Spectrophotometric Study of Acid-Base Equilibria of Sudan Green in Mixed Water-Organic Solvent Media

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The visible absorption spectra of 1,4-bis(4'-methylanilino)-8-hydroxyanthraquinone (Sudan Green or SG) have been recorded in water-organic solvent mixtures in the pH range 0.2-11.5. The organic solvents used are ethanol, dimethylformamide and acetone. The spectral changes have been explained in terms of shifts in equilibria amongst different molecular and ionic species of SG existing in solution. The $pK_a$ values corresponding to the different ionisation steps have been determined at $25^\circ C$ and $I = 0.1\, \text{mol dm}^{-3} (\text{NaClO}_4)$ by graphical analysis of the absorbance-pH curves. The elimination of proton from the $\alpha$-OH group of the nonionised species of SG occurs below pH 4.5 as a result of mesomeric interaction between the imino nitrogen and the $C=O$ group. The results obtained are discussed in terms of molecular structure of the reagent and the nature of the organic cosolvent used.

Our continued interest in the solution equilibria of arylaminoanthraquinones$^1-5$, led us to undertake the title study. The main objectives have been to study pH-dependence of the spectral changes and to obtain a desirable level of control over the various acid-base equilibria existing in solution. The values of the acid dissociation constants have also been determined whenever possible from the graphical analysis of the spectral data.

Materials and Methods
Stock solution of Sudan Green (SG) ($2 \times 10^{-4}\, \text{mol dm}^{-3}$) was prepared by dissolving accurately weighed amount of the purified reagent in spectrograde ethanol, dimethylformamide or acetone. Other chemicals used were of AR grade. Standard carbonate-free NaOH and perchloric acid solutions were prepared. Deionized water was used throughout the present study. The working solutions were prepared by accurate dilution. The acidity of the solutions was adjusted by adding dilute HClO$_4$ or NaOH solution. The ionic strength was maintained constant at $I = 0.1\, \text{mol dm}^{-3} (\text{NaClO}_4)$. The absorption spectra were recorded on a Pye-Unicam SP 8000 spectrophotometer at $25^\circ C$ using 1 cm matched stopped quartz cells. pH measurements were made using a Radiometer pH-meter (model M63) equipped with Radiometer combined glass electrode. The pH values in mixed media were corrected as described by Douheret$^6$.

Results and Discussion
Depending on the acidity of the medium, the solution of SG contains four different acid-base forms, viz., $LH_2^+$, $LH_4^+$, $LH_4^0$, and $LH_7^2$. The diprotonated species $LH_2^+$ (520 nm) is present in strong acid medium ($pH < 0.3$). In the pH range of 0.5-1.2 the solution contains the purple monoprotonated $LH_4^+$ form (550 and 630 nm) together with the neutral $LH_4^0$ form ($pH > 1.5$). Above $pH_2$, the spectrum of SG exhibits a new absorption band in the region 440-460 nm. The longer wavelength band appears as split bands with $\lambda_{max}$ at 590 and 640 nm. The intensity of 440 nm band increases rapidly with rise in $pH$ whereas that of 550 nm band decreases gradually until it vanishes completely at $pH \approx 4.5$. These spectral changes are assumed to accompany the transformation of the neutral form $LH_4^0$ to the monoanionic species $LH_7^0$. The red $LH_3^+$. The red $LH_3^0$ species existing in the pH range of 2-3.5 is converted into the pale green monoanionic $LH_7^0$ at $pH \approx 4.0$, above which the spectrum of the solution exhibits no band shift or intensity changes.

The acid-base equilibria of SG have been studied in media containing 20%, 50% and 75% of the organic cosolvent (ethanol, DMF or acetone). The absorbance of $4 \times 10^{-5}\, \text{mol dm}^{-3}$ SG solution in the presence of various concentrations of HClO$_4$ or NaOH was recorded. The spectral changes of SG with $pH$ (Fig. 1 as a representative case) show three isosbestic points (near 575, 560 and 495 nm) indicating the presence of different equilibria in solution.

