromide, nitrate, nitrite, phosphate, acetate, sulphate, sulphide, sulphite, cyanide, thiosulphate, thiocyanate, oxalate, tartrate, borate (upto 1000 fold) calcium, niobium, tantalum, aluminium (upto 100 fold) do not interfere.

The tolerance of other anions and cations which do not cause deviation of ±2% in absorbance and which interfere seriously are listed in Table V.3

Table V.3: Effect of diverse ions.

<table>
<thead>
<tr>
<th>Foreign Ions</th>
<th>Tolerance limits</th>
<th>Masking agent, if any</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>20 fold</td>
<td></td>
</tr>
<tr>
<td>Iodide</td>
<td>100 fold</td>
<td></td>
</tr>
<tr>
<td>Thiourea</td>
<td>S.I</td>
<td></td>
</tr>
<tr>
<td>Thiosemicarbazide</td>
<td>S.I</td>
<td></td>
</tr>
<tr>
<td>Silver(I)</td>
<td>6 fold</td>
<td></td>
</tr>
<tr>
<td>Gold(III)</td>
<td>8 fold</td>
<td></td>
</tr>
<tr>
<td>Element</td>
<td>Value</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Manganese(II)</td>
<td>25 &quot;</td>
<td></td>
</tr>
<tr>
<td>Iron(II)</td>
<td>15 &quot;</td>
<td></td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>20 &quot;</td>
<td></td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>15 &quot;</td>
<td></td>
</tr>
<tr>
<td>Copper(II)</td>
<td>4 &quot;</td>
<td></td>
</tr>
<tr>
<td>Zinc(II)</td>
<td>4 &quot;</td>
<td></td>
</tr>
<tr>
<td>Cadmium(II)</td>
<td>5 &quot;</td>
<td></td>
</tr>
<tr>
<td>Palladium(II)</td>
<td>10 &quot;</td>
<td></td>
</tr>
<tr>
<td>Chromium(III)</td>
<td>50 &quot;</td>
<td></td>
</tr>
<tr>
<td>Lead(II)</td>
<td>55 &quot;</td>
<td></td>
</tr>
</tbody>
</table>

SI - Serious interference

A.2. Spectrophotometric determination of mercury(II) with PBS.

1. Spectral characteristics of the complex and effect of pH:

A series of solutions containing known amount of mercury(II) (1.0ml of 2x10^-4M) and excess of PBS (2.0ml of 5x10^-4M) was prepared and pH's were adjusted at different levels in a total volume of 25ml. Absorption spectra of the solutions were recorded against the corresponding reagent blank. The absorption spectra of the complex formed at different pH levels are recorded in Fig. V.6 and showed the maximum absorbance at 568nm.
Plots of pH vs. absorbance of the complex at the $\lambda_{\text{max}}$ show that constant absorbance is exhibited in the pH range 4.5-6.5 (Fig V.7). An acetate buffer was found suitable for this purpose and the subsequent studies have been carried out at pH 5.5 using 2ml of acetate buffer.

2. **Effect of reagent concentration:**

   The effect of reagent concentration on complex formation was studied by preparing a series of solutions containing a fixed amount of mercury(II) (1.0ml of $2\times10^{-4}$M) and increasing amounts of the reagent PBS at pH 5.5. It was found that 3 times molar excess of PBS per mole of mercury(II) was needed for maximum colour development (Fig. V.8). In subsequent studies, however, 5 times molar excess of PBS was maintained.

3. **Physico-chemical characteristics of the complex:**

   Beer's law range and sensitivity: In Table V.4, Beer's law validity, optimum concentration range calculated from Ringbom plot (Fig. V.9), sandell's sensitivity and molar extinction coefficient of the complex are summarised.

<table>
<thead>
<tr>
<th>Table V.4. Physico-chemical characteristics of the mercury(II)-PBS complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. $\lambda_{\text{max}}, \text{nm}$</td>
</tr>
<tr>
<td>2. Beer's law validity range, ppm</td>
</tr>
</tbody>
</table>
Fig Y.6. ABSORPTION SPECTRA OF Hg (II) - PBS COMPLEX AT DIFFERENT pH.

Fig Y.7. EFFECT OF pH ON Hg(II) - PBS COMPLEX.
Fig. 8. Effect of reagent concentration on Hg(II) - PBS complex.

