CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

All chemicals used were of Analytical Reagent (AR) grade. Double distilled water from an all glass still was used for preparing reagents.

3.2 INSTRUMENTS

The instruments used for the study were pH meter (LI 120 ELICON make), conductivity meter (CF 197, WTW), high speed stirrer (model RW 16 B IKA Labortechnik), magnetic stirrer (model RH IKA Labortechnik), Ion Chromatograph (IC) (DX-120 DIONEX) and Atomic Absorption Spectrophotometer (AAS) (Analytic Jena (AJ) Vario 6).

3.3 ANALYSIS

Heavy metals such as cadmium, chromium, copper, lead, nickel and zinc were analysed by atomic absorption spectrophotometer. The measurements were done against calibration curves produced using the standard solutions prepared according to the American Public Health Association (1998).
3.3.1 Preparation of Standards

3.3.1.1 Stock solutions

The stock solutions (1000 mg/L) were prepared by dissolving the corresponding salts in 0.5 M HNO₃ and making up to 1 litre.

**Cadmium**: 2.2819 g of $\text{CdS}_\text{O}_4\cdot 8\text{H}_2\text{O}$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

**Chromium**: 2.8289 g of $\text{K}_2\text{Cr}_2\text{O}_7$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

**Copper**: 3.9292 g of $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

**Lead**: 1.5985 g of $\text{Pb(NO}_3)_2$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

**Nickel**: 4.7853 g of $\text{NiSO}_4\cdot 7\text{H}_2\text{O}$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

**Zinc**: 4.3976 g of $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ was taken in one litre volumetric flask, dissolved with 0.5 M HNO₃ and made up to the mark.

3.3.1.2 Working standards

The intermediate stock and working standards were prepared by diluting the 1000 mg/L stock with 0.5 M HNO₃ as detailed in Table 3.1.
Table 3.1 Details of working standards

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Heavy metal</th>
<th>Concentration range, (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cadmium</td>
<td>0.05 – 2</td>
</tr>
<tr>
<td>2</td>
<td>Chromium</td>
<td>0.2 – 10</td>
</tr>
<tr>
<td>3</td>
<td>Copper</td>
<td>0.2 – 10</td>
</tr>
<tr>
<td>4</td>
<td>Lead</td>
<td>1.0 – 20</td>
</tr>
<tr>
<td>5</td>
<td>Nickel</td>
<td>0.3 – 10</td>
</tr>
<tr>
<td>6</td>
<td>Zinc</td>
<td>0.05 – 2</td>
</tr>
</tbody>
</table>

3.3.2 Calibration Curve

The Atomic Absorption Spectrophotometer (AAS) was calibrated for each metal using the standard solutions and following the procedure given by the instrument manual.

3.3.3 Estimation of Purity of Sodium Sulphide

The sulphide content of sodium sulphide (Na₂S·9H₂O) was estimated using iodometric method (Jeffery et al 1989).

\[ \text{H}_2\text{S} + \text{I}_2 \rightarrow 2 \text{H}^+ + 2 \text{I}^- + \text{S} \]  (3.1)

The sulphide solution at concentration of about 0.02N was added to a known excess of acidified 0.01N iodine solution. The excess iodine left over was then titrated against standard thiosulphate solution using starch as indicator. The purity of Na₂S in the sample was found to be 55%.
3.3.4 Estimation of Purity of Hydrogen Peroxide

The purity of hydrogen peroxide was estimated using acidified KMnO₄ (Jeffery et al 1989).

\[
2 \text{KMnO}_4 + 3\text{H}_2\text{SO}_4 + 5 \text{H}_2\text{O}_2 \rightarrow \text{K}_2\text{SO}_4 + 2\text{MnSO}_4 + 8\text{H}_2\text{O} + 5\text{O}_2 \quad (3.2)
\]

A solution of potassium permanganate of about 0.1M was added dropwise to an acidified solution of hydrogen peroxide; each drop was decolorized until all the hydrogen peroxide was consumed.

