CHAPTER 3

EXPERIMENTAL PROCEDURE AND ANALYSIS

3.1 INTRODUCTION

In this chapter, the experimental procedures used for determination of solubility and mass transfer coefficient of acids and alizarin in different hydrotrope solutions are described. The procedure for the measurement of various properties including viscosity, specific gravity, surface tension, specific conductance and refractive index of hydrotrope solution are discussed. The important physical properties of acids, alizarin and hydrotropes used in this work are also given.

3.2 MATERIALS USED

3.2.1 Acids

A series of organic acids studied in this work is listed in Table 3.1. The aqueous solubility of acids and alizarin under normal conditions are also presented in the same table as reported by Dean (1987) and Perry (1997).

3.2.2 Hydrotropes

The hydrotropes used in this work are listed in Table 3.2. The solubility of hydrotropes in the aqueous phase under normal conditions is also given in the same table as reported by Dean (1987). All organic acids, alizarin
and hydrotropes used in this work were Analar grade. Double distilled water was used for the preparation of hydrotrope solutions.

### Table 3.1 Details of organic acids and alizarin studied

<table>
<thead>
<tr>
<th>Solutes</th>
<th>Molecular weight gm/mol</th>
<th>Solubility* in 100 parts of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>122.12</td>
<td>0.26</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>138.12</td>
<td>0.53</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>167.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>148.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Alizarin</td>
<td>240.21</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Table 3.2 Details of hydrotropes used

<table>
<thead>
<tr>
<th>Hydrotropes</th>
<th>Molecular weight gm/mol</th>
<th>Solubility* in 100 parts of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium salicylate</td>
<td>160.11</td>
<td>60.00</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>144.11</td>
<td>62.90</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>122.12</td>
<td>69.10</td>
</tr>
<tr>
<td>Urea</td>
<td>60.06</td>
<td>51.80</td>
</tr>
<tr>
<td>Potassium p-toluene sulfonate</td>
<td>211.18</td>
<td>Highly soluble</td>
</tr>
<tr>
<td>Sodium cumene sulfonate</td>
<td>222.24</td>
<td>40.00</td>
</tr>
</tbody>
</table>

* at ambient temperature

### 3.3 SELECTION CRITERIA

#### 3.3.1 Acids

The acids selected for this study are either sparingly soluble or insoluble in water in the absence of any hydrotrope. All the acids selected find
extensive applications in many chemical and pharmaceutical industries. The separation of such acids from any mixture is also found to be difficult at present.

3.3.2 Alizarin

Alizarin is one of the important industrial dyes originating from the roots of the madder plant (Rubia tinctorum) exhibiting bactericidal, antifungal and spasmyloytic activities. Consequently, it facilitates the loosening of kidney concretions containing calcium and magnesium phosphates. For this purpose, alizarin and its derivatives are widely used in pharmaceutical industry. The separation of alizarin from the roots of madder plant is found to be difficult at present.

3.3.3 Hydrotropes

The hydrotropes selected for this study are freely soluble in water. All the hydrotropes used are non-reactive, non-toxic and do not produce any temperature effect when dissolved in water. The presence of a hydrophilic group such as -OH, -NH₂, -Na, -K, H⁺ which normally enhance the solubility substantially, cheapness and the easy availability of hydrotropes are the other factors considered in the selection of hydrotropes.

3.4 PARAMETERS VARIED

3.4.1 Acids and Alizarin

For the determination of solubility, the parameters varied were temperature (303-333K) and the hydrotrope concentration (0-3.00 mol/L). The determination of mass transfer coefficient was carried out under the influence of different hydrotrope concentrations.
3.5 EXPERIMENTAL PROCEDURE AND ANALYSIS

3.5.1 Solubility Determination

3.5.1.1 Acids

The experimental setup for the determination of solubility values consists of a thermostatic bath with a temperature range of 30-100°C and a separating funnel as shown in Figure 3.1.

