CHAPTER-2

MATERIALS AND METHODS

2.1 Instruments:

i) A UV -1700 Pharmaspec UV – Visible Spectrophotometer (Shimadzu) with matched quartz cells of 10 mm path length were used for absorbance measurements.

ii) Eutech Cyberscan pH 310 digital pH meter with glass electrodes of 0-14 pH range were used for pH adjustments. Standardisation of the pH meter was carried out from time to time with standard buffer solutions.

iii) Pre calibrated pipettes, burettes, measuring flasks, conical flasks etc (Borosil make) were used for volume measurement.

2.2 Preparation of 2,5-dimethyl-4-(2-quinolylazo)phenol (DM-p-QAP):

For the present study 2,5-dimethyl-4-(2-quinolylazo)phenol, DM-p-QAP, a new heterocyclic azo dye has been synthesised and investigated for its use as an analytical reagent for spectrophotometric determination of metal ions in ppm level. Synthesis of the ligand involves the following steps.

2.2.1 Preparation of 2-quinolylhydrazine:

2-quinolylhydrazine was prepared by the method of Perkin and Robinson [1].

6.0 gm of 2-chloroquinoline was refluxed with 25 gm of hydrazine hydrate (80%) on a sand bath for 2 hours, when a reddish solid separated.

\[
\begin{align*}
\text{2-quinolylhydrazine} & \quad \text{Reflux} \quad \text{2-quinolylhydrazine} \\
\text{Cl} & \quad \text{H}_{2}\text{O} \\
\text{2-quinolylhydrazine} & \quad \text{H}_{2}\text{O} + \text{HCl}
\end{align*}
\]

The excess of hydrazine hydrate was removed by distillation under reduced pressure. The solid was then washed with hot water four times and recrystallized with benzene.
2.2.II Recrystallization of 2,5-dimethyl-1,4-benzoquinone:

2,5-dimethyl-1,4-benzoquinone was purified [2] by placing it in a flask attached to a condenser and passing a rapid current of steam into the flask. The said benzoquinone sublimed and was collected in the receiver. It was separated from water by filtration, dried and then recrystallized from petroleum ether.

2.2.III Condensation of 2,5-dimethyl-1,4-benzoquinone with 2-quinolylhydrazine:

2,5-dimethyl-1,4-benzoquinone and 2-quinolylhydrazine condense in 1:1 ratio.

\[ \text{2,5-dimethyl-1,4-benzoquinone} + \text{2-quinolylhydrazine} \rightarrow \text{2,5-dimethyl-4-(2-quinolylazo)phenol} \]

1.36 g (0.01 mole) of the said benzoquinone was dissolved in pure ethanol. A solution of 2-quinolylhydrazine (1.59 g; 0.01 mole in minimum volume of dilute hydrochloric acid) was added to this solution. The resulting solution was kept for 20 minutes and then neutralised with ammonia solution when an orange red precipitate of 2,5-dimethyl-4-(2-quinolylazo)phenol was obtained. The solid was filtered, recrystallized from ethanol and dried over phosphorus pentoxide in vacuum.

2.3 Characterization of the ligand DM-p-QAP:

Purity of the ligand was checked by paper chromatography. The melting point of the ligand could not be ascertained as it got blackened above 208°C.
2.3.1 Elemental Analysis:

Elemental analysis data for the ligand are reported below:

<table>
<thead>
<tr>
<th>Element</th>
<th>Calculated %</th>
<th>Found %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>73.64</td>
<td>73.92</td>
</tr>
<tr>
<td>N</td>
<td>15.16</td>
<td>15.28</td>
</tr>
<tr>
<td>H</td>
<td>5.41</td>
<td>5.33</td>
</tr>
<tr>
<td>O</td>
<td>5.79</td>
<td>5.47 (by difference)</td>
</tr>
</tbody>
</table>

The molecular mass of the ligand is calculated to be 277.

