Selection of Best pH Range for Extraction of Amine–Bromthymol Blue Complexes

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Abstract □ This paper describes the determination of the best pH range for the extraction of amine–bromthymol blue complexes with chloroform. The experimental data on eight amine salts indicate that the best pH range is about 5.2–6.4 for a greater sensitivity in the analytical technique. A theoretical equation was derived and solved using a computer. The experimental results are in fairly good agreement with the theoretical results.

Keyphrases □ Amine–bromthymol blue complexes, extraction—theoretical and experimental determination of optimum pH range □ Bromthymol blue complexes with amines, extraction—theoretical and experimental determination of optimum pH range □ Extraction of amine–bromthymol blue complexes—determination of optimum pH range, theoretical, experimental □ pH range, extraction of amine–bromthymol blue complexes—theoretical and experimental determination

The variation in the amount of amine–dye complexes extracted from an aqueous solution by an organic solvent is dependent on the pH of the aqueous phase (1, 2). The selection of pH for the quantitative determination of amines has been arbitrary (3). Eight amine salts, atropine sulfate (I), codeine phosphate (II), emetine hydrochloride (III), ephedrine hydrochloride (IV), pilocarpine nitrate (V), procaine hydrochloride (VI), quinine hydrochloride (VII), and strychnine sulfate (VIII), were investigated both theoretically and experimentally to determine the best pH range for the extraction of amine–dye complexes. Bromthymol blue (IX) was selected as the dye and chloroform as the organic solvent for the extraction of amine–bromthymol blue complexes since they have been reported to be the best for the analysis of amines (4). The purpose of this paper is to report these studies.

EXPERIMENTAL

Reagents and Materials—All chemicals and reagents used were USP, NF, or ACS grade. Bromthymol blue[1] was used as supplied without further purification. Procaine hydrochloride was purchased from one source[2], and all other amine salts (I–V, VII, and VIII) were purchased from another[3].

Preparation of Solutions—Buffered (0.05 *M* phosphate) aqueous solutions of bromthymol blue (1×10^{-4} *M*) of various pH values (3.0, 4.0, 4.6, 5.2, 5.8, 6.4, 7.0, 7.6, and 8.4) were prepared according to the procedure reported earlier (5). Aqueous solutions of various amine salts of various concentrations were prepared using a simple solution method.

Determination of Best pH Ranges—A 5.0-ml. quantity of the amine salt solution (10 mcg./ml.) was mixed with 5.0 ml. of the dye solution of appropriate pH value in a 125-ml. separator. A 10.0-ml. quantity of chloroform was added, and the mixture was shaken for 1 min. The polar and nonpolar phases were allowed to separate, and the organic phase was collected. The absorbance of the amine–IX complex in the organic phase was measured[4] at 420 nm., the wavelength of maximum absorbance. A blank was prepared by substituting 5.0 ml. of distilled water for 5.0 ml. of the amine salt solution. All extraction curves were similar to the ones reported earlier (1, 6–9) under different experimental conditions. The best pH ranges (maximum absorbance ±5%) are presented in Table I.

Table I—Best pH Ranges for Extraction of Various Amine–Bromthymol Blue Complexes

Amine Salt	Best pH Range, Experimental	Best pH Range, Theoretical
I	5.2–6.4	5.6–6.8
II	5.2–5.8	5.8–6.0
III	4.0–5.8	5.8–6.2
IV	5.2–6.4	6.0–6.6
V	5.2[a]	5.2–5.8
VI	5.2–5.8	5.8–6.0
VII	3.0–4.6	4.2–6.4
VIII	3.0–4.6	4.4–6.0

[a] No range; this appears to be the optimum pH.

Determination of Partition Coefficients of Various Amine Salts—A 5.0-ml. quantity of the aqueous solution of the amine salt of appropriate concentration (I, 2.0 mg./ml.; II, 0.2 mg./ml.; III, 0.1 mg./ml.; IV, 0.6 mg./ml.; V, 0.75 mg./ml.; VI, 0.01 mg./ml.; VII, 0.06 mg./ml.; and VIII, 0.02 mg./ml.) was mixed with 5.0 ml. of pH 7.0 phosphate buffer (0.05 *M*) in a 125-ml. separator. A 10.0-ml. quantity of chloroform was then added, and the mixture was shaken for 1 min. (same time as was given for the extraction of amine–IX complexes). The clear aqueous layer was collected and measured at the wavelength of maximum absorbance (I, 257.5 nm.; II, 284 nm.; III, 282 nm.; IV, 257 nm.; V, 239 nm.[5]; VI, 290 nm.; VII, 330 nm.; and VIII, 254 nm.) using a spectrophotometer[6]. A blank was prepared by mixing 5 ml. of distilled water with 5.0 ml. of the buffer solution. The initial reading of each mixture (equal volumes of buffer and the aqueous solution of the amine salt) was also recorded. The partition coefficients of various amines were then calculated according to the procedure reported (1) earlier for thiamine, except that the undissociated fractions of four amines (III, V, VII, and VIII) were determined using Eq. 1 instead of the Henderson equation since two pKb values for these amines were reported (10, 11) previously. The pKb values and the results are presented in Table II. Equation 1 is:

$$(F)_{Aua} = \frac{(OH^-)^3}{(OH^-)^3 + (OH^-)K_{b1} + K_{b1}K_{b2}} \qquad \text{(Eq. 1)}$$

where $(F)_{Aua}$ is the fraction of the undissociated amine in the aqueous phase.

[1] Eastman Organic Chemicals.
[2] Mallinckrodt Chemical Works.
[3] Merck & Co.
[4] Using a Bausch & Lomb Spectronic 20.
[5] Not the wavelength of maximum absorbance. No sharp peak was recorded.
[6] Beckman DK.

Table II—Partition Coefficients of Various Amine Salts

Amine Salt	Partition Coefficient	pKb Values Used (10, 11)
I	154.0	4.35
II	57.3	6.10
III	1252.0	5.73, 6.74
IV	2.7	4.64
V	2.1	7.2, 12.5
VI	262.7	5.2
VII	116.8	6.0, 9.89
VIII	145.3	6.0, 11.7

Determination of Equilibrium Constant (K)—The equilibrium constants, K, of the amine–IX complexes were determined using Eqs. 2 and 3. Equation 2 was used for 1:1 complexes and Eq. 3 for 1:2 (amine–dye) complexes. The K values of four amines (III, V, VII, and VIII) were determined using Eq. 3. The assumption of 1:2 (amine–dye) complexes for these amines appears to be reasonable and was reported (12) earlier. For the determination of the K values, the experimental data at pH 7.6, as recorded under the *Determination of Best pH Ranges*, were used. A pH value of 7.6 was preferred since at lower pH values the concentration of free dye in the organic phase could not be assumed to be zero. At a pH value higher than 7.6, the extraction of amine complexes was either very poor or nil. The equations are:

$$K = \frac{(C)_{co}}{(A)_{ua}(D)_{ua}} \qquad \text{(Eq. 2)}$$

$$K = \frac{(C)_{co}}{(A)_{ua}(D)_{ua}^2} \qquad \text{(Eq. 3)}$$

where $(C)_{co}$ is the concentration of the amine–IX complex in the organic phase in moles per liter, $(A)_{ua}$ is the concentration of the undissociated amine in the aqueous phase in moles per liter, and $(D)_{ua}$ is the concentration of the undissociated dye in the aqueous phase in moles per liter.

To determine the concentration of the complex in the organic phase, an absorbance value of 0.285 was shown to represent a 1.6×10^{-5} M concentration of the 1:1 complex (12) and a 8×10^{-6} M concentration of the 1:2 (amine–dye) complex (12). The concentrations of the amine and dye in water were calculated by difference. The undissociated fractions of the amines in the aqueous phase were determined according to the procedure reported above (Eq. 1 and the Henderson equation). The undissociated fraction of IX in the aqueous phase was determined according to the procedure reported earlier (5). The calculated equilibrium constant values are presented in Table III.

THEORETICAL

By assuming the following reaction, an equation was derived to calculate the amount of amine–IX complexes extracted by the organic solvent:

$$n(A)_{ua} + n(D)_{ua} = (A^{+n} - D^{-n})_o \qquad \text{(Eq. 4)}$$

where n represents the number of moles of each reacting. Assuming the above reaction and using the symbol $(C)_{co}$ for the concentration of the complex in the organic phase:

$$K = \frac{(C)_{co}}{(A)_{ua}^n(D)_{ua}^n} \qquad \text{(Eq. 5)}$$

since in both types of complexes the n value for amines is 1; therefore, the equation simplifies to:

$$K = \frac{(C)_{co}}{(A)_{ua}(D)_{ua}^n} \qquad \text{(Eq. 6)}$$

In Eq. 6, the value of n is 1 for 1:1 complexes (I, II, IV, and VI) and 2 for the other four amines (III, V, VII, and VIII).