The dissociation constants of SG in various media were evaluated from the individual formation regions of the absorbance-pH curves by graphical analysis. The principles of the graphical treatment of data have been given elsewhere$^7$. For the sake of brevity we shall give only the final equations. The transformations are derived from the equation for the equilibrium constant and mass balance equations, combined...
with the equation for total absorbancy of the solution
(assuming the validity of Beer's law). After simple
rearrangement one obtains for the equilibrium (A) the
transformations (1-4)

\[ K = \frac{[\text{HL}][H^+]}{[\text{LH}][\text{H}^+]}; \quad K_a \]

\[ \epsilon_1 = \epsilon_2 \]

\[ A = \epsilon_1 C_1 + K_a (\epsilon_2 C_1 - A) /[H^+] = A_{11} + F_1 K_a \]

\[ A = \epsilon_2 C_2 - (A - \epsilon_1 C_1) K_a^{-1} [H^+] = A_{02} - F_2 K_a^{-1} \]

\[ C_{1/A} = \frac{1}{\epsilon_1 - (C_1 \epsilon_2 - A)} [H^+]^{-1} A^{-1} \epsilon^{-1} K_a \]

\[ C_{1/A} = \frac{1}{\epsilon_2 + (A - C_1 \epsilon_1)} [H^+] A^{-1} \epsilon^{-1} K_a \]

(1)

(2)

(3)

(4)

where \( A \) is the absorbance, \( C_1 \) is the total reagent concentration, \( \epsilon_1 \) and \( \epsilon_2 \) are molar absorption coefficients, \( K_a \) is the equilibrium constant of the reaction (A), and the other symbols are self-explanatory. The plots of \( A = \epsilon_1 C_1 + K_a (\epsilon_2 C_1 - A) /[H^+] \) versus \( C_1/A \) were linear. The values of the dissociation constants were obtained from the slopes of these linear plots. More precise values of \( K \) could be evaluated using "the graphical logarithmic analysis". The corresponding Eq. (5) can be derived from any one of the Eqs (1)-(4).

\[ \log \frac{A - \epsilon_1 C_1}{\epsilon_2 C_1 - A} = q p H - p K_a \]

The slope of the plot \( \log [(A - A_{11})/(A_{02} - A)] \) versus \( f(pH) \) gives the number of protons \( q \) liberated in the acid-base equilibrium \( (q = 1 \text{ for reaction A}) \). The value of \( p H \) at which \( \log [(A - A_{11})/(A_{02} - A)] = 0 \) determines the value of \( p K_a / q \).

The procedure described above was applied separately to each wavelength and from the series of \( p K_a \) values mean values were calculated. Deviation from the mean \( p K \) values were evaluated using Eq. (6).

\[ \sigma(pK) = \left[ \frac{1}{N} \sum_{n=1}^{N} (pK - pK)^2 \right]^{1/2} \]

where \( pK \) is the mean value calculated from \( pK \) values obtained from curves for individual wavelength \( n \); \( N \) is the number of wavelengths used. The acid-base and spectral characteristics of SG are given in Table 1.

Below \( pH \) 0.4, the spectrum of SG consists mainly of a symmetrical band with \( \lambda_{max} \) at \( \sim 520 \text{ nm} \). This band undergoes red shift and an apparent decrease in intensity on decreasing the acidity of the medium. At \( pH \) 2.5-4, the spectra of the reagent display an absorption at \( \sim 460 \text{ nm} \) which is blue shifted with rise in \( pH \). In addition, two overlapped bands are well defined at 590 and 640 nm. The latter band makes its first appearance at \( pH \sim 1.2 \).

The appearance of only one absorption band at lower \( pH \) values can be ascribed to the localisation of the \( \pi \)-electrons of the imino nitrogen whereas the separation into two bands is due to the interaction of these electrons with the \( \pi \)-electrons of the aromatic system. The band at \( \sim 460 \text{ nm} \) can be assigned to \( \pi-\pi^* \) intramolecular charge transfer associated with the charge migration from the substituents to the an-

\[ \text{Table 1 — Acid-Base and Spectral Characteristics of SG in Various Media.} \]