2.3.11 IR spectral data of DM-p-QAP:

The IR spectrum of DM-p-QAP in KBr was recorded in a Fourier Transform Infrared spectrometer (Shimadzu FTIR-8400S). It showed the following results:

- $\nu$ – OH stretching = 3441 cm$^{-1}$
- $\nu$ – N=N- stretching = 1616 cm$^{-1}$
- $\nu$ – C-O stretching = 1099 cm$^{-1}$

The above fact suggests that in the solid state DM-p-QAP exists in the enol form. This form can act as a bidentate ligand, the two coordinated sites being the N atom of the quinolyl ring and the N atom of the azo group away from the heterocyclic ring.

2.3.111 $^1$H NMR spectra of DM-p-QAP:

The $^1$H-NMR spectra of the ligand recorded in a Brooker Advance 300MHz spectrophotometer using DMSO as the solvent shows the peaks at the following $\delta$ values.
The singlet at δ3.5 is assigned to residual proton of DMSO. The singlet at δ3.1 is assigned to phenolic –OH group. The singlet at δ2.5 is assigned to DMSO. The singlet at δ2.1 is assigned to –CH$_3$ group. The aromatic protons appear between δ7.6 to δ8.5.

2.4 Preparation of the ligand solution:

The ligand is highly soluble in ethanol but insoluble in water. A 1x10$^{-3}$M solution of the ligand is prepared by dissolving 0.0277g of 2,5-dimethyl-4-(2-quinolylazo)phenol in 100mL ethanol. The solution is found to be stable for more than one month. The solution is diluted to get working solutions of appropriate strength whenever required. Reagent solutions older than a week were discarded.

2.5 Effect of pH on DM-p-QAP solution:

Effect of pH on the absorption behaviour of the alcoholic solution of DM-p-QAP was studied both in the ultraviolet and the visible region. The spectra of the ligand solution at different pH were recorded. It was found that in highly acidic medium the
The colour of the solution is yellow which may be due to protonation of N atoms of the heterocyclic ring and that in the azo group. As the pH of the solution is gradually increased the colour of the ligand changes to light orange and then to intense orange at pH around 8.06. This may due to deprotonation of the N atoms of the heterocyclic ring and the azo group. When the pH of the solution is raised to 11 or above colour of the solution gradually changes to red suggesting ionization of the phenolic OH group. The wavelengths of maximum absorption ($\lambda_{\text{max}}$) and molar absorptivities ($\varepsilon$) at different pH’s are given in the table below.

<table>
<thead>
<tr>
<th>pH</th>
<th>$\lambda_{\text{max}}$</th>
<th>$\varepsilon \times 10^4$ Lmol$^{-1}$cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.03</td>
<td>528</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>428</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td>385</td>
<td>5.89</td>
</tr>
<tr>
<td>6.04</td>
<td>529</td>
<td>3.17</td>
</tr>
<tr>
<td></td>
<td>383</td>
<td>5.77</td>
</tr>
<tr>
<td>7.02</td>
<td>529</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>6.05</td>
</tr>
<tr>
<td>8.06</td>
<td>504</td>
<td>5.72</td>
</tr>
<tr>
<td></td>
<td>387</td>
<td>4.51</td>
</tr>
<tr>
<td>9.01</td>
<td>505</td>
<td>5.79</td>
</tr>
<tr>
<td></td>
<td>387</td>
<td>4.51</td>
</tr>
<tr>
<td>10.02</td>
<td>500</td>
<td>9.65</td>
</tr>
<tr>
<td>11.05</td>
<td>500</td>
<td>13.08</td>
</tr>
<tr>
<td></td>
<td>362</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>274</td>
<td>4.5</td>
</tr>
<tr>
<td>12.09</td>
<td>499</td>
<td>13.09</td>
</tr>
<tr>
<td></td>
<td>274</td>
<td>4.63</td>
</tr>
<tr>
<td>13.08</td>
<td>500</td>
<td>13.12</td>
</tr>
<tr>
<td></td>
<td>274</td>
<td>4.65</td>
</tr>
</tbody>
</table>
Absorption spectra of DM-p-QAP at different pH’s
2.6 Solutions of diverse ions:

For preparation of solutions of metal ions chlorides, nitrates or sulphates were used. The respective salts of analytical grade were dissolved in doubly distilled water. Whenever necessary the solutions were standardized by well known methods. For solution of anions sodium, potassium or ammonium salts were used in most of the cases.