Fig. 9. Ringbom plot for Hg(II) - PBS complex.
4. Recommended procedure:

To an aliquot containing 15-100 μg of mercury(II) ion, add sufficient excess of PBS solution (5 molar times) followed by 2.0ml acetate buffer of pH 5.5, dilute to 25ml with double distilled water. Measure the absorbance of the complex formed at λmax against a reagent blank prepared under similar conditions. Calculate the unknown amount mercury from a standard calibration curve drawn under identical conditions.

5. Absorbance deviations and accuracy of the method:

The mean absorbance of a series of eight solutions containing 1ml of 2x10^{-4}M of mercury(II) and excess of PBS at pH 5.5 in a total volume of 25ml was found to be 0.355 with a standard deviation of 0.004.

The accuracy of the method was checked by preparing a series of solutions containing different amount of mercury(II) and sufficient excess but fixed amount of the reagent. Mercury(II) content was determined in these solutions, following the recommended procedure. Mercury(II) contents determined with %error are listed in Table V.5.
Fig V10. COMPOSITION OF Hg(II)-PBS COMPLEX BY JOB'S METHOD.
Table V.5. Accuracy of the method

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Amount of mercury(II)/25ml taken (ppm)</th>
<th>Mercury(II) found (ppm)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>21.8</td>
<td>21.6</td>
<td>-0.91</td>
</tr>
<tr>
<td>2.</td>
<td>32.7</td>
<td>32.52</td>
<td>-0.55</td>
</tr>
<tr>
<td>3.</td>
<td>43.6</td>
<td>43.6</td>
<td>0.0</td>
</tr>
<tr>
<td>4.</td>
<td>54.5</td>
<td>55.0</td>
<td>+0.91</td>
</tr>
<tr>
<td>5.</td>
<td>65.4</td>
<td>66.6</td>
<td>+1.8</td>
</tr>
</tbody>
</table>
6. Effect of diverse ions:

In the determination of mercury(II) at 1.6 μg/ml level, fluoride, chloride, bromide, nitrite, acetate, phosphate, sulphate, sulphite, sulphide, oxalate, borate, cyanide (upto 1000 fold), calcium niobium, tantalum, aluminium (upto 100 fold) do not interfere, while EDTA, nitrate, iodide, thiourea, thiocyanate, thiosulphate, thiosemicarbazide and citrate interfere seriously.

Tolerance limits of other anions and cations which do not cause deviation in absorbance of ±2% and which interfere seriously, in addition to the masking possibilities are summarised in Table V.6.

Table V.6: Effect of diverse ions

<table>
<thead>
<tr>
<th>Foreign Ions</th>
<th>Tolerance limits</th>
<th>Masking agent, if any</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese(II)</td>
<td>20 fold</td>
<td>masked by O₂⁻</td>
</tr>
<tr>
<td>Iron(II)</td>
<td>4 fold</td>
<td>masked by NO₂⁻</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>10 fold</td>
<td>masked by NO₂⁻</td>
</tr>
<tr>
<td>Nickel(II)</td>
<td>8 fold</td>
<td></td>
</tr>
<tr>
<td>Copper(II)</td>
<td>6 fold</td>
<td></td>
</tr>
<tr>
<td>Zinc(II)</td>
<td>6 fold</td>
<td></td>
</tr>
<tr>
<td>Cadmium(II)</td>
<td>8 fold</td>
<td></td>
</tr>
<tr>
<td>Chromium(III)</td>
<td>12 fold</td>
<td></td>
</tr>
</tbody>
</table>
Discussion:

Amongst the various spectrophotometric reagents used for mercury, dithizone (1) is the most widely used. In acid medium, mercury(II) forms a yellow-orange mercury dithizonate Hg(HD$_2$)$_2$, extractable in CHCl$_3$ or CCl$_4$. A violet mercury dithizonate, HgD$_2$ is formed in neutral or alkaline medium with a deficiency of dithizone. Dithizone also forms a large number of coloured complexes with different metal ions and this lead to a greater likelihood of interferences in analysis. The reagent is highly sensitive for the metal, however, due to instability of both the reagent and the complex, there has been a search for alternative procedures. The 2-naphthyl analogue of dithizone, viz., di-2-naphthylthiocarbazone has been used for the determination of mercury in air (5).

Mercury discharges the colour of 1,3-bis(4-nitrophenyl)triazine (20). This observation was utilized to develop a method for the determination of the metal. However Cd, Ag, Sb(V), and As(III) were found to interfere seriously.