By a process reported earlier (1), it can be shown that:

$$(A)_{ua} = \frac{(C)_A - (C)_{co}}{(P)_A + [1/(F)_{Aua}]} \qquad \text{(Eq. 7)}$$

Table III—Equilibrium Constant (K) Values of Various Amine–Bromthymol Blue (IX) Complexes

Amine–IX Complex	K Value
I–IX	2.32×10^{16}
II–IX	3.16×10^{13}
III–IX	2.87×10^{27}
IV–IX	1.22×10^{16}
V–IX	8.83×10^{24}
VI–IX	2.4×10^{14}
VII–IX	3.21×10^{27}
VIII–IX	1.10×10^{27}

where $(C)_A$ is the total concentration of the amine in both phases (free as well as in the complex), $(P)_A$ is the partition coefficient of the amine, and $(F)_{Aua}$ is the undissociated fraction of the amine in the aqueous phase and equals:

$$(F)_{Aua} = \frac{(OH^-)^2}{(OH^-)^2 + (OH^-)K_{b1} + K_{b1}K_{b2}} \qquad \text{(Eq. 8)}$$

If the amine has only one K_b value, K_{b2} equals zero and:

$$(D)_{ua} = \frac{(C)_D - n(C)_{co}}{(P)_D + [1/(F)_{Dua}]} \qquad \text{(Eq. 9)}$$

where $(C)_D$ is the total concentration of the dye in both phases (free as well as in the complex), $(P)_D$ is the partition coefficient of the dye, and $(F)_{Dua}$ is the undissociated fraction of the dye in the aqueous phase and equals:

$$(F)_{Dua} = \frac{(H^+)^2}{(H^+)^2 + (H^+)K_{a1} + K_{a1}K_{a2}} \qquad \text{(Eq. 10)}$$

With the values for $(A)_{ua}$ and $(D)_{ua}$ derived, the values of $(C)_{co}$ in Eq. 6 were determined at various pH values (3–9 at intervals of 0.2) using a computer[7].

To solve Eq. 6, the following information was used:

K_{a1} and K_{a2} = first and second acid dissociation constants of IX, respectively (5)
K_{b1} and K_{b2} = first and second base dissociation constants of the amines, respectively (Table II) (If the amine has only one K_b value, K_{b2} equals zero)
$(P)_A$ = partition coefficient value of the amine (Table II)
$(P)_D$ = partition coefficient value of the dye (5)
K = equilibrium constant (Table III)
$(OH^-) = \frac{10^{-14}}{(H^+)}$

All curves, as drawn by the computer[8], were similar to the one reported earlier (1) which was drawn using a slightly different equation. The best pH range (maximum extraction ±2%) is presented in Table I.

In the derivation of Eq. 5, all of the complex in the aqueous phase was assumed to be completely dissociated since calculations using a typical value of +1.0 for the dissociation constant of the complex gave negligible concentrations of the undissociated complex in water as compared with the concentrations in the organic phase. Also, to keep the model as simple as possible, the use of an average value for the partition coefficient of bromthymol blue was preferred in these calculations. The incorporation of the effect of pH on the partition coefficient into the model will shift the extraction curves slightly but will not affect the general conclusions of this study. For example, at pH 5.5, the average $(P)_D$ value is 1.27 times the experimental value (5). In the case of codeine phosphate, it will cause an error of about 8% in the concentration of $(C)_{co}$ extracted. At higher pH values, the error is of a smaller magnitude but opposite in sign. The net effect of this correction will shift the theoretical

[7] IBM 360/75.
[8] Using RANGEF and PLOTF plotting subroutines as described in memorandum number LS-30-1 from Triangle Universities Computation Center, Research Triangle Park, N. C. A complete computer program is available on request.

optimum to lower pH values and this will result in increased overlap of the theoretical and experimental optimum ranges.

