# Acid Dye Method for the Analysis of Thiamine

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**An accurate, precise, and simple method for the analysis of thiamine has been developed. The method is based on the salt formation of thiamine with an acid dye, bromothymol blue. There is no interference from other vitamins, hormones (ethinyl estradiol and progesterone), and excipients. The nature of reaction between thiamine and bromothymol blue appears to be a simple acid–base reaction. The stoichiometric balance has been established to be one part of dye to one part of thiamine.**

THIAMINE is an essential nutritional or therapeutic component of a great many oral and injectable pharmaceutical preparations. Many biological and chemical methods for the quantitative determination of thiamine have been proposed. Chemical assay methods are usually preferred to biological methods, since analysis can be performed rapidly and economically, and they are usually more reliable for routine determinations.

Although a number of chemical assay methods of thiamine are known, only the coupling reaction with diazotized 6-aminothymol (1) and thiochrome reaction (2) have been developed as precise methods of assay. In recent years the thiochrome method has been used almost exclusively, but close attention to details makes this method unsuitable for rapid control work. The development of an accurate colorimetric method of analysis for thiamine is the object of this investigation. Schill and Marsh (3) and Schill (4–6) have studied the photometric determination of amines and quaternary ammonium compounds with bromothymol blue. They reported that thiamine reacts with bromothymol blue to form a salt which can be determined photometrically. The present investigation includes studies pertinent to the nature of the salt, factors influencing its formation, and the stoichiometric balance.

## EXPERIMENTAL

**Reagents**—All the chemicals and reagents used were either USP, NF, or A.C.S. grade. Bromothymol blue was purchased from Fisher Scientific Co. and was used without further purification.

**Preparation of Solutions**—A chloroform solution of the dye ($4 \times 10^{-4}$ *M*) was prepared by dissolving 62.44 mg. of bromothymol blue in enough chloroform to make 250 ml. of solution. This solution was diluted with chloroform to obtain various other strengths as needed. These solutions were stable for at least 1 month.

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An aqueous buffer solution of bromothymol blue ($2 \times 10^{-4}$ *M*) was prepared by dissolving 62.44 mg. of the dye in approximately 0.5 ml. of 0.2 *M* sodium hydroxide solution and then adding enough pH 6.0 buffer solution to make 500 ml. The pH of the solution was determined with a Beckman Zeromatic pH meter. This solution was stable for at least 2 weeks.

Aqueous solutions of thiamine were prepared by dissolving 40.0 mg. of either thiamine hydrochloride or thiamine mononitrate in enough buffer solution to make 100 ml. These stock solutions could be used to prepare solutions of lower thiamine concentrations by diluting with appropriate buffer solutions. Solutions with pH 6.6 or lower were prepared fresh daily while those with values higher than pH 6.6 were prepared immediately before use, since thiamine is unstable at higher pH values.

Buffer solutions were prepared according to directions in the USP (7). Buffer solutions between pH 4.0 and 8.0 were prepared by taking 50 ml. of 0.2 *M* potassium dihydrogen phosphate solution, adding an appropriate amount of 0.2 *M* sodium hydroxide solution or 0.2 *M* hydrochloric acid solution, and then adding enough distilled water to make 200 ml. Solutions between pH 1.0 and 4.0 were prepared by adding an appropriate amount of 0.2 *M* hydrochloric acid to distilled water, and solutions between pH 9.0 and 11.5 were prepared by adding 0.2 *M* sodium hydroxide to distilled water. The pH of each solution was determined with a Beckman Zeromatic pH meter.

**Determination of Thiamine Dye Salt in Organic Phase**—*Procedure A*—A 10.0-ml. quantity of buffer solution containing an appropriate amount of thiamine alone or with other substances was placed in a 125-ml. separator. A 10.0-ml. quantity of dye solution in chloroform ($1 \times 10^{-4}$ *M* unless otherwise indicated) was added to 10.0 ml. of the aqueous solution, and the separator was shaken vigorously by hand for 1 min. The nonpolar and polar phases were allowed to separate and the chloroform layer was collected. The aqueous phase was extracted with 2 additional 10.0-ml. quantities of chloroform and the 3 chloroform extracts were combined. The chloroform solution was evaporated in a 50-ml. beaker using low heat (60°) to a volume less than 10 ml. After the solution had cooled to room temperature, chloroform was added to make 10.0 ml. of solution. A portion of this solution was centrifuged for 5 min. at 2500 r.p.m., and the absorbance of the clear solution was read at 420 mμ (slit width 0.03 mm.) using a Beckman DU spectrophotometer. A blank was prepared by substituting 10.0 ml. of plain buffer solution for thiamine solution in the above procedure.

*Procedure B*—This procedure is the same as above

except that only one 10.0-ml. quantity of chloroform was used for extraction instead of three.

*Procedure C*—A 5.0-ml. quantity of pH 6.0 buffer solution containing 10.0 mcg./ml. of thiamine hydrochloride was combined with 5.0 ml. of buffered dye solution in a 125-ml. separator. A 10.0-ml. quantity of organic solvent was added and the separator was shaken vigorously by hand for 1 min. The polar and the nonpolar phases were allowed to separate, and the organic phase was collected and centrifuged at 2500 r.p.m. for 5 min. The absorbance of the clear solution was read at 420 mμ. A blank was prepared by substituting plain buffer solution (5.0 ml.) for thiamine solution in the above procedure.