2.7 Theoretical treatment of the methods used:

2.7.1 Beer-Lambert’s Law:

Lambert’s law states that when monochromatic light is passed through a transparent medium, the intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically. This can be expressed as follows:

\[ \frac{dI}{dt} = KI \] ..................(1)

where \( I \) is the intensity of the incidental light of wavelength \( \lambda \), ‘\( t \)’ is the thickness of the medium and \( K \) is the proportionality factor. Integrating equation (1) and putting \( I = I_0 \), we get

\[ \ln \frac{I_0}{I_t} = Kt \]

or \( I_t = I_0 e^{kt} \) ..................(2)

Beer studied the effect of concentration of the coloured constituent in the solution upon the light transmission or absorption. He found the same relation between transmission and concentration as Lambert found between transmissions and thickness of the layer, i.e., the intensity of a beam of monochromatic light decreases exponentially as the
concentration of the absorbing substance increases arithmetically, and in combined
simplified form we have,

\[ I_t = I_0 \times 10^{-\varepsilon ct} \] .................(3)

Or \[ \log \frac{I_0}{I_t} = \varepsilon ct \] .............(4)

This is the fundamental equation of spectrophotometry, known as Beer-
Lambert’s law. When ‘c’ is expressed in gram moles per litre & ‘t’ in centimetres, ε is
the molar extinction coefficient in Lmol\(^{-1}\)cm\(^{-1}\).

**2.7.II Ringbom plot:**

Ayers [3] pointed out that a straight line obtained in Beer’s law does not show
directly the concentration range within which accurate determination of the coloured
species is possible. The optimum range for highest precision is determined by plotting
percent transmittance against the log of the metal ion concentration in ppm (Ringbom
plot). By plotting this ‘ʃ’ shaped curve is obtained and the optimum range for accurate
determination of metal ion is shown by the linear portion of the curve having maximum
slope [4].

**2.7.III Molar Composition of the complex:**

Various methods have been employed to ascertain the molar composition of the
complex in solution. Out of these mole ratio method and Job’ methods of continuous
variation are very useful and have been employed in the present investigations, are
given below:

**2.7.III.a. Job’s method of continuous variation [5, 6]:**

Though the principle of continuous variation was first applied by Ostromisslensky
[7] and Denison [8], nevertheless the discovery of the method is frequently attributed to
Job who applied it to complex system. This is one of the most generally applicable and widely used methods for elucidating the formula of the complex ion.

Suppose that a metal ion ‘M’ and a ligand ‘L’ react to form a complex $ML_n$,

$$M + nL \rightarrow ML_n$$

Then instability or the dissociation constant, $K_{ins}$ is given by the expression,

$$K_{ins} = \frac{[ML_n]^n}{[M][L]^n}$$

Or $$[ML_n] = \frac{[M][L]^n}{K_{ins}}$$

A series of solution is prepared in which the sum of the formal concentration of ‘M’ and ‘L’ is kept constant, while their ratio is varied. Repeating these formal concentrations by $C_M$ and $C_L$ respectively, we have

$$C_M + C_L = C$$

A quantity ‘f’ is defined in such a way that in any one of the series of solutions,

$$C_L = fC \quad \text{and} \quad C_M = (1-f)C$$

In any mixture of ‘M’ and ‘L’

$$[M] = (1-f)C - [ML_n]$$

And $$[L] = fC - n[ML_n]$$

Where $[M]$, $[L]$ are free metal and ligand concentration at equilibrium. Obviously, the concentration of the complex will change as ‘f’ is varied. It will be zero when ‘f’ is either zero or unity and will have a maximum value in between. If absorbance is measured at a wavelength at which the absorption is primarily due to $[ML_n]$, the value of ‘f’ at the point of maximum absorbance will correspond to the maximum
concentration of \([\text{ML}_n]\). This is in turn, will be the value of ‘f’ at the point where 
\(d/df[\text{ML}_n]\) is zero since,

\[
[\text{ML}_n] = \frac{(1 - f)C - n[\text{ML}_n]}{f C - n[\text{ML}_n]} K_{\text{ins}}
\]

