469. *The Determination of the Dissociation Constants of Very Sparingly Soluble Weak Electrolytes.*

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A differential method is proposed for the spectrophotometric determination of the dissociation constants of very sparingly soluble electrolytes, which is applicable where the solubility of the un-ionised compound is so low that its absorption spectrum cannot be determined.

The method has been tested and confirmed for 4-dimethylamino-2′-methylstilbene, the solubility of which (in water) is $\sim 2 \times 10^{-6}$M.

During investigations of the physicochemical properties of a series of carcinogenic aminostilbene derivatives, requiring determination of their dissociation constants, it immediately became apparent that direct potentiometric titration or conductance measurements would fail completely owing to the low solubilities in water (*ca.* 10^{-6}M.)

Other methods have been proposed, *e.g.*, titration with perchloric acid in glacial acetic acid (Hall and Conant, *J. Amer. Chem. Soc.*, 1927, **49**, 3047, 3062) or titration in aqueous organic solvents, in which the solubility of the base is somewhat higher than in water (Albert and Goldacre, *Nature*, 1942, **149**, 245; Bell and Roblin, *J. Amer. Chem. Soc.*, 1942, **64**, 2905; Kumler and Daniels, *ibid.*, 1943, **65**, 2190; Stockton and Johnson, *J. Amer. Pharm. Assoc.*, Sci. Ed., 1944, **33**, 383; Cook, Heilbron, Reed, and Strachan, *J.*, 1945, 861). The method used by Cook *et al.* (*loc. cit.*) was not applicable for all the compounds which they investigated. Furthermore, extrapolation of data obtained by these methods to pure aqueous solutions has been shown (Gorvin, *J.*, 1949, 3309) to be unreliable. A considerable number of dissociation constants of bases, reported by Hall and Sprinkle (*J. Amer. Chem. Soc.*, 1932, **54**, 3469), were obtained from e.m.f. measurements of a concentration cell containing the half-neutralized base. This method, however, is not applicable to aminostilbene derivatives, because of their low solubilities and because hydrolysis and other corrections would be of such a high order as to render the results of doubtful accuracy. Krebs and Speakman (*J.*, 1945, 593) have proposed a method for slightly soluble bases depending on the solubility at a number of pH values. The method is limited by three factors: (*a*) the base should be capable of being determined in the presence of the buffer solution; (*b*) the limiting solubility of the base must not be immeasurably small; in the example quoted (sulphadiazine), it was of the order of $2{\cdot}5 \times 10^{-4}$M.; (*c*) the electrolyte should not decompose in the solutions employed during the time taken to reach equilibrium (cf. dithizone; Irving, Cooke, Woodger, and Williams, *J.*, 1949, 1847).

Spectrophotometric techniques have been used for the determination of the dissociation constants of organic compounds in the visible (Brode, *J. Amer. Chem. Soc.*, 1924, **46**, 581; Holmes and Snyder, *ibid.*, 1925, **47**, 2232), and in the ultra-violet region (*inter alii* Flexser, Hammett, and Dingwall, *ibid.*, 1935, **57**, 2103; Clark, "The Determination of Hydrogen Ions," MacMillan and Co., 3rd Edition, 1928). In all of these a knowledge of the extinction coefficients both of the ion and of its conjugate base at a number of wave-lengths is necessary. Such spectrophotometric methods are particularly suitable for solutions of low concentrations (10^{-4}—10^{-5}M.) if $\varepsilon_{\text{max.}}$ is large, (*e.g.*, $\sim 10^4$, 1-cm. cell). It was considered possible, therefore, to apply the methods to the sparingly soluble aminostilbenes which absorb strongly in the ultra-violet region. However, because of the immeasurably small limiting solubility in water of the free bases, it was found impossible to measure their absorption spectra, even in a cell of 5-cm. light path. It was therefore necessary to devise an alternative procedure, taking advantage of the greater solubility of the ion at lower pH values.

For substances which obey Beer's law, the usual spectrophotometric determination of dissociation constants (Clark, *op. cit.*, p. 154) consists in measuring, at a particular wave-length, the optical density of absorption of a series of solutions of known total concentration with different pH values. If α is the degree of *ionisation* of the base in any one of these solutions, then the observed optical density D is given by the equation

$$D = \varepsilon_0 lc\alpha + \varepsilon_b lc(1 - \alpha) \quad \ldots \ldots \quad (1)$$

where ε_0 and ε_b are respectively the molar extinction coefficients of the ion and of the base, c is the molar concentration, and l the cell-length in cm.; ε_0 and ε_b are previously determined by measurements of solutions of known concentration and of sufficiently high and low pH values, where α is virtually zero and unity respectively (for a base). Equation (1) then reduces to

either $$D_0 = \varepsilon_0 lc \quad \ldots \ldots \quad (2a)$$

or $$D_b = \varepsilon_b lc \quad \ldots \ldots \quad (2b)$$

It follows that (1) may also be written as

$$D = D_0\alpha + D_b(1 - \alpha) \quad \ldots \ldots \quad (1a)$$

It is frequently possible to find a suitable wave-length at which ε_0 or ε_b is zero, and this is the normal practice. One of the terms on the right-hand side of (1*a*) then disappears and α is immediately determinable. In the present work, ε_b, as previously indicated, could not be determined; and for no wave-length can it be certain that absorption of light is due to the ion only, so that (1*a*) cannot be applied directly. It is possible, however, to determine α at any suitable pH value by the following procedure, involving a knowledge of the extinction coefficient of one species only.