Mercury(II) forms anionic complex with Cl$^-$, Br$^-$, I$^-$ and CN$^-$ ions which react with basic dyes to give ion-association complexes which are extractable in organic solvents. Crystal violet (12)
has been used to determine 0.1 µg of mercury. Other dyes such as methylene blue (16) and bindschedler's green (21) have been recommended as colorimetric reagents, but claimed to be less sensitive than dithizone.

Rhodamine 6-G (37) determines mercury at 200-1000 ppm. EDTA masks cupric, lead, bismuth and ferric ions. Stannous and arsenate ions are oxidised with bromine. Platanic ion is masked with sulphite and palladous ion by ammonia. Silver is precipitated as the iodide. When an aqueous solution of mercury is added to aqueous cupric diethyldithiocarbamate (38), the reduction in absorption by displacement of the copper is a measure of the mercuric ion. The same reaction is given by silver. The combined value for mercury and silver less that for mercury with the silver masked gives silver by difference. Copper thiuramate (39) is also known as mercupral. In benzene solution it is bleached by displacement of copper by mercuric ion. Cerium decolourised the reagent. Although mercuric ion is determined by displacement of cupric ion from copper diethyldithiocarbamate, mercuric diethyldithiocarbamate is also read in ultraviolet. The latter can be extracted with carbon tetrachloride from pH 4-11. If iron is present it coprecipitates mercury above pH 10. By the technique specified, there is no interference by 1mg of the majority of common elements. It tolerates 0.5mg of silver, 0.2mg of ruthenium, 0.1mg of lead, 0.01 mg of thallous ion and 2 µg of copper.
Mercuric ion reacts with the ferric complex of ferric tris-2-mercaptopyridine-1-oxide (42) to give a determination by difference. There is interference by cupric, bismuth, stannic, palladium, molybdenum (VI), nitrate and thiocyanate ions.

The bromomercurate ion is extractable with 1,2-dichloroethane as a complex with 2,2'-bipyridyl (44) at pH 4-5. There is no interference by zinc, cobalt, ferric ion, lead or bismuth. Upto 0.01mg per ml of copper and cadmium can be masked with 0.004 M nitrilotriacetic acid. While the complex of neutral red (45) with mercuric ion is extractable into nitrobenzene. Silver, stannous and stannic ions, cyanide, iodide, iodate, thiocyanate, dichromate and nitrate interfere. Whereas methyl violet (46) is read directly in aqueous solution. The method is applicable for upto 0.03mg of mercuric ion in the sample. Cadmium, cupric and ferric ions interfere. Replacement of potassium iodide by potassium bromide avoids this upto 0.06mg of the interfering ion per ml. In the acid medium mercuric ion forms a 1:2 complex with nitrofurazone (47), which is 5-nitro-2-furaldehyde semicarbazone. Oxalic, ascorbic, and citric acids, hydroxyl amine sulphate and stannous ion interfere. The reagent 5-nitro-2-furaldehyde semicarbazone (48) also called furacine, gives an orange complex with mercuric ion at pH 3.5-7.6. Beer's law applies upto 0.2 mM. The colour is destroyed by iodide, bromide, sulphide, sulphite, and thiosulphate ions. The complex of mercury with thiamine (49) is read by fluorescence. Beer's law is followed for 0.05-1.6 µg/ml. There
is interference by silver, stannous, bismuth, ferric, iodide, thiosulphate, sulphite, sulphide, ferrocyanide, ferricyanide, manganate, chromate, and dichromate ions.

Mercuric ion produces an intense yellow colour with titan yellow (50). The desirable pH of the solution is 6.8-9.5. For 0.1 mg of mercuric ion, add 4 ml of 1% titan yellow solution. Read at 456nm. Beer's law applies for 1-28 μg of mercury per ml. Mercury at 0.022-3.2mM can be determined by variamine blue B (51). The resulting complex is extracted with nitrobenzene and read at 605nm. For maximum colour development, the dye must be at least in three fold molar excess over the metal ion. Serious interference occurs with stannous, stannic, antimonous, iodide, cyanide and thiocyanate ions. With caprolactam (52), mercuric ion gives turbidity which can be read photometrically. Beer's law holds for 0.03-0.1mg of mercuric ion in the final suspension.

4-(2-Pyridylazo)resorcinol (53) is a highly sensitive reagent but lacks selectivity. 1-(2-Lepidylazo)-2-acenaphthylenol (74) has been reported to be more sensitive than dithizone. However, thiosulphate, oxalate, citrate, zinc, cadmium and cobalt interfere seriously.