DISCUSSION

Many authors have recommended (3) a pH range of 7–7.5 for the extraction of amine–IX complexes with chloroform or other organic solvents. The experimental results of these studies (Table I) indicate that the best pH range for these extractions is from 5.2 to 6.4 for 1:1 complexes (I, II, IV, and VI) and from 3.0 to 5.8 for 1:2 (amine–dye) complexes (III, V, VII, and VIII). The theoretical results (Table IV) indicate a best pH range of 5.6–6.8 for 1:1 complexes and of 4.2–6.4 for 1:2 complexes. The best pH range was determined from the maximum absorbance value ±5% for the experimental data and from the maximum theoretical extraction ±2% for the computer data. Since experimental data are not as precise as theoretical data, a wider range was allowed on experimental values. Both the experimental and the theoretical data (Tables I and IV) show that the arbitrarily reported pH range of 7–7.5 (3) is not the optimum for a maximum sensitivity in the assay technique. Nevertheless, sometimes it may be preferable to sacrifice the sensitivity in favor of eliminating the interferences from other ingredients such as in the biological systems. At too low pH values (about 3–4.6), the chances of forming hard-to-break emulsions are greater than at higher pH values. The blank absorbance value against pure chloroform is zero above a pH value of 6.4. At lower pH values, the blank increases with a decrease in the pH, becoming significantly high (0.04–0.05) below pH 5.

It appears that a pH range of about 5.2–6.4 is the best for the extraction of amine–IX complexes. For 1:2 (amine–dye) complexes, the lower value (5.2) should be preferred.

REFERENCES

(1) V. D. Gupta, D. E. Cadwallader, H. B. Herman, and I. L. Honigberg, *J. Pharm. Sci.*, **57**, 1199(1968).

(2) P. Mukerjee, *Anal. Chem.*, **28**, 870(1956).

(3) T. Higuchi and J. I. Bodin, "Pharmaceutical Analysis," Interscience, New York, N. Y., 1961, pp. 414–417.

(4) V. D. Gupta, *Can. J. Pharm. Sci.*, **5**, 44(1970).

(5) V. D. Gupta and D. E. Cadwallader, *J. Pharm. Sci.*, **57**, 2140 (1968).

(6) V. D. Gupta and N. M. Ferguson, *Amer. J. Hosp. Pharm.*, **26**, 408(1969).

(7) *Ibid.*, **26**, 168(1969).

(8) V. D. Gupta, *Amer. J. Hosp. Pharm.*, **28**, 55(1971).

(9) *Ibid.*, **28**, 285(1971).

(10) E. L. Parrott, "Pharmaceutical Technology," Burgess, Minneapolis, Minn., 1970, pp. 219–221.

(11) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," 2nd ed., Lea & Febiger, Philadelphia, Pa., 1969, p. 195.

(12) V. D. Gupta, *J. Pharm. Sci.*, **60**, 1584(1971).

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Mississippi-Grown *Cannabis sativa* L. III: Cannabinoid and Cannabinoid Acid Content

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Abstract □ A procedure for the assay of acidic and nonacidic cannabinoids qualitatively and quantitatively by chemical fractionation prior to TLC and GLC analyses is reported. Various samples of foreign and domestic, wild and cultivated marijuana were analyzed. It was demonstrated that cannabinoids occur largely as acids, that these acids undergo decarboxylation upon storage, and that plants vary significantly in their cannabinoid composition due to heredity.

Keyphrases □ *Cannabis sativa* L.—analysis, acidic and nonacidic cannabinoids by chemical fractionation prior to GLC and TLC, hereditary plant composition and effect of storage on cannabinoid content □ Cannabinoids, acidic, nonacidic—analysis, chemical fractionation prior to GLC and TLC, hereditary plant composition, decarboxylation during storage □ Marijuana—analysis, acidic and nonacidic cannabinoids by chemical fractionation prior to GLC and TLC, hereditary plant composition, and effect of storage on cannabinoid content

The major cannabinoids and cannabinoid acids that occur naturally in the various strains of *Cannabis sativa* L. (marijuana) plants included in this investigation are Δ^9-tetrahydrocannabinol (I), its two corresponding acids Δ^9-tetrahydrocannabinoic acid A (II) and Δ^9-tetrahydrocannabinoic acid B (III), cannabidiol (IV), and its corresponding acid cannabidiolic acid (V) (1–3). It has been established that I is the principal psychotomimetic compound in marijuana (4). When marijuana is smoked, II and III undergo decarboxylation and are inhaled as I (5, 6). This is one reason why marijuana exerts higher activity when smoked than when eaten.

GLC is being used for the quantitative analysis of marijuana (7–9), but these procedures do not distinguish between I and its respective acids. The acids decarboxylate rapidly to I in the injection port. Thus, the data determined by GLC analysis represent I plus its two respective acids.

The Department of Pharmacognosy at the University of Mississippi is growing *C. sativa* L. in order to supply analyzed marijuana and marijuana extracts to the National Institute of Mental Health (NIMH) for