3.4 PREPARATION OF SYNTHETIC SAMPLES

Synthetic samples containing heavy metals, namely, cadmium, chromium, copper, lead, nickel and zinc were prepared separately by dissolving an accurately weighed quantity of the respective heavy metal salts in distilled water. The initial concentrations of standard metal solutions used in the investigations were 500 mg/L. The metal salts used were as follows:

<table>
<thead>
<tr>
<th>Metal</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>3CdSO₄.8H₂O</td>
</tr>
<tr>
<td>Chromium</td>
<td>K₂Cr₂O₇</td>
</tr>
<tr>
<td>Copper</td>
<td>CuSO₄. 5H₂O</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb(NO₃)₂</td>
</tr>
<tr>
<td>Nickel</td>
<td>NiSO₄. 7H₂O</td>
</tr>
<tr>
<td>Zinc</td>
<td>ZnSO₄. 7H₂O</td>
</tr>
</tbody>
</table>
The complexing agents used in this study were ammonium chloride, tartrate and citrate as these are commonly used in the electroplating baths. Since they are weak complexing agents, the concentration taken for the study was 1000 mg/L.

3.5 METHODOLOGY
3.5.1 Removal of Heavy Metals

Batch experiments were conducted for the removal of heavy metals by employing univariant approach of varying one parameter at a time.

3.5.1.1 Hydroxide precipitation technique

For hydroxide precipitation, pH of 200 mL of heavy metals solution (500 ppm) was raised from 7 to 11 using 1N NaOH. The contents were allowed to settle for 30 minutes. The supernatant was filtered through a Whatman No.42 filter paper and analysed using atomic absorption spectrophotometer for the presence of various heavy metals. The precipitation was carried out in the presence and absence of complexing agents such as ammonium chloride, tartrate and citrate. The removal of chromium needed reduction of hexavalent form to trivalent form before precipitation as hydroxide. Ferrous sulphate was used as reducing agent at pH 2. In addition to pH, the ferrous sulphate dose was also optimized for chromium.

3.5.1.2 Sulphide precipitation technique

For sulphide precipitation, variation of pH, sulphide dose and reaction time were done on 200mL of heavy metal solution (500 ppm). Initially
H was optimized using 1N sodium hydroxide or 1N sulphuric acid with heavy metals solution by the addition of sulphide under rapid mixing conditions. The contents were allowed to settle for 30 minutes. The supernatant was filtered through a Whatman No.42 filter paper and analysed using atomic absorption spectrophotometer for the presence of various heavy metals. Similarly, the sulphide dose was also optimized. The precipitation was carried out in the absence and presence of complexing agents such as ammonium chloride, tartrate and citrate.

5.5.2 Recovery of Heavy Metals

Batch experiments were conducted for the recovery of heavy metals by varying the volume of hydrogen peroxide, reaction time and pH under sunlight. The variables were optimized by employing the univariant approach of varying one parameter at a time. The sulphide precipitate was filtered through Whatman No.1 filter paper, washed, collected and made up to a volume of 100 mL with distilled water. To this hydrogen peroxide was added and kept in sunlight under mixing conditions. The volume of sample was maintained to prevent the loss of water due to evaporation. Then, the sample was filtered using Whatman No.1 filter paper and the concentration of sulphate was analyzed using Ion Chromatograph (IC).

3.5.3 Analysis of Industrial Effluents

Effluents from industries [Brakes India Ltd, Padi, Chennai (effluent No.1 and 7); Guindy metal finishing industries, Chennai (effluent No.2); Metal Alloy Industries, Ambattur, Chennai (effluent No.3); Royal Electroplaters, Chennai (effluent No.4); Exide battery, Guindy, Chennai (effluent No.5); Tami]
Nadu Akaline Batteries Ltd., Ambattur, Chennai (effluent No.6)] were collected and characterized.

In addition to heavy metals, pH, electrical conductivity (EC), total dissolved solids (TDS), chloride, sulphate, nitrate, phosphate, fluoride, bromide, and chemical oxygen demand (COD) were estimated for the industrial effluents. Various parameters analyzed and the method adopted are listed in Table 3.2.