Amongst the recently introduced reagents for mercury ammonium(2'-amino-3'-hydroxypyridyl-4'-azo)benzene-4-arsonate
(AHP-4A) (68) has been found to be a sensitive one. The Hg(II)-(AHP-4A) complex has also been used for indirect determination of cyanide ions.

Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine, trisodium salt (74) forms two complexes at 550nm and 633nm. In the determination of mercury by this method manganese(II), iron(II), cobalt(II), nickel(II), zinc(II), cadmium(II), silver(I) and lead(II) interfere.

The present method using PBA reagent at pH 10 is a sensitive one but is not very selective method. However most of the metals either do not develop colour with PBA or absorb weakly at 520nm (λmax for mercurry(II)-PBA complex). PBS forms a water soluble and stable 1:2 complex at 570nm. Manganese(II) is masked by oxalate, iron(II) and cobalt(II) are masked by nitrite. However attempts to mask cadmium(II), nickel(II) copper(II), zinc(II) failed. However sensitivities of these methods are comparable to some of the well known reagents for the determination of mercury(II). (Table V.7)
Table V.7: Sensitivities of some known methods for mercury

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Molar absorptivity (ε)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalazinylformezans</td>
<td>6.4x10⁴/520nm</td>
<td>30</td>
</tr>
<tr>
<td>4-(2-Pyridylazo)resorcinol</td>
<td>6.8x10⁴/500nm</td>
<td>53</td>
</tr>
<tr>
<td>Azoxine ash</td>
<td>3.54x10⁴/540nm</td>
<td>60</td>
</tr>
<tr>
<td>Azoxine Ts</td>
<td>4.38x10⁴/540nm</td>
<td>60</td>
</tr>
<tr>
<td>1-(2-Thiazolylazo)-2-naphthol</td>
<td>5x10³/580nm</td>
<td>61</td>
</tr>
<tr>
<td>2-(4-Antipyrylazo)-5-diethylaminophenol</td>
<td>(1.6-2.1)x10⁴/600nm</td>
<td>63</td>
</tr>
<tr>
<td>1-(4-Methyl-2-thiazolylazo-2-naphthol</td>
<td>(5.8±1.2)x10³/580nm</td>
<td>61</td>
</tr>
<tr>
<td>Ammonium(2'-amino-3'-hydroxypyridyl-4'-azo) benzene-4'-arsenate</td>
<td>3.63x10⁶/535nm</td>
<td>68</td>
</tr>
<tr>
<td>Tris-(2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo))-s-triazine, trisodium salt (TMT).</td>
<td>6.69x10⁵/633nm</td>
<td>75</td>
</tr>
<tr>
<td>2,6-Bis[(7-hydroxy-acenaphthyl-8-azo)pyridine</td>
<td>4.0x10⁴/520nm</td>
<td>Present method</td>
</tr>
<tr>
<td>2,6-Bis[4-sulpho-1-hydroxy-2-naphthylazo]pyridine, sodium salt.</td>
<td>4.43x10⁴/568nm</td>
<td>do</td>
</tr>
</tbody>
</table>

B.1. Spectrophotometric determination of Thiourea and Thiosemicarbazide with Hg(II)-PBA Complex

Thiourea and its derivatives possess several medicinal and industrial applications. Very few methods for their spectrophotometric determination are known. Sodium nitroprusside alone or with Grote's reagent has been used for their spectrophotometric determination (78). Folin-Ciocalteu reagent is used to determine
1,3-diphenyl thiourea (79). Tewari and Pande (80), reported the determination of substituted thioureas in the UV region. Hutchinson and Boltz method is by converting thiourea to thio­cyanate and finally determining as $[\text{Fe(SCN)}]^{2+}$ (81).

Thiosemicarbazide shows pharmacological activity, which is enhanced by presence of small amounts of metal ions (82). The metal-TSC complexes were found to be active against tuberculosis (82), small pox (83), influenza (84), protozoa (85), and act as good fungicides (86) and pesticides (87). The methods reported for their estimation so far are based upon their oxidation by alkali metal hypohalities (88,89), lead tetraacetate (90), chloramine-T(91,92) and by mercurimetry (93). These methods are limited to estimation of small quantities and are slow. Only a few colorimetric methods are known. Sodium nitroprusside alone or with Gorte's reagent (78,94,95), glyoxylic acid (96) is also another method for the photometric determination of thiosemi­carbazide. Another method for indirect determination of thiourea and thiosemicarbazide is with the reagent, tris[2,4,6-(2­-hydroxy-4-sulpho-1-naphthylazo)-s-triazine, trisodium salt (74).