For each solubility test, 100 ml of a solution of the hydrotrope of known concentration was taken in a separating funnel and an excess amount of acid was added. Hydrotrope solutions of different concentration were prepared by dilution with distilled water. The separating funnel was immersed in a constant-temperature bath fitted with a temperature controller which could control the temperature within ± 0.1 °C. The setup was kept overnight for equilibration. After equilibrium was attained, the solution was filtered from the remaining solid.

The concentration of the dissolved organic acid in aqueous hydrotrope solutions was analyzed by titration using standardized NaOH solutions with phenolphthalein as an indicator. All the solubility experiments were conducted in duplicate to check the reproducibility. The observed difference was < 2 %.

3.5.1.2 Alizarin

For each solubility test, excess amount of alizarin was added to the hydrotrope solutions of known concentration in the separating funnel. The separating funnel was sealed to avoid evaporation of the solvent at higher temperatures. The separating funnel was immersed in a constant temperature bath fitted with a temperature controller which could control the temperature...
within ±0.1 °C. The setup was kept overnight for equilibration. After it attained equilibrium, the solution was filtered using whatman filter paper to remove the excess undissolved alizarin.

![Experimental setup for the determination of solubility](image)

**Figure 3.1 Experimental setup for the determination of solubility**

The concentration of dissolved alizarin was determined by titrating against standardized NaOH. No indicator was used since alizarin is a natural acid-base indicator. The color change is from reddish orange to violet. Blank titrations for the prepared hydrotropic solution were also carried out. Some of the solubility experiments were conducted in duplicate to check the reproducibility. The observed error in the reproducibility is < 2%.

### 3.5.2 Determination of Mass Transfer Coefficient

The experimental setup for the determination of the mass transfer coefficient consists of a vessel provided with baffles and a turbine impeller run by a motor to agitate the mixture. The speed of the impeller was selected in such a way to get effective mixing, which was maintained at the same
value for all experiments. The schematic diagram of the experimental setup is shown in Figure 3.2. The vessel is of 40 cm height and 15 cm inner diameter. The turbine impeller has a diameter of 5 cm, a width of 1 cm, and a length of 1.2 cm. It has four blades and is made to rotate at 600 rpm.

![Schematic diagram of the experimental setup](image)

**Figure 3.2** Schematic diagram of the experimental setup for the determination of mass transfer coefficient 1-Agitated vessel; 2-Baffle; 3-Thermometer; 4-Electric motor; 5-Water bath; 6-Peristatic pump; 7-Rotometer; 8-Outlet valve

For each test, to determine the mass transfer coefficient, an excess amount of acid was added to the aqueous solution of the hydrotrope of known concentration. This sample was agitated for a known time of 600, 1200, 1800 and 2400 seconds. After the end of fixed time, ‘t’, the entire mixture was transferred to a separating funnel. After allowing the sample to stand for some time, the solution was filtered from the remaining solid.

The concentration of the solubilized organic acid and alizarin in aqueous hydrotropic solutions at time ‘t’ was analyzed as was done for
solubility determinations. A plot of \(-\log_{10} [1 - \frac{C_b}{C^*}]\) vs ‘t’ is drawn, where ‘C_b’ is the concentration of acids and alizarin at time ‘t’ and C* is the equilibrium solubility of the acid and alizarin at the same hydrotrope concentrations (taken from the solubility determination part).

The slope of the graph gives \(k_{L,a}/2.303\), from which \(k_{L,a}\), the mass transfer coefficient was determined. Duplicate runs were made to check the reproducibility. The observed difference was < 2%.

### 3.5.3 Determination of Properties of Hydrotrope Solution

The properties of hydrotrope solution, such as viscosity, specific gravity, surface tension, specific conductance and refractive index were determined for a range of hydrotrope concentration between 0.10 and 2.00 mol/L. This study on properties of hydrotrope solution is undertaken to propose a possible mechanism of hydrotropy.