<table>
<thead>
<tr>
<th>% of organic solvent</th>
<th>( pK_a )</th>
<th>( \lambda_{max} )</th>
<th>( \lambda_{max} \text{ nm; } 10^{-4} \int \epsilon_{max} \text{ mmol}^{-1} \text{ cm}^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(LH/( \text{LH} ))</td>
<td>(LH/( \text{LH} ))</td>
<td>( \lambda_{max} )</td>
<td>( \lambda_{max} \text{ nm; } 10^{-4} \int \epsilon_{max} \text{ mmol}^{-1} \text{ cm}^{-2} )</td>
</tr>
<tr>
<td>Ethanol-water media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.85 ± 0.03</td>
<td>3.72 ± 0.01</td>
<td>573</td>
</tr>
<tr>
<td>50</td>
<td>2.33 ± 0.01</td>
<td>3.75 ± 0.01</td>
<td>570</td>
</tr>
<tr>
<td>75</td>
<td>2.45 ± 0.02</td>
<td>3.85 ± 0.03</td>
<td>570</td>
</tr>
<tr>
<td>DMF-water media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.75 ± 0.02</td>
<td>3.67 ± 0.03</td>
<td>573</td>
</tr>
<tr>
<td>50</td>
<td>2.18 ± 0.01</td>
<td>3.65 ± 0.01</td>
<td>574</td>
</tr>
<tr>
<td>75</td>
<td>2.38 ± 0.02</td>
<td>3.55 ± 0.02</td>
<td>576</td>
</tr>
<tr>
<td>Acetone-water media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.04 ± 0.03</td>
<td>3.74 ± 0.01</td>
<td>575</td>
</tr>
<tr>
<td>50</td>
<td>2.34 ± 0.03</td>
<td>3.85 ± 0.02</td>
<td>575</td>
</tr>
<tr>
<td>75</td>
<td>3.88 ± 0.01</td>
<td>3.88 ± 0.01</td>
<td>572</td>
</tr>
</tbody>
</table>

† Mean value obtained from the values calculated for each wavelength using Eq. (5).
The blue shift of this band with increase in pH is due to blocking of the lone pair of electrons of N atom, leading to hindrance of the charge transfer resulting in higher excitation energy.

As referred elsewhere in the paper, the appearance of three distinct isosbestic points (Figs 1 and 2) indicates the existence of different equilibria. These equilibria can be represented as shown in Scheme 1.

The appearance of the two overlapped bands on the longer wavelength side at pH ~ 1.2 and the obvious decrease in intensity of the π-π* transition band at 280 nm corresponding to the quinonoid system suggest that SG in its monocationic form (LHH+) is involved in a resonance interaction of the type I = II. Such equilibrium seems to be shifted to the right at pH ~ 2. This assumption provides evidence for the spectral changes accompanying liberation of a proton from the α-(OH) group, which takes place in the pH range of 3.5 to 4.0. The band at 520 nm becomes ill-defined at pH ~ 4 presumably due to the transformation of LH3H to LH5. The removal of the proton from the α-(OH) group hinders the ionisation of the mesomeric form (II) of SG. This is evidenced by the non-existence of spectral changes above pH ~ 5.
An attempt was made to ascertain the ability of proton dissociation from the LH$_2^-$ species pH-metrically. Titration of HClO$_4$ (50 ml, $2.5 \times 10^{-3}$ mol dm$^{-3}$) at $I = 100$ mmol dm$^{-3}$ (NaClO$_4$) was performed in 50% (v/v) ethanol, DMF or acetone-water medium. The pH-titration curves of SG in its monoanionic form (LH$_2^-$) gives only one steep inflection at $C_{OH}/C_L = 1$, where $C_{OH}$ is the equivalent base added and $C_L$ is the total initial concentration of SG. Multi-titrations were carried out in the media investigated. The inflection obtained was always found to start at pH 8.8-8.5. This inflection was unambiguously due to dissociation of the proton from LH$_2^-$ species and the production of doubly ionised form (LH$^{2-}$) of the compound.

It is therefore not surprising that the LH$_2^-$ species in the prevalent form of SG in the pH range of 4.5-8. In this respect, it is worthy of mentioning that the last proton elimination is not accompanied by measureable changes in the spectra of SG in different media (cf. Figs. 1 and 2). This behaviour can be explained on the basis that the N-phenyl moiety is twisted out-of-plane of the rest of the molecule$^{10,11}$, resulting in a weak interaction of the N-phenyl group with the anthraquinone nucleus. Accordingly one can assume that the acid dissociation occurring at pH > 8.5 is due to liberation of proton from the anilino group. The above conclusion may give us an insight into the complex forming ability of SG when present in its divalent anionic form.

The $pK_a$ values obtained in solutions containing the same percentage of the different organic solvents follow the order: DMF (9.35) < ethanol (9.5) < acetone (9.79). This is presumably due to a decrease in the tendency of the solvent to associate with the solute through H-bond. Acetone is characterized by a very weak tendency to donate hydrogen bond$^{12}$. The lowest $pK_a$ value in DMF is because of its high basic character facilitating the ionisation process.

References