**Present Work:**

In the present work, analytical potentiality of mercury(II)-PBA complex has been investigated in the indirect determination of thiourea and thiosemicarbazide. Both compounds decompose the mercury(II)-PBA complex quantitatively and decrease
in absorbance of mercury complex with the increase in concentration of thiourea or thiosemicarbazide has been made basis of determination of these two compounds.

The details of the method are given as under.

**Reagents:**
Thiourea and Thiosemicarbazide solutions: Stock solutions of thiourea and thiosemicarbazide were prepared by dissolving appropriate amount of the compounds of the analytical grade in double distilled water.

Borate buffer, pH 10: A borate buffer was prepared as described earlier in this chapter.

**EXPERIMENTAL**

1. **Spectral behaviour of the colour system:**

   Solutions containing 1.0ml of $2 \times 10^{-4} M$ mercury(II) and 4ml of $5 \times 10^{-4} M$ PBA was prepared and dilute solutions of thiourea or thiosemicarbazide were added followed by 2.0ml of borate buffer. The mixtures were extracted in 10ml of chloroform. Absorbance of each solution was measured against the corresponding reagent blank ($4.0 \text{ml of } 5 \times 10^{-4} M \text{ PBA + 2.0ml of borate in 10ml of chloroform}$). The nature of spectral curves was found similar to that obtained in case of mercury(II)-PBA complex absorbing at $\lambda_{\text{max}}$ 520nm.
2. Effect of pH:

The reaction between mercury(II)-PBA complex and thiourea or thiosemicarbazide has been studied at different pH values. Best results were obtained in the pH range 9.5-11.0. In subsequent studies, 2.0ml of borate buffer of pH 10, which was found to be suitable was used to adjust the appropriate pH.

3. Effect of reagent concentration:

The best results were obtained when at least 5 fold molar excess of PBA to mercury was used and in subsequent studies 10 times molar excess of PBA was used.

4. Calibration curve:

The decomposition curves drawn by measuring absorbances at 520nm of a series of solutions containing 1ml of 2×10^{-4}M of mercury(II) [50 μg in 10ml is the maximum limit for determination of mercury(II)] with 4.0ml of 5×10^{-4}M PBA and varying amounts of thiourea or thiosemicarbazide at pH 10. The calibration curves showed linearity up to 1.70 μg/ml of thiourea and 1.85 μg/ml of thiosemicarbazide. Similarly a calibration curve was drawn taking 1.25ml of 2×10^{-4}M mercury(II) (maximum limit for mercury(II) determination with PBA i.e. 50 μg in 10 ml.) and following the above procedure. The calibration curve showed linearity up to 2.10 μg/ml of thiourea and 2.30 μg/ml for thiosemicarbazide (Fig. V.11, Fig. V.12).
**Fig. 11. Decomposition Behaviour of Hg(II)-PBA Complex by Thiourea.**

Hg(II) = $2 \times 10^{-5}$ M
PBA = $2 \times 10^{-4}$ M

**Fig. 12. Decomposition Behaviour of Hg(II)-PBA Complex by Thiosemicarbazide.**

Hg(II) = $2 \times 10^{-5}$ M
PBA = $2 \times 10^{-4}$ M
5. Recommended procedure:

To a solution containing 50 µg of mercury(II), add 5 ml of 5x10^-4 M PBA solution followed by the sample solution containing upto 21.0 µg of thiourea or 23.0 µg of thiosemicarbazide. Add 2.0 ml of borate buffer to each solution. Extract the complex in 10 ml of chloroform. Measure the absorbance against the corresponding reagent blank at 520 nm.