Consider a base ionizing according to

$$\mathrm{BH^+} \rightleftharpoons \mathrm{B} + \mathrm{H^+} \quad \ldots \ldots \quad (3)$$

Then application of the law of mass action and rearrangement gives

$$\mathrm{p}K_a = \mathrm{pH} + \log \alpha/(1 - \alpha) - \log f_{\mathrm{BH^+}}/f_{\mathrm{B}} \quad \ldots \ldots \quad (4)$$

$$\mathrm{p}K_a' = \mathrm{pH} + \log \alpha/(1 - \alpha) \quad \ldots \ldots \quad (5)$$

where f refers to an activity coefficient and K_a' is Brønsted's acidity constant (*Chem. Reviews*, 1928, **5**, 293).

At two values of the hydrogen ion activity $\{\mathrm{H^+}\}_1$ and $\{\mathrm{H^+}\}_2$, measured by pH_1 and pH_2, it follows from (5) that

$$\Delta\mathrm{pH} = \mathrm{pH}_1 - \mathrm{pH}_2 = \log \frac{\alpha_2(1 - \alpha_1)}{\alpha_1(1 - \alpha_2)} = \log R_{\mathrm{H}} \text{ (say)} \quad \ldots \quad (6a)$$

Then $$R_{\mathrm{H}} = \alpha_2(1 - \alpha_1)/\alpha_1(1 - \alpha_2) \quad \ldots \ldots \quad (6b)$$

and from (6*a*) $$R_{\mathrm{H}} = \{\mathrm{H^+}\}_2/\{\mathrm{H^+}\}_1 \quad \ldots \ldots \quad (7)$$

From (6*b*) also, $$\alpha_1 = \alpha_2/[R_{\mathrm{H}} + (1 - R_{\mathrm{H}})\alpha_2] \quad \ldots \ldots \quad (8)$$

For any wave-length we have, from (1*a*):

At pH_1, $$D_1 = D_0\alpha_1 + D_b(1 - \alpha_1) \quad \ldots \ldots \quad (9)$$

At pH_2, $$D_2 = D_0\alpha_2 + D_b(1 - \alpha_2) \quad \ldots \ldots \quad (10)$$

whence $$(D_1 - D_0\alpha_1)/(D_2 - D_0\alpha_2) = (1 - \alpha_1)/(1 - \alpha_2) \quad \ldots \ldots \quad (11)$$

From (8) and (11) it can be shown that

$$\alpha_1 = (D_1 - D_2)/(D_0 - D_2)(1 - R_{\mathrm{H}}) \quad \ldots \ldots \quad (12)$$

$$\alpha_2 = (D_1 - D_2)R_{\mathrm{H}}/(D_0 - D_1)(1 - R_{\mathrm{H}}) \quad \ldots \ldots \quad (13)$$

From (7), (12), and (13) it follows that

$$\alpha_1 = \frac{D_1 - D_2}{D_0 - D_2} \cdot \frac{\{\mathrm{H^+}\}_1}{\{\mathrm{H^+}\}_1 - \{\mathrm{H^+}\}_2} \quad \ldots \ldots \quad (12a)$$

$$\alpha_2 = \frac{D_1 - D_2}{D_0 - D_1} \cdot \frac{\{\mathrm{H^+}\}_2}{\{\mathrm{H^+}\}_1 - \{\mathrm{H^+}\}_2} \quad \ldots \ldots \quad (13a)$$

Substitution of α from (12) or (13) in (5), the appropriate pH value being used, gives the value of $\mathrm{p}K_a'$.

EXPERIMENTAL.

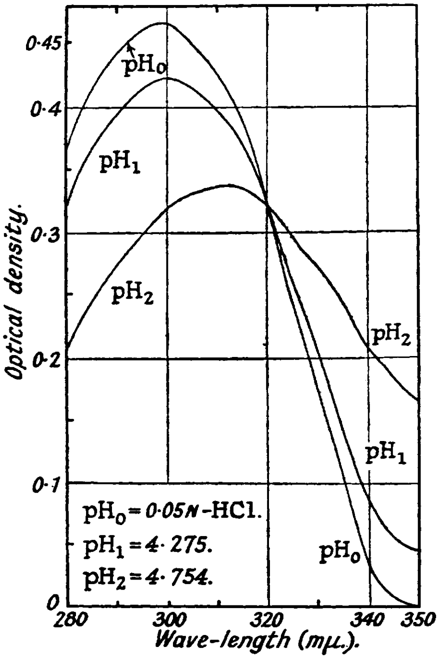
The procedure was used for the determination of the dissociation constant of 4-dimethylamino-2′-methylstilbene. Measurements of optical density were made on the " Uvispek " spectrophotometer adapted to take 5-cm. Beckman cells. Thermostatic control was not attempted, although the temperature of the room was maintained as nearly as possible at 20° ±1°. Measurements of pH were made with the glass electrode standardised with potassium hydrogen phthalate, and the temperature thermostatically controlled at 20° ±0·2°, in well-buffered solutions, over the range required.

