Aggregation of Neutral Red

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The absorbances of solutions of different concentrations of the dye, Neutral Red have been measured at 543 nm at different intervals of time and from the observed deviation from Beer's law, the aggregation number has been calculated. It is observed that the aggregation number increases with (i) increasing concentration of the dye, (ii) time of storage, and (iii) increasing pH.

Several methods have been proposed for the study of aggregation of dyes and according to Pugh et al.,1 and Lemin and Vickerstaff2, spectrophotometric method is very convenient for this purpose. Recently, Malik and Gupta3 have investigated the aggregation of some solochrome mordant dyes and Coates4 has studied the effect of standing time on the aggregation of 1-(2'-hydroxy-4'-sulpho-5'-nitronaphthylazo)-2-hydroxynaphthalene. We present here the results of our studies on the aggregation of Neutral Red, an azine dye (Colour Index No. 50040).

A Chroma sample of Neutral Red was employed in the present study. The solvents used were of reagent grade quality. A Shimadzu double beam spectrophotometer (U.V.-140.02) was used for spectral studies. The dye was purified chromatographically3, dried in vacuo and tested for purity by TLC and analysis4.

Solutions of different concentrations of the dye were prepared by suitable dilution, with deionised water, of 3.462 \times 10^{-3} \, M (0.1%) solution of the dye. The absorption spectra of these solutions were recorded immediately after preparation and at different intervals of time. The effect of pH was studied using Clark and Lubs buffers. The solutions were kept in dark except during measurements. The validity of Beer's law was verified from plots of absorbance against concentration of the dye.

The average aggregation number was calculated using Eqs 1-3 proposed by Pugh et al.,1

\[
(1 - \alpha) = A \cdot a \cdot \Delta A
\]

\[
M = M_0 / (1 - \alpha)
\]

\[
N = M / M_0
\]

where \( \alpha \) = fraction of the monomer in the combined state; \( A \) = observed absorbance; \( a \) = probability of photon being absorbed by a monomer; \( \Delta A \) = deviation in absorbance from that predicted by Beer's law; \( M \) = average molecular weight of the aggregated molecule; \( M_0 \) = molecular weight of the monomer; and \( N \) = average aggregation number.

According to Pugh et al.,1, the probability of a photon being absorbed by a monomer, \( a \), is given by:

\[
a = e_{\text{max}} \times 10^{-6}
\]

where \( e_{\text{max}} \) is the molar absorptivity of the dye. The value of \( e_{\text{max}} \) for Neutral Red is 2.025 \times 10^4. Substituting this value in Eq. 4, value of \( a \) comes out to be 0.02025.

The average aggregation number of Neutral Red was calculated from the above data. Taking the value of \( M_0 \) as 288.8, the results obtained are given in Table 1 when the absorbances were measured after a time interval of 35 hr. These results indicate that the average aggregation number increases with increasing concentration of the dye. In this connection, it may be mentioned that Malik and Gupta3 have noticed a similar behaviour with solochrome dyes.

Coates4 has observed that the aggregation of the dye is dependent upon time. A similar behaviour is observed in the case of Neutral Red also and the values of the average aggregation number at different times are presented in Table 2.

The effect of pH on the aggregation of Neutral Red was studied spectrophotometrically in the pH range 1-6 employing Clark and Lubs buffers. The values of the average aggregation number of the dye are 2, 3 and

<table>
<thead>
<tr>
<th>[Dye] \times 10^4 (M)</th>
<th>( a )</th>
<th>( A )</th>
<th>( \Delta A )</th>
<th>( (1 - \alpha) )</th>
<th>( M )</th>
<th>( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.925</td>
<td>0.0203</td>
<td>1.82</td>
<td>0.055</td>
<td>0.6717</td>
<td>429.9</td>
<td>1</td>
</tr>
<tr>
<td>1.00</td>
<td>0.0205</td>
<td>1.925</td>
<td>0.10</td>
<td>0.3898</td>
<td>740.89</td>
<td>3</td>
</tr>
<tr>
<td>1.155</td>
<td>0.0202</td>
<td>2.15</td>
<td>0.18</td>
<td>0.2413</td>
<td>1197</td>
<td>4</td>
</tr>
<tr>
<td>1.60</td>
<td>0.02031</td>
<td>2.75</td>
<td>0.5</td>
<td>0.1117</td>
<td>2585</td>
<td>9</td>
</tr>
<tr>
<td>1.845</td>
<td>0.02019</td>
<td>2.98</td>
<td>0.745</td>
<td>0.0808</td>
<td>3574</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Dye] \times 10^4 (M)</th>
<th>Storage time (hr)</th>
<th>( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.155</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>4</td>
<td></td>
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<tr>
<td>45</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1.60</td>
<td>5</td>
<td>7</td>
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<tr>
<td>10</td>
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<td>45</td>
<td>12</td>
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</tbody>
</table>

995
5 respectively at pH values 2.2, 3.2 and 5.2, at a concentration of $1.155 \times 10^{-4} M$, of the dye solution; thus the aggregation number increases with increasing pH. The $\lambda_{\text{max}}$ of the solution in this pH range (1-6) varies from 535 to 545 nm. In strong acidic solutions, the dye attains a blue colour ($\lambda_{\text{max}} = 595$ nm) which may be due to protonation. Beyond pH 6.8, the colour of the dye solution becomes yellow ($\lambda_{\text{max}} = 460$ nm) which is perhaps due to the formation of the leucobase of the dye.

In freshly prepared solutions, the absorbance-concentration plot is linear up to a concentration of $1.2 \times 10^{-4} M$, and beyond this concentration, there is deviation. This shows that the dye does not aggregate up to this concentration but does so at concentrations beyond this value. The concentration of the dye beyond which aggregation does not occur decreases with increasing storage time. Further, it has been observed that at concentrations beyond $1.845 \times 10^{-4} M$, there is a shift in the absorption maximum towards shorter wavelengths, namely 532 nm, from the original value of 543 nm. However, for concentrations which lie in the range of validity of Beer's law, the absorption maximum is in the range 540-546 nm.

The spectral behaviour of two different concentrations of the dye solutions is shown in Fig. 1. From this, it is evident that for solutions of concentrations above $1.845 \times 10^{-4} M$ the $\lambda_{\text{max}}$ occurs at lower wavelengths. In this connection, it may be mentioned that Moulik et al.\(^8\) have observed a similar behaviour with Acridine Orange. According to these authors, the presence of a negative ionic species helps the dye association in water, which in turn brings about changes in the spectral behaviour. Such an association may play a significant role in the present study involving Neutral Red also, and may lead to dye-dye interactions. Since there is no other external species present in the solution, the presence of the chloride ion in the dye molecule may prevent the solvation of the cation with water molecules, thus facilitating aggregation, which leads to deviation from Beer's law. We believe that the aggregation of Neutral Red involves formation of hydrogen bonding.

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References