6. Optimum range for determination and accuracy of the method:

The optimum range of concentration to determine mercury(II) with PBA is 1.5-5.0 µg/ml. Calibration curves for the determination of thiourea or thiosemicarbazide are drawn by taking 5.0 µg/ml of mercury(II). (Fig. V.11, Fig. V.12.). Similar calibration curves were drawn by taking different aliquots of mercury(II) in the range 1.5-5.0 µg/ml, which are the minimum and maximum determinable amounts of mercury(II). The calibration curves showed that the optimum concentration range for determination has been 1.8-17.5 µg/10 ml for thiourea and 3.2-18.2 µg/10 ml for thiosemicarbazide. The recovery data obtained for the determination of thiourea and thiosemicarbazide are recorded in Table V.8, which shows that accuracy of the method is high.
Table V.8: Recovery data of thiourea and thiosemicarbazide by decomposition of mercury(II)-PBA complex

[Conc. of mercury(II) = 5.0 µg; at 520nm and pH = 10]

<table>
<thead>
<tr>
<th>Conc. of thiourea taken per 10ml (µg)</th>
<th>Average conc.* of thiourea found (µg/10ml)</th>
<th>Conc. of thiosemicarbazide taken per 10ml(µg)</th>
<th>Average conc.* of Thiosemicarbazide found (µg/10ml)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>3.82</td>
<td>4.5</td>
<td>4.48</td>
<td>0.14</td>
</tr>
<tr>
<td>5.7</td>
<td>5.73</td>
<td>6.75</td>
<td>6.76</td>
<td>0.026</td>
</tr>
<tr>
<td>7.6</td>
<td>7.62</td>
<td>9.0</td>
<td>9.12</td>
<td>0.45</td>
</tr>
<tr>
<td>9.5</td>
<td>9.54</td>
<td>11.25</td>
<td>1116</td>
<td>0.05</td>
</tr>
<tr>
<td>11.4</td>
<td>11.39</td>
<td>13.5</td>
<td>13.59</td>
<td>0.06</td>
</tr>
<tr>
<td>13.3</td>
<td>13.28</td>
<td>15.25</td>
<td>15.16</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Average of four experimental values

7. Stoichiometric behaviour of the reaction:

Addition of dilute solutions of thiourea and thiosemicarbazide solution to mercury(II)-PBA complex causes decrease in absorbance which falls proportionally with the concentration of these compounds, when recorded at 520nm. To determine the stoichiometry of the reaction between thiourea or thiosemicarbazide and
mercury(II)-PBA complex, a mole ratio study was made. Suitable aliquots of the compound were added to a known amount of mercury(II)-PBA complex. Reactions of the compounds with mercury(II)-PBA complex indicate that the decrease in absorbance reached a maximum at molar ratio of 1:1 of thiourea or thiosemicarbazide to mercury(II) (Fig. V.11, Fig. V.12). The overall reaction may be represented as:

\[
[Hg(II)-(PBA)_2] + TU \rightarrow [Hg(TU)]^{2+} + 2[PBA]^-
\]

\[
[Hg(II)-(PBA)_2] + TSC \rightarrow [Hg(TSC)]^{2+} + 2[PBA]^-
\]

Where TU and TSC represent the thiourea and thiosemicarbazide respectively.

8. Effect of foreign ions:

The effect of foreign ions was studied by taking 5.0 μg/ml mercury(II) and 0.76 μg/ml of thiourea or 0.91 μg/ml of thiosemicarbazide, adding varying amounts of interfering ions and determining the concentration of the compounds following the recommended procedure. Interferences were identical to those in case of determination of mercury(II) by PBA.

Discussion:

Colorimetric methods for determination of thiourea, based on colour formation with sodium nitroprusside alone or with Grote's reagent, are sensitive but colours are affected by light (78). Folincio-Ciocalteu reagent (79) has been used in the determination
of 10–60 μg of 1,3-diphenylthiourea. Spectrophotometric determination of substituted thioureas in the UV region was reported by Tiwari and Pande (80), but the other ions interfere in this region. Hutchinson and Boltz reported the determination of thiourea after its conversion to thiocyanate with nitrous acid and finally determining as [Fe(SCN)]²⁺ (81).

Very few methods are known for determination of thiosemicarbazide. Sodium nitroprusside alone or with Grote's reagent has been used but the colours are affected by light (78,94,95). Photometric methods based on the reaction between glyoxylic acid and thiosemicarbazide has been used in determination of 0.9–15 μg of the semicarbazide at 285nm (96). This method is subjected to interference by various cations and anions.

The method using tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthyl-azo)]-s-triazine, trisodium salt (THT) is again indirect one (74). Thiourea and thiosemicarbazide decompose the mercury(II)-THT complex in the ratio 1:1 and has been used for determination of 2.25–28.5 μg/25ml of thiourea or 2.75–31.75 μg/25ml of thiosemicarbazide.

