PROCEDURE FOR MERCURY ESTIMATION

Preparation of Reagents

1) Standard 0.0001M mercuric chloride solution
   Molecular weight of HgCl₂ : 271.5g
   0.1M HgCl₂ = 20.06mg mercury /ml
   0.01M HgCl₂ = 2.006mg mercury/ml
   0.001 M HgCl₂ = 0.2006 mg mercury/ml
   0.0001M HgCl₂ = 20 µg/ml

2) 0.001M KI solution
   Molecular weight of KI = 166.01g
   ∴ 0.166g in 1000 ml distilled water gives 0.001M KI solution

3) 0.001M malachite green solution
   Molecular weight of malachite green = 346.5g
   ∴ 0.346g in 1000 ml distilled water gives 0.001M malachite green solution

4) Acetate Buffer (stock solution)
   A = 0.2M acetic acid
   11.55ml of glacial acetic acid in 1000ml distilled water
   B = 0.2 sodium acetate solution
   16.4g of C₂H₃O₂ Na in 1000ml distilled water
   For working solution: 2ml of A and 48 ml of B are mixed together

5) Benzene
   Commercially available AR grade (BDH)

Method

1) Suitable aliquots of mercuric chloride containing 0 to 20 µg of mercury were taken.

2) 2ml of KI solution followed by 2ml of malachite green solution were added in each tube.

3) 10 ml of buffer solution to adjust the pH of the system to 6 was added to each tube.

4) The tubes were shaken vigorously in separating funnel with 15 ml
benzene for 5 minutes.
5) The extract in form of organic solvent was collected.
6) The colour intensity of organic layer was measured at 630 nm.
7) A standard curve of OD versus mercury concentration was plotted.

COMPOSITION OF BUFFER USED FOR pH ADJUSTMENT.

**Citrate phosphate Buffer**

Stock solutions
- A : 0.1M solution of citric acid (19.21 g in 1000 ml D/W)
- B : 0.2M solution of dibasic sodium phosphate
  \((28.39 \text{ g Na}_2\text{HPO}_4 \text{ in 1000 ml D/W})\)
- \(x \text{ ml of A} + y \text{ ml of B} ; \text{diluted to total of 100 ml with distilled water}\)

<table>
<thead>
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<th>pH</th>
<th>x</th>
<th>y</th>
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<tr>
<td>3.0</td>
<td>39.8</td>
<td>10.2</td>
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<tr>
<td>4.0</td>
<td>30.7</td>
<td>19.3</td>
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<td>5.0</td>
<td>24.3</td>
<td>25.7</td>
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<td>6.0</td>
<td>17.9</td>
<td>32.1</td>
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<td>7.0</td>
<td>9.1</td>
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**Boric Acid Borax Buffer**

Stock solutions
- A : 0.2M Boric acid solution (12.4 g in 1000 ml D/W)
- B : 0.5M Borax solution (19.05 g in 1000 ml D/W)
- 50 ml of A + x ml of B; diluted to 200 ml with D/W

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<td>9.0</td>
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GRAPH : 40
STANDARD CURVE FOR MERCURY ESTIMATION

OD 630 nm

MERCURY CONCENTRATION (µg)
**IDENTIFICATION OF BACTERIAL ISOLATE**

<table>
<thead>
<tr>
<th>Number</th>
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<tbody>
<tr>
<td>Isolate 6</td>
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<tr>
<td>Isolate 12</td>
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<tr>
<td>Isolate 15</td>
<td><em>Bacillus</em></td>
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<tr>
<td>Isolate 17</td>
<td><em>Serratia marcescens</em></td>
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<tr>
<td>Isolate A</td>
<td><em>Pseudomonas</em></td>
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<tr>
<td>Isolate a₆</td>
<td><em>Pseudomonas</em></td>
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<tr>
<td>Isolate Unk</td>
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