Table 3.2 Analytical parameters and methods adopted

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>pH meter (pH – 197, WTW)</td>
</tr>
<tr>
<td>2</td>
<td>Electrical Conductivity (EC)</td>
<td>Conductivity meter (LF – 197, WTW)</td>
</tr>
<tr>
<td>3</td>
<td>Total Dissolved Solids (TDS)</td>
<td>Gravimetric method</td>
</tr>
<tr>
<td>4</td>
<td>Chloride</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>5</td>
<td>Sulphate</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>6</td>
<td>Nitrate</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>7</td>
<td>Phosphate</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>8</td>
<td>Fluoride</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>9</td>
<td>Bromide</td>
<td>Ion Chromatograph (DIONEX)</td>
</tr>
<tr>
<td>10</td>
<td>Chemical Oxygen Demand (COD)</td>
<td>Open dichromate reflux method</td>
</tr>
<tr>
<td>11</td>
<td>Heavy metals</td>
<td>Atomic Absorption Spectrophotometer (AAS) (Analytic Jena (AJ) Vario 6).</td>
</tr>
</tbody>
</table>
The in-situ parameters such as pH and electrical conductivity were measured in the field at the time of sample collection using the pH and EC probes, respectively.

3.6 QUALITY CONTROL

3.6.1 Outlier Test (rm test)

The outliers were identified by the Maverick test (rm-test). This test helps to identify the values, which deviate significantly from the true value.

In this test, the standard deviation (SD) was first calculated using the formula,

$$SD = \sqrt{\frac{\sum(X_m - X_0)^2}{N-1}}$$  \hspace{1cm} (3.3)

where 'X_0' is the actual (Theoretical) concentration and 'X_m' is the measured concentration and 'N' is the number of samples.

The Maverick value was calculated using the formula

$$r_m = |X_m - \bar{X}| / SD$$  \hspace{1cm} (3.4)

The calculated value was compared with rm table values (Table A.1). When the calculated value was greater than the table value, then a maverick was given and that sample was eliminated from the set. The new SD and rm were calculated with the remaining values until no maverick appeared.
5.6.2 Detection and Determination Limits

In order to assess whether the method is suitable for trace analysis, it is necessary to determine the sensitivity or detection limit. The detection limit \( D_L \) is the minimum concentration of determinant that can be detected with 95% certainty. This was calculated using the formula

\[
D_L = \frac{2 \ SD * C}{X} \tag{3.5}
\]

where 'C' is the analyte concentration and 'X' is the mean of the values. The determination limit or the limit of quantification \( X_D \) was calculated using the formula

\[
X_D = \sqrt{2 \ t \ SD} \tag{3.6}
\]

where 't' is the student t (Table A.2).

5.6.3 Warning and Control Limits (Control Charts)

The warning limit (WL) was estimated for 99% level of significance and (N-1) degree of freedom using the formula

\[
WL = 3.499 * SD \tag{3.7}
\]

The control limit (CL) was calculated for 99.9% level of significance using the formula

\[
CL = 5.408 * SD \tag{3.8}
\]
5.6.3.1 X – Control chart

The X – control chart gives the precision of the method; in this case the AAS measurement. The X – control chart was drawn for each set of values. The values out-ranging the warning limit, but within the control limit were accepted with caution to attend to some analytical problems. The values out-shooting the control limit were rejected and considered as failure of the analysis. It was checked immediately.

5.6.3.2 S – Control chart

The S – control chart gives the bias of the method between the two sets. 32 samples were divided into 16 replicates. They were considered as two sets and each set was treated separately. The deviations within each set were estimated and the S – control chart was drawn. The warning limit was calculated using the formula

\[
WL = \frac{3.499 \times w_m}{d_2}
\]

(3.9)

where ‘\(w_m\)’ is the mean width between the two sets of values and \(d_2\) factor depends on the degree of freedom (Table A.3).

Similarly, the control limit was calculated using the formula

\[
CL = \frac{5.408 \times w_m}{d_2}
\]

(3.10)
The S-control chart was drawn for the two sets of values. The values which were out-ranging the warning limit but within the control limit were accepted with caution while values out shooting the control limit were rejected and considered as biased. It was checked immediately.