The present method is also indirect. The decrease in absorbance of the mercury(II)-PBA complex is proportional to the concentration of thiourea or thiosemicarbazide. These compounds
decompose mercury(II)-PBA complex in the ratio 1:1. The methods are highly sensitive and 1.8-17.5 µg/10ml of thiourea or 3.2-18.2 µg/10ml of thiosemicarbazide can be accurately determined.

B.2 Spectrophotometric determination of thiosulphate:

Thiosulphate can be determined spectrophotometrically by its reaction with cyanide in presence of copper(II) to form thiocyanate ions, which are determined with iron(III) (97,98) or thiocyanate can be extracted in 1,2-dichloroethane as ion-pair with methylene blue (99). Thiosulphate can be oxidised with iron(III) to tetrathionate and excess of iron(III) can be determined spectrophotometrically (100). Other methods which deserve mention are: using the reaction between thiosulphate and p-benzoquinone (101), bleaching of methylene blue with thiosulphate (102), extracting thiosulphate with basic dyes (Rhodamine G or Crystal violet) (103), and indirect methods using ligand exchange reactions with complexes, viz., mercury(II)-diphenylcarbazone (104,105), mercury(II)-chloranilate (106) and mercury(II)-tris [2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine (107).

Present work:

The complex formed by 2,6-bis(4-sulpho-1-hydroxy-2-naphthylazo)pyridine, sodium salt (PBS) with mercury is decomposed quantitatively and therefore this complex can be utilized for indirect determination of thiosulphate ions. In the present work, thus, potentialities of mercury(II)-PBS complex has been
investigated for the determination of thiosulphate ions. The analytical method involves the ligand exchange reaction between thiosulphate and mercury(II)-PBS complex in solution.

**EXPERIMENTAL**

**Reagents:**

Thiosulphate solution: A stock solution of thiosulphate was prepared by dissolving an appropriate amount of Na$_2$S$_2$O$_3$.5H$_2$O in double distilled water and it was standardized iodometrically.

Acetate buffer solution, pH 5.5: A buffer solution of this pH was prepared as described earlier in this chapter.

1. **Spectral behaviour of the colour system:**

A series of solutions containing 1.0ml of 2x10$^{-4}$M mercury(II) and 5.0ml of 5x10$^{-4}$M of PBS was prepared and dilute solutions of thiosulphate were added, followed by 2.0ml of acetate buffer. All the solutions were finally diluted to 25ml with distilled water and absorbances were recorded against a corresponding reagent blank. The nature of the spectral curves were found to be similar to that obtained in case of mercury(II)-PBS complex (Fig. V.6) with $\lambda_{\text{max}}$ at 570nm, with decreasing absorbance with the increase in thiosulphate concentration showing thereby the decomposition of mercury(II)-PBS complex by thiosulphate ions with the formation of a stable mercury salt. The complexed reagent molecules were therefore set free.
2. **Effect of pH:**

The reaction between mercury(II)-PBS complex and thiosulphate has been studied at different pH levels. Best results were obtained in the pH range 4.5-6.5. In subsequent studies 2.0ml of acetate buffer solution (pH 5.5) has been used for adjusting appropriate pH.

3. **Effect of reagent concentration:**

Effect of reagent concentration has been studied by the addition of varying amounts of PBS to a known amount of mercury(II) and fixed amount of thiosulphate. Highest sensitivity is exhibited when at least 5 fold molar excess of PBS to mercury(II) is used.

4. **Calibration curves:**

The decomposition curves were drawn by measuring absorbance at 570nm of a series of solutions containing 4.0 μg/ml mercury(II), complexed with 5ml of PBS (5x10^{-4}M) and varying amounts of thiosulphate at pH 5.5. The calibration curves show linearity upto 1.87ppm of thiosulphate ions. Similar decomposition behaviour of the complex by the anions was studied by taking different aliquots between 0.6-4.0 μg/ml of mercury(II) (optimum concentration range for determination of mercury by PBS) and Beer's law was followed in the range of 0.18-1.87ppm for thiosulphate ions.

5. **Recommended procedure:**

To 2.5ml of 2x10^{-4}M mercury(II) (100 μg, maximum limit for determinable value in 25ml of total volume) add 5.0ml of 5x10^{-4}M
PBS solution, followed by suitable aliquots containing up to 47 μg of thiosulphate ions. Add 2.0 ml of acetate buffer and dilute to 25 ml with distilled water. Measure the absorbance against a corresponding reagent blank [without mercury(II) and thiosulphate ions] at 570 nm and deduce the amount of the thiosulphate from the standard calibration curves drawn under similar conditions.