The pure 4-dimethylamino-2′-methylstilbene, previously synthesised in this Institute, was recrystallised from light petroleum (b. p. 40—60°) immediately before use, and sufficient was dissolved in N-hydrochloric acid to give a final concentration of approximately 2×10^{-4}M.

Equal aliquot portions of this solution were added to suitable amounts of N-hydrochloric acid and of M-sodium acetate, and the whole diluted so that the total concentration of the stilbene was of the order of 4×10^{-6}M. at the required pH value. The solution was also maintained at an ionic strength $I = 0{\cdot}05$ by suitable additions of 0·1M-sodium chloride.

For measurement of the optical density of absorption of the ion (D_0), an aliquot was taken and added to sufficient hydrochloric acid so that on dilution the final acid concentration was 0·05N. After optical measurements of the buffered solutions the pH values of the cell contents were taken.

In the earliest experiments anomalous absorption spectra were obtained. This was thought to be due to adsorption of positive stilbene ions on the fused quartz walls of the cells (which would seriously affect solutions of such low concentrations), because this effect was particularly noticeable after cleaning the cells with chromic–sulphuric acid, even after prolonged washing with water. It was inferred that the cell walls were still negatively charged, and that the neutralization or reversal of this charge by use of a cationic detergent would lead to more consistent results. Therefore, the cells were rinsed with 0·01% aqueous trimethylcetylammonium bromide, followed by thorough washing with distilled water. After such " lining " of the cell with a detergent multilayer, the stilbene solutions were found to obey Beer's law at any pH value, and it was concluded that this treatment was sufficient to inhibit the adsorption of the positive stilbene ions.



In the figure are shown the absorption spectra of the basic stilbene in 0·05N-hydrochloric acid and in acetate buffers at pH $4{\cdot}27_5$ and pH $4{\cdot}75_4$. The accuracy of any experiment may be gauged by the concurrence of the spectra at the isobestic point, the wave-length at which the extinction coefficients of the base, its ion, or any mixture of the two, are equal. In the figure, the isobestic point is seen to be at 321 mμ. (See Clark, *op. cit.*, pp. 153, 687.)

In another experiment, in which additions of the cationic detergent at a concentration of 1 in 10^7 were made, very good agreement was obtained; an identical isobestic point was found. The table summarises the results obtained in a typical experiment.

Spectrophotometric determination of dissociation constant of
4-dimethylamino-2′-methylstilbene.

λ (mμ.).	D_0.	D_1.	D_2.	a_1.	a_2.	λ (mμ.).	D_0.	D_1.	D_2.	a_1.	a_2.
350	0·002	0·046	0·165	0·848	0·446	300	0·465	0·422	0·3175	0·823	0·392
345	0·008	0·057	0·184	0·838	0·418	295	0·459	0·412	0·296	0·827	0·398
340	0·0395	0·0855	0·2085	0·845	0·432	290	0·443	0·394	0·272	0·829	0·402
335	0·108	0·145	0·244	0·845	0·432	285	0·413	0·365	0·242	0·835	0·414
330	0·182	0·207	0·275	0·849	0·439	280	0·367	0·323	0·209	0·838	0·418
305	0·451	0·416	0·330	0·825	0·397	—	—	—	—	—	—
									Means	0·836	0·417
										±0·010	±0·018

Mean $\mathrm{p}K_a' = 4{\cdot}59 \pm 0{\cdot}03$.

A series of experiments with the same compound gave a mean value for $\mathrm{p}K_a'$ of 4·58 ± 0·04 at 20°. As previously mentioned, the solutions were of ionic strength $I = 0{\cdot}05$ for all experiments. With the conventional assumption that the activity coefficient of the un-ionised base is unity, the calculation of the mean activity coefficient of the ion is given with sufficient accuracy from the approximate Debye–Hückel equation $-\log f_{\mathrm{BH^+}} = 0{\cdot}50Z^2\sqrt{I}/(1 + \sqrt{I})$. For the uni-univalent electrolyte employed, this gives $-\log f_{\mathrm{BH^+}} = 0{\cdot}09$. Hence the thermodynamic $\mathrm{p}K_a$ is 4·67 ± 0·04 at 20°.

The treatment has been confined to a sparingly soluble monoacid base, but it is clear that with suitable modification it can equally well be applied to a monobasic acid. It is also possible that this method may find application in cases where, although the limiting solubility of the compound is higher, it is nevertheless unstable in acid or alkaline medium.

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