SPECTROPHOTOMETRIC DETERMINATION OF
THE DISSOCIATION CONSTANTS OF
4-(p-NITROPHENYL-AZO)-8-HYDROXY-QUINOLINE*

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Received June 6, 1956
(Communicated by K. S. G. Doss, F.A.S.C.)

Azo dyes have been employed extensively as analytical reagents for metal ions. Gutzeit and Monnier¹, ² studied a large number of azo dyes containing the oxine nucleus and concluded that they gave specific tests for Hg⁺⁺, Pd⁺⁺, Ni⁺⁺, etc. Bogel Desering and Shreve³ estimated the relative values of the azo dyes as analytical reagents for the varied metal ions. Of these azo dyes, 4-(p-nitrophenyl-azo)-8-hydroxy-quinoline in alcoholic medium appeared to be important as an analytical reagent especially for Ca⁺⁺.⁴, ⁵ During an examination of its applicability to the estimation, with ease, of calcium content in sugarcane juices it appeared desirable to determine the dissociation constant(s) of the dye; the knowledge of which is of marked importance for clear understanding the use of the dye in analytical work. No data on this aspect exist in the literature. The present communication reports the determination of the dissociation constant(s) by employing the characteristic absorption spectra of the substance in the visible region.

EXPERIMENTAL

The starting material employed by the authors for the preparation of the azo dye were acetanilide and 8-hydroxy-quinoline; and these were of the following quality: 8-hydroxy-quinoline used was of B.D.H. quality. Acetanilide was a Bush product; and was converted into p-nitroaniline in the usual manner⁶; this last was purified by recrystallisation in rectified spirit till it gave a constant melting point 147-5⁰ (148⁰) C. p-nitroaniline (1 mol.) was dissolved in N-HCl acid (2½ mol.). The solution was cooled to 5⁰ C. and then treated with 10% solution of NaNO₂ (1 mol.). The diazotised solution was added dropwise to alkaline solution of 8-hydroxy-quinoline (2½ mol. in N-NaOH) maintained at 5⁰ C.; the reaction mixture was stirred for one hour to ensure complete coupling.⁷ The dye was then precipitated by acidifying it with 2 N acetic acid; filtered and washed several times with water. It was crystallised 4-5 times in rectified spirit; a product with a melting point of 258⁰ C. was obtained.

* A preliminary note was published in Naturwiss., 1956, 43, 179.
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The absorption spectra of the alcoholic solution of the azo dye at different pH values was studied with a Unicam S.P. 350 D.G. Spectrophotometer; this had one cm. effective light path. The buffers used were due to Clark and Lubs and to Sörensen; the actual pH of the solution (1 vol. of solution + 1 vol. of buffer) was checked by a Beckmann pH meter.

**RESULTS AND DISCUSSIONS**

Figure 1 gives a typical series of results on the absorption spectra of 4-(p-nitrophenyl-azo)-8-hydroxy-quinoline in alcoholic solution at different pH in the range 4–12. These data refer to a concentration of 0·002 mM of the azo

![Absorption spectra](image)

**Fig. 1.** Absorption spectra of 4-(p-nitrophenyl-azo)-8-hydroxy-quinoline.

Curve 1 refers to pH=6; 2 to pH=7·0; 3 to pH=8·0; 4 to pH=8·2 and 5 to pH=10·5.
dye. It was interesting to note that the dye exhibited absorption maximum at \( \lambda = 600 \text{ m\(\mu\)} \) in alkaline solution and another maximum at \( \lambda = 480 \text{ m\(\mu\)} \) in the neutral and acidic solutions. It is to be remarked that freshly prepared solution of the dye exhibited an absorption maximum at 560 \( \text{m\(\mu\)} \) in alkaline solutions, which on long standing, however, shifted permanently to 600 \( \text{m\(\mu\)} \). The experiments were conducted only when the system exhibited stable and perfectly reproducible characteristics.

It was interesting to observe that, while the absorption at \( \lambda = 480 \text{ m\(\mu\)} \) was not affected appreciably, the same at \( \lambda = 600 \text{ m\(\mu\)} \) was, however, variant markedly with the change of the pH of the solution especially in the range 5–12 pH. The system exhibited an isobestic point, though not well defined in character, in the neighbourhood of \( \lambda = 510 \text{ m\(\mu\)} \) (Fig. 1). The data in Fig. 2 show the variation of the optical density of the system at \( \lambda = 600 \text{ m\(\mu\)} \) with pH of the solution. It was interesting to note that at \( \lambda = 600 \text{ m\(\mu\)} \) the optical density was almost unaltered with pH from 4–6·5; and a further change in pH from 6·5–7, increases the optical density markedly. Thus, e.g., optical density was 0·05, 0·08 and 0·2 at pH = 6·0, 6·5 and 7·5 respectively. Later in the pH range 7·5 and 8, the optical density of the system did not vary appreciably; in fact, it tended to saturate in the sense that the curve representing the variation of optical density with pH appeared to run parallel to the pH axis (see Fig. 2). Further increase of pH again increased optical density, which was unaffected by pH > 9·0 (see Fig. 2). Thus there appeared to exist two inflexions in the curve representing the variation of optical density with pH of the solution, indicating that 4-(p-nitrophenyl)-8-hydroxy-quinoline possess two dissociation constants. The data given in Fig. 2 are therefore not amenable for the familiar calculations, from spectrophotometric data, of the dissociation constant of the substance, which take into consideration that the optical density at different hydrogen concentrations is dependent on the proportion of the substance in the acid or base form (molecule or ion). The present circumstance is apparently complicated as in the case of the calculation of the dissociation constant of a dibasic acid by potentiometric titration method. The existence of two dissociation processes in the system under investigation is presumably due to the presence of the -OH and \( \geq N \) in the molecule of the dye (NQOH)