Where \(K_{\text{ins}}\) is the dissociation constant of the complex, it can be shown that,

\[
K_{\text{ins}} \frac{d}{df}[\text{ML}_n] = \{fC - n[\text{ML}_n]\}^n \{C - \frac{d}{df}[\text{ML}_n]\} \{fC - n[\text{ML}_n]\} \{fC - n[\text{ML}_n]\}
\]

The above equation reduces to \(n = f/(1 - f)\), when \(\frac{d}{df}[\text{ML}_n]\) is equal to zero.

Accordingly the absorbance of the solution is plotted against ‘f’, the mole fraction of the ligand in the mixture. For a 1:1 complex ML, the curve will pass through a maximum of \(f = 0.5\), if the composition of the complex is 1:2 (ML\(_2\)), the maximum will occur at \(f = 0.66\), if the formula is M\(_2\)L, the maximum will be found at \(f = 0.33\) and so on.

The simplicity of the method makes it very attractive and in spite of the criticism of the method by a large number of workers [9-13] it is agreed that the results are reliable when absorbance measurements are carried out. The method, however, is not applicable when two or more complexes are formed and it is also not suitable when L:M ratio is greater than 3.

2.7.11.b. Mole ratio method:

This is another popular and convenient method for investigation of coloured complexes in solution, worked out by Yoe and Jones [14]. Here the total concentration of the metal ion is kept constant while the total concentration of the ligand is increased. The curve of absorbance plotted against the concentration ratios rises linearly from the origin and breaks sharply to a horizontal straight line at the mole ratio of the components in the complex. However, a complex that undergoes appreciable dissociation in solution gives a continuous curve, which only becomes parallel to the
mole ratio axis when an excess of the variable component is added. It is often seen that such a curve breaks sharply at the correct molar ratio, if the ionic strength of the solution is adjusted to a suitable value by addition of an indifferent electrolyte. Thus in such cases, it is possible to get the true composition of the complex. Mayer and Ayres [15] gave a mathematical treatment for deducing the composition of several complexes formed in a system under a given set of condition.

### 2.8 Preliminary Investigations:

During preliminary investigations on 2,5-dimethyl-4-(2-quinolylazo)phenol, DM-p-QAP, as a possible chelating agent for the spectrophotometric determination of micro amounts of metal ions, qualitative tests of the colour reactions were carried out. The results are summarized below.

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Colour of the complex</th>
<th>$\lambda_{\text{max}}$(nm)</th>
<th>Stability of the complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>Purple</td>
<td>569</td>
<td>Stable for 48 hours</td>
</tr>
<tr>
<td>Cu(I)</td>
<td>Yellowish green</td>
<td>452</td>
<td>Stable for 24 hours</td>
</tr>
<tr>
<td>Ag(I)</td>
<td>Dark red</td>
<td>574</td>
<td>Stable for 48 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not sensitive to light.</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>Blood red</td>
<td>617</td>
<td>Stable for 48 hours</td>
</tr>
<tr>
<td>Co(II)</td>
<td>Purple violet</td>
<td>584</td>
<td>Stable for 48 hours</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>Maroon</td>
<td>593</td>
<td>Stable for 48 hours</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>Blood red</td>
<td>582</td>
<td>Stable for 03 hours</td>
</tr>
</tbody>
</table>

On the basis of these investigations it was considered to explore the possibilities of this ligand as a spectrophotometric reagent for trace determination of Cu(I), Cu(II), Ag(I), Ni(II), Co(II), Cd(II) and Hg(II).
Reference:


7. Ostromissensky J; *Ber.* 1911, **44**, 268


