

Determination of First pKa' Value and Partition Coefficients of Bromothymol Blue

By V. DAS GUPTA and D. E. CADWALLADER*

The first pKa' value and partition coefficients (between buffered aqueous phases and organic solvents; benzene, carbon tetrachloride, chloroform, ethylene dichloride, methylene chloride, and toluene) of bromothymol blue have been determined. The pKa' value was determined using a method similar to that of Reilley and Swayer. Chloroform showed the highest affinity for bromothymol blue, followed by methylene chloride, ethylene dichloride, benzene, toluene, and carbon tetrachloride. Even at low concentrations ($6 \times 10^{-5} M$) bromothymol blue appears to undergo polymerization, since partition coefficient values change with pH.

MANY AMINES FORM SALTS with acid dyes. These salts can be extracted by organic solvents and the dye component measured spectrophotometrically. This method has been used to assay many drugs (1). The amount of amine-dye salt extracted by an organic solvent is dependent upon the pH of the aqueous phase (2). Gupta *et al.* (3) derived an equation which can be used to predict the optimum assay conditions, such as pH, of the aqueous phase. To solve this equation, the first pKa value and the partition coefficient of the dye between the organic solvent to be used for extraction and the buffered aqueous phase are required. In the authors' previous paper (3) on the effect of pH and dye concentration on the extraction of a thiamine-dye salt by an organic solvent, they had reported the determination of the partition coefficient of bromothymol blue only between chloroform and water, using the approximated pKa value. The approximated pKa value did not agree with the reported value of Schill (4).

This paper describes the determination of the first pKa' value and the partition coefficients of bromothymol blue between buffered aqueous phases and the organic solvents (benzene, carbon tetrachloride, chloroform, ethylene dichloride, methylene chloride, and toluene) which are most commonly used for the extraction of amine-dye salts (1).

EXPERIMENTAL

Reagents—All chemicals and reagents used were either USP, NF, or ACS grade. Bromothymol blue was purchased from W. H. Curtin & Co. and used without further purification.

Preparation of Solutions—*Bromothymol Blue Solutions for the Determination of pKa' Value*—Bromothymol blue (100.0 mg.) was dissolved in

10 ml. of ethyl alcohol in a 50-ml. volumetric flask and enough distilled water added to make up to volume. Solutions of different pH values were prepared by diluting 0.5 ml. of this solution to 100 ml. with hydrochloric acid of various concentrations. All the solutions contained 0.001% of bromothymol blue. Hydrochloric acid (12.0 N) was standardized according to USP procedure (5). This hydrochloric acid solution was diluted with distilled water to obtain various other strengths as needed.

Bromothymol Blue Solutions for the Determination of Partition Coefficients—Aqueous buffer solutions of bromothymol blue ($6 \times 10^{-5} M$) were prepared by dissolving 18.7 mg. of dye in approximately 0.5 ml. of 0.2 M sodium hydroxide solution and then adding enough 0.05 M phosphate buffer solutions of various pH values to make 500 ml. The pH of each solution was determined with a pH meter (Corning 12). Buffer solutions of various pH values were prepared according to the directions in the USP (6).

Determination of pKa' Value—The pKa' value was determined according to the procedure of Reilley and Swayer (7). Solutions of bromothymol blue (0.001%) in hydrochloric acid solutions of various concentrations were scanned between 360 and 600 $m\mu$ using a Beckman DK 2 spectrophotometer. Distilled water was used as the blank. Two wavelengths were chosen where the H_2I and HI^- forms of the dye showed a maximum difference in their absorbance. Plots of absorbance *versus* theoretical pH concentration ($-\log [H^+]$) were prepared for each of the two wavelengths selected. Two horizontal lines across each of the plots were drawn; one corresponding to the absorbance of the dye solution of theoretical pH -1.08 , and the other corresponding to the absorbance of solution of 0.92 pH value (Figs. 1 and 2). The first line (A in Fig. 1 and B in Fig. 2) gave the absorbance of the dye when present entirely in the H_2I form. The second line (A in Fig. 2 and B in Fig. 1) gave the absorbance of the dye when present entirely in the HI^- form. The midpoint of each curve between the Lines A and B represented the equal concentration of H_2I and HI^- form of bromothymol blue. This was marked as the pKa' value on each curve.