![Chemical structure](image)
which acts presumably as an ampholytic substance exhibiting the following equilibria:

\[
\begin{align*}
\text{H}_2\text{O} + \text{HNQOH} & \rightleftharpoons \text{NQOH} \rightleftharpoons \text{NQO}^- + \text{H}^+ \\
& \quad \text{(b)} \quad \text{(a)}
\end{align*}
\]

It can be shown that at half-transformation points of the inflexions in the curve given in Fig. 2, the pH is roughly equivalent to \( pK \) of the system; this leads to the deduction from data in Fig. 2 that \( pK_b \) and \( pK_a \) are roughly 7·3 and 8·1. It is to be noted that the system is complicated not only by the two dissociation processes but also by close proximity of the values of \( pK_a \) and \( pK_b \), indicating that at any pH value, the optical density of the system is contributed not only by the cation or anion and neutral molecule, but by all the three forms of the substance.

The following considerations employed for calculation of the optical density at any pH value based on the values of 7·3 and 8·1 for \( pK_b \) and \( pK_a \)
referring to the basic and acidic dissociation processes respectively, are instructive: Let \( \epsilon \) be the optical density of the system at any pH value. As indicated above, this is contributed by the three components of the system, viz., NQOH, NQO\(^-\) and +HNQOH, the exact concentration of any one of these components being dependent upon the pH value of the solution. Thus in highly alkaline solutions (at pH \( \geq 10 \)), it can be assumed that the entire substance (relative concentration = 1) exists in the form of anion, while in strongly acid solutions (at pH \( \leq 5 \)) the cation form of the substance predominates; at any intermediate pH, it is apparent that the optical density is contributed by the appropriate portions, controlled by the dissociation constants \( K_a \) and \( K_b \) (vide infra) of the three forms of the substance. Let \( C_1, C_2 \) and \( C_0 \) be the concentrations of the cation, anion and uncharged components of the substance at any pH value; and \( E_1, E_2 \) and \( E_0 \) represent the relative extinction coefficients, constant characteristic quantities of the three forms, respectively, the optical density \( \epsilon \) at any pH is given by

\[
\epsilon = E_1C_1 + E_0C_0 + E_2C_2
\]

Further, from the observed optical densities at pH = 4 and 12, where the entire substance (relative concentration = 1) is in the cation and anion forms respectively, \( E_1 \) and \( E_2 \) can be assumed to be 0·035 and 1·1. The value of \( E_0 \) can be computed from the relationships that

\[
pH = pK_b \log \frac{C_0}{C_1} = pK_a \log \frac{C_2}{C_0}
\]

and from \( \epsilon = 0·8 \) at pH = 8·1 where \( C_2 = C_1 \); we have therefore from eq. (1) that

\[
\epsilon = 0·035 \; C_1 + 0·55 \; C_0 + 1·1 \; C_2
\]

The values of \( C_1, C_2 \) and \( C_0 \) for any pH value can be computed from eq. (2) and from that \( C_1 + C_0 + C_2 = 1 \). The values of \( \epsilon \) calculated at different pH values are indicated by crosses in Fig. 2. It was instructive to note that despite the fact that the variation of the optical density with pH was not simple, the calculated values of the optical density agreed closely with the observed data.

**SUMMARY**

Absorption spectra of 4-(p-nitrophenyl-azo)-8-hydroxy-quinoline (NQOH) in the visible region was studied at different pH values in the range 4–12. NQOH exhibited two absorption maxima, one at \( \lambda = 480 \; m\mu \) in acid solution and the other at \( \lambda = 600 \; m\mu \) in alkaline solution. The absorption at
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\( \lambda = 600 \text{ m} \mu \) was markedly variant with the pH of the solution; the data indicated two dissociation processes presumably due to the ampholytic character of NQOH

\[
-NQOH + \text{HNQOH} \rightleftharpoons NQOH \rightleftharpoons NQO^- + H^+ \quad (\text{H}_2\text{O})
\]

with 7.3 and 8.1 for \( pK_b \) and \( pK_a \) referring in acidic and basic dissociation processes, respectively. The optical density values calculated from these constants agreed closely with the observed data.

Acknowledgement

Authors’ grateful thanks are due to Professor K. S. G. Doss, D.Sc., F.R.I.C., F.Inst.P., F.A.Sc., Director, Indian Institute of Sugar Technology, for his interest in the work.

References