**Determination of Dye in Aqueous and Organic Phases**—To determine the amount of dye in the aqueous phase of an experimental run, the aqueous layer was collected and centrifuged for 5 min. at 2500 r.p.m., and the absorbance was measured at 503 mμ (slit width 0.015 mm.), the isosbestic point of bromothymol blue, with a Beckman DU spectrophotometer, and compared with a standard.

To determine the amount of dye in the organic phase of an experimental run, the organic layer was collected and a portion added to an equal volume of alkaline aqueous solution (pH 11.5) in a 125-ml. separator. The funnel was vigorously shaken by hand for 1 min., the aqueous phase was collected, and the absorbance of the solution measured at 503 mμ as described above.

The isosbestic point is that wavelength where the absorption of a dye in solution is dependent only on the concentration of dye and not the pH. The isosbestic point of bromothymol blue was determined according to the procedure of Reilley and Swayer (8). A stock solution of bromothymol blue was prepared by dissolving 100 mg. of the dye in enough 20% v/v ethyl alcohol to make 100 ml. Two milliliters of the stock solution was placed in a 50-ml. volumetric flask, 20 ml. of distilled water and 8 drops of concentrated hydrochloric acid were added, and the volume was brought to 50 ml. with distilled water. The pH of this solution was approximately 1. A pH 6.9 solution was prepared by placing 2.0 ml. of the stock solution in a volumetric flask and adding sufficient quantity of pH 6.9 phosphate buffer to make 50 ml. A solution having pH 13 (approximately) was prepared by placing 2.0 ml. of the stock solution in a 50-ml. volumetric flask, adding 28 drops of 3.5 *M* sodium hydroxide solution, and diluting to 50 ml. with distilled water. An absorption spectrum of each of the 3 solutions between 365 to 600 mμ was recorded on the same graph paper using a Perkin-Elmer 202 spectrophotometer. The point of intersection where the 3 curves have the same absorbance is the isosbestic point for bromothymol blue (Fig. 1).

**Determination of Thiamine in Aqueous and Organic Phases**—The determination of thiamine in aqueous phase was carried out according to the USP assay method (2). The fluorescence of each solution was measured using a Coleman model 12 C fluorophotometer and thiamine content was determined by comparison with standard readings.

The determination of thiamine in an organic solvent was carried out by first mixing the organic phase with an equal volume of 0.2 *M* hydrochloric acid in a 100-ml. beaker and evaporating the organic

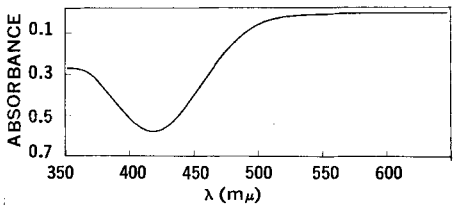


*Fig. 1—Absorption spectrum of bromothymol blue.*

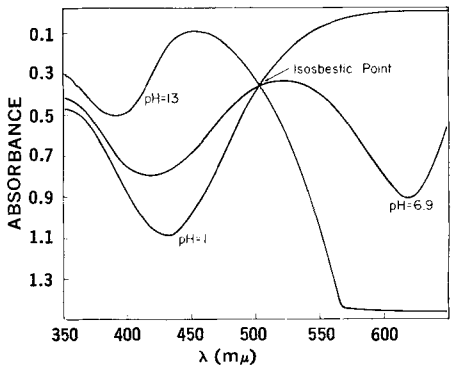


*Fig. 2—Absorption spectra of thiamine dye salt.*

phase by heating at 80°. After the solution was cooled to room temperature, 0.2 *M* hydrochloric acid was added to give a thiamine salt concentration of approximately 0.2 mcg./ml. The thiamine assay was then carried out as described above.

**Determination of the Wavelength of Maximum Absorption**—A 10.0-ml. quantity of pH 6.6 buffer solution containing approximately 8.5 mcg./ml. of thiamine hydrochloride was placed in a 125-ml. separator. A 10.0-ml. quantity of bromothymol blue solution ($1 \times 10^{-4}$ *M*) in chloroform was added to the aqueous solution, and the separator was shaken vigorously for 1 min. The two phases were allowed to separate, the chloroform phase was collected, and the absorption spectrum of the thiamine dye salt in solution was obtained by scanning on a Perkin-Elmer 202 spectrophotometer. The absorption spectrum is shown in Fig. 2.

**Determination of Maximum Dye Concentration**—Ten-milliliter quantities of pH 6.0 buffer solution containing 4.0 mcg./ml. of thiamine hydrochloride were placed in 125-ml. separators. Ten-milliliter quantities of dye in chloroform solutions (various concentrations of dye) were added to the separators, and each mixture was treated in the manner described under procedure *B* for the determination of thiamine dye salt. The results are presented in Table I.

**Preparation of Calibration Curves**—Using procedure *A*, pH