From each figure, the ratio of (HI^-/H_2I) at various pH values was obtained by measuring the relative distances of the point from HI^- and H_2I lines. Plots of $\log (HI^-/H_2I)$ (vertically) *versus* pH concentration were prepared (Figs. 3 and 4) for each of

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* Present address: University of Georgia, School of Pharmacy, Athens, GA 30601

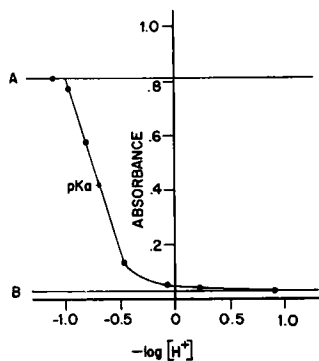


Fig. 1—Determination of pK_a' value using absorbance values at 558 $m\mu$.

the two wavelengths selected. The point where the curve crossed the pH axis corresponded to pK_a' value, since $H_2I \rightleftharpoons HI^- + H^+$ (dissociation of HI^- to I^{2-} and H^+ can be neglected in the pH range of -1.08 to 0.92 since K_2 is 1×10^{-7}). Therefore, $K_1 = \frac{[HI^-][H^+]}{[H_2I]}$, or $\log K_1 = \log HI^- + \log H^+ - \log H_2I$; hence, first $pK_a' = pH - \log HI^- + \log H_2I$, or $\log (HI^-/H_2I) = pH - pK_a'$. Therefore, when $HI^- = H_2I$, $\log (HI^-/H_2I) = 0$ and $pH = pK_a'$. Results from Figs. 1-4 are presented in Table I.

Determination of Partition Coefficients—To determine the partition coefficients (D) of bromothymol blue, 25.0 ml. of dye solution ($6 \times 10^{-6} M$) of appropriate pH value was mixed with 25.0 ml. of an organic solvent in a 250-ml. conical flask, and the flask shaken at high speed (temperature $24.5 \pm 0.5^\circ$) for 30 min. using an Eberbach shaking machine. Preliminary investigations indicated that an equilibrium between the aqueous and organic phases was established within 30 min. After shaking, the mixture was placed in a 125-ml. separator and polar and nonpolar layers were allowed to separate and the organic phase collected. A portion of the organic phase was centrifuged at high speed for 10 min. using an International clinical centrifuge. The absorbance of clear solution so obtained and of the original dye solution used in each experiment was read at 503 $m\mu$, the isosbestic point of bromothymol blue (7) using a spectrophotometer (Bausch & Lomb Spectronic 20). Distilled

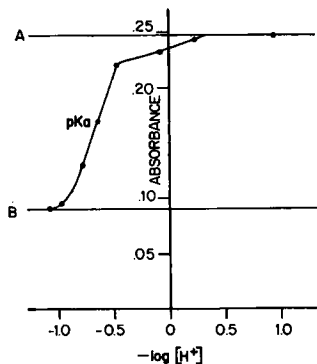


Fig. 2—Determination of pK_a' value using absorbance values at 470 $m\mu$.

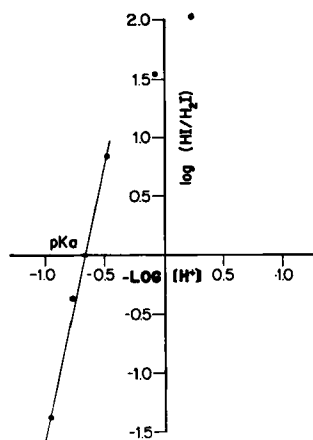


Fig. 3—Determination of pK_a' value using $\log (HI^-/H_2I)$ (from Fig. 1) versus pH concentration plot.

water was used as the blank. The amount of dye present in the organic phase was calculated by difference between the original and final absorbance values. To determine the partition coefficient $D = \frac{[(H_2I)_0]}{[(H_2I)_a]}$ the amount of dye present in the organic phase was assumed to be undissociated and therefore represented $(H_2I)_0$. The fraction of undissociated dye present in the aqueous phase, that is $(H_2I)_a$, at each pH value was calculated as follows (8):

$$(H_2I)_a = \frac{(H^+)^2}{(H^+)^2 + K_1(H^+) + K_1K_2} (C_{H_2I})_a$$

Results are presented in Table II.

DISCUSSION

pK_a' Value—The pK_a' value of -0.662 (Table I) did not compare with the value (1 to 1.48) reported by Schill (4). Three-point straight lines crossing the axis (Figs. 3 and 4) predict that the pK_a' value lies between -0.95 and -0.47 . Two points did not

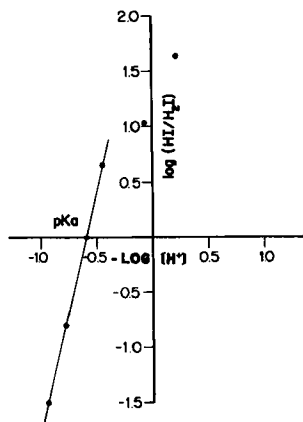


Fig. 4—Determination of pK_a' value using $\log (HI^-/H_2I)$ (from Fig. 2) versus pH concentration plot.