#### 3.5.3.1 Viscosity

The viscosity of hydrotrope solution was measured using Ostwald viscometer (0 to 5 cP) immersed in the water bath at 303 K. The viscometer was thoroughly cleaned and dried before immersing it in the water bath. The hydrotrope solution under test was introduced into bulb ‘A’ and then forced under pressure into bulb ‘B’ until its meniscus was just above the upper mark (Figure 3.3). The liquid was then allowed to fall freely back into bulb ‘A’ and the time interval, ‘t’, which elapses between the meniscuses passing upper mark and lower mark was measured with a stop watch and recorded. The tube was then cleaned again and the procedure was repeated for different concentrations of hydrotrope solution.
3.5.3.2 Specific gravity

An empty specific gravity bottle was weighed and filled with hydrotrope solution at a temperature of 303 K up to the volume marker. The specific gravity which is the ratio between the weight of hydrotrope solution and an equal volume of water is determined.

3.5.3.3 Surface tension

Surface tension of hydrotrope solution was measured by capillary rise method at 303 K. The end of a capillary was immersed into the solution. The height at which the solution reaches inside the capillary was observed and the same is related to the surface tension by the equation

\[
\text{Surface tension} = \frac{(\Delta h \times \rho \times r \times g)}{(2 \times \cos\theta)}
\]

3.5.3.4 Specific conductance

The resistance of hydrotrope solution was measured using a conductivity bridge. Measurements were recorded after the system had
attained equilibration at 303 K. The specific conductance was then calculated using the conductance cell constant.

### 3.5.3.5 Refractive index

Refractive index of hydrotrope solution was measured using traveling microscope at 303 K. The real depth was the depth of an object placed at the bottom of an empty beaker. The apparent depth was the depth of the object placed at the bottom of the beaker containing hydrotrope solution. The ratio between the real depth and apparent depth is the refractive index of hydrotrope solution.

### 3.6 EXPERIMENTAL PROCEDURE FOR EXTRACTION OF MANGIFERINS

#### 3.6.1 Raw Materials

Mangifera indica L. leaves were washed thoroughly with distilled water, shade dried, cut into small pieces and crushed to coarse powder. The hydrotropes such as sodium salicylate and sodium cumene sulfonate were of high purity and procured from HiMedia Chemicals, Mumbai. Mangiferin standard was purchased from Sigma-Aldrich Co. (St Louis, U.S.A). Methanol with purity of 99.5% was used.

#### 3.6.2 Extraction Procedure

The experimental setup for the extraction of mangiferin from mango leaves consisted of cylindrical glass vessel equipped with a four blade impeller placed in a thermostatic bath with a temperature range of 30-100°C as shown in Figure 3.4.
Two hydrotropes namely sodium salicylate and sodium cumene sulfonate were selected for extraction of mangiferin from mango leaves. Extraction was conducted by suspending raw material of known quantity in 100ml of hydrotrope solution of known concentration, maintained at predetermined temperature. Speed of the agitation was maintained at 1000rpm. Extraction time was maintained as 6hrs and after extraction the solution was allowed to settle for 30minutes followed by vacuum filtration.

From each set of experiment 10ml of the extract was withdrawn and was diluted below Minimum Hydrotrope Concentration (MHC) without adjusting pH, which results in brownish precipitate. Further the precipitate was centrifuged and dissolved in 90% methanol for analyzing mangiferin content using HPLC.

Figure 3.4   Experimental setup for the extraction of mangiferin
3.6.3 Analytical Method

The mangiferin content in the extract was analyzed using High Performance Liquid Chromatography (HPLC) (Shimadzu Prominence, Isocratic pump, LC-20AT and UV/Vis Detector). Chromatographic separation was achieved by C18 column (250mm × 4.6mm, 5µ particle size). The column was eluted at a flow rate of 1.5mL/min using mobile phase prepared from 0.1% (v/v) aqueous phosphoric acid and acetonitrile. The injection volume was 20µL and the detection wavelength was 258nm. The column was maintained at ambient temperature throughout analysis.