6. Optimum range for determination and accuracy of the method:

Optimum concentration range for determination of thiosulphate ions was ascertained by taking known and fixed aliquots of mercury(II) in the range 0.60–4.0 μg/ml [optimum concentration range for mercury(II)] adding 5 ml of 5x10⁻⁴ M PBS and varying amounts of thiosulphate. The absorbance was recorded at 570 nm against the reagent blank. Calibration curves were obtained by plotting absorbance vs. concentration of thiosulphate (Fig. V.13). The amount of thiosulphate which can be determined accurately using 4.0 μg/ml of mercury(II) is 0.18–2.0 ppm. The recovery data for thiosulphate ions obtained from synthetic solutions using 4.0 μg/ml of mercury(II) are tabulated in Table V.9, which show good agreement between the amounts taken and found.
Fig. 13. Decomposition behaviour of Hg(II)-PBS complex by thiosulphate ions.

Hg(II) = 2 × 10^{-5} M
PBS = 5 × 10^{-4} M
Table V.9 Recovery data of thiosulphate by decomposition of mercury(II)-PBS complex.

[Conc. of mercury(II) = 4.0 μg/ml, at 570nm and pH 5.5]

<table>
<thead>
<tr>
<th>Conc. of $S_2O_3^{2-}$ per 25 ml(μg)</th>
<th>Average Conc.* of thiosulphate found in 25 ml (μg)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.82</td>
<td>4.85</td>
<td>8.03</td>
</tr>
<tr>
<td>9.64</td>
<td>9.55</td>
<td>0.06</td>
</tr>
<tr>
<td>14.46</td>
<td>14.42</td>
<td>0.06</td>
</tr>
<tr>
<td>19.28</td>
<td>19.37</td>
<td>0.14</td>
</tr>
<tr>
<td>24.10</td>
<td>24.43</td>
<td>0.27</td>
</tr>
<tr>
<td>28.92</td>
<td>28.82</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* Average of 4 experimental values.

7. Stoichiometric behaviour of the reaction:

Addition of dilute solution of thiosulphate to a solution of mercury(II)-PBS complex, causes decrease in absorbance which falls proportionally with the concentration of thiosulphate, recorded at 570nm.

To determine the stoichiometry of the reaction between thiosulphate and mercury(II)-PBS complex, a mole ratio study was made. Suitable aliquots of thiosulphate were added to known solutions containing a fixed amount of mercury(II)-PBS complex.
Reaction of thiosulphate with mercury(II)-PBS complex, indicates that displacement of PBS with thiosulphate ions is in stoichiometric ratio 1:1, with the formation of mercuric thiosulphate, as shown by the following equation.

\[
[Hg(PBS)_2] + S_2O_3^{2-} \rightarrow [Hg(S_2O_3)] + 2PBS^{-}
\]

8. Effect of foreign ions:

The effect of diverse ions was studied in the determination of 1.0 μg/ml of thiosulphate [using 4.0 μg/ml of mercury(II)]. The tolerance limits of various ions were found to be same as reported in case of mercury(II) determination by PBS.

Discussion:

Thiosulphate reacts with cyanide in presence of copper(II) to form thiocyanate, which can be determined as iron(III)-thiocyanate complex at 496nm (97,98). Koh's method is based on the reaction of cyanide with thiosulphate to form thiocyanate, which is extracted in 1,2-dichloroethane as ion-pair with methylene blue (99). Thiosulphate can also be oxidised by ferric ions to tetrathionate and excess of ferric ions determined photometrically as thiocyanate complex (100). Other methods known for determination are by extraction of thiosulphate with basic dyes viz. rhodamine-G and crystal violet (103) in benzene and nitrobenzene (3:1) or (4:1) at 536nm and 600nm respectively. Thiosulphate has also been determined indirectly by ligand exchange reaction, using mercury(II)-diphenylcarbazone (105) and
mercuric(II)-chloranilide (106), $\text{Hg}^{2+}\text{tris}[2,4,6-(2\text{-hydroxy}-4\text{-}
\text{sulpho}-1\text{-naphthlazo})]-\text{s-triazine}, \text{trisodium salt} (107)$. The present method is sensitive one and is used to determine thiosulphate indirectly at 570nm. The range for accurate determination of thiosulphate is 0.18-2.0 ug/ml.
REFERENCES


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75. S. Chilov, Talanta, 1975, 22, 205.