TABLE I—pKa' VALUES AS DETERMINED FROM FIGURES ONE THROUGH FOUR

Figure No.	First pKa' Value	K ₁
1	-0.670	4.68
2	-0.648	4.45
3	-0.668	4.66
4	-0.600	3.98
	Av. -0.662 ^a	Av. 4.60 ^a

^a Average of first three values.

TABLE II—EFFECT OF CHANGES IN pH ON THE PARTITION COEFFICIENTS OF BROMOTHYMOL BLUE^a

Solvent	Partition Coefficient × 10 ⁻⁵ at pH			
	5.0	5.5	6.0	6.4
Benzene ^b	2.18	2.81	5.61	—
Carbon tetrachloride ^b	0.69	1.26	1.46	—
Chloroform ^c	—	12.15	18.70	27.42
Ethylene dichloride ^c	—	8.11	10.32	16.13
Methylene chloride ^c	—	10.03	15.00	23.95
Toluene ^b	1.29	1.98	2.50	—

^a Each value is an average of two experiments. ^b Not done at pH 6.4 due to lack of sensitivity in the method to calculate the amount of dye in the organic phase. ^c Not done at pH 5.0 due to lack of sensitivity in the method to calculate the amount of dye in the aqueous phase.

fall on these straight lines, probably due to lack of sensitivity in the method to calculate either H₂I or HI⁻. It was not possible to take into account the activity coefficients at these high concentrations of hydrochloric acid. This will, no doubt, cause some error in the determination of the pKa' value.

Partition Coefficients—Partition coefficients varied with changes in pH (Table II). This may be due to the formation of polymers. The HI⁻ has been reported (4) to form dimers and tetramers. Error will probably be higher at higher pH value since the concentration of HI⁻ (present only in the aqueous phase) will increase with the pH. An error in the first pKa' value does not appear to affect the relative differences between the partition coefficient values with changes in pH (Table III).

It is apparent that chloroform has the highest partition coefficient value (Table II), followed by methylene chloride, ethylene dichloride, benzene, toluene, and carbon tetrachloride (arranged in order

TABLE III—EFFECT OF CHANGES IN pKa' VALUE ON THE RATIO OF PARTITION COEFFICIENTS AT pH 5.0 AND 5.5 USING BENZENE AS THE MODEL

pKa'	Partition Coefficient × 10 ⁻⁵ at pH		Ratio ^a
	5.0	5.5	
-0.845	3.37	4.33	1.29
-0.662	2.18	2.81	1.29
-0.544	1.68	2.16	1.29
-0.301	0.954	1.24	1.30
1.0	0.482	0.619	1.28

^a Calculated taking pH 5.5 value and dividing by pH 5.0 value.

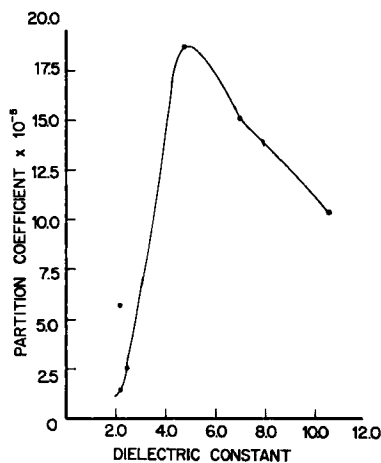


Fig. 5—Plot of partition coefficient versus dielectric constant.

of decreasing partition coefficient values). This is true when partition coefficient values are compared at any particular pH. Further analytical application of this information will be discussed in another report.

A plot of partition coefficient at pH 6.0 (Table II) versus dielectric constant (9) at 20° (benzene, 2.28; carbon tetrachloride, 2.24; chloroform, 4.81; ethylene dichloride, 10.65; methylene chloride, 9.08; and toluene, 2.39) passes through a maximum (Fig. 5). The maximum occurs at a dielectric constant value of 4.81 which corresponds to chloroform.

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Keyphrases

Bromothymol blue—acid dye
 Partition coefficients—bromothymol blue
 pKa' value, first—bromothymol blue
 Colorimetric analysis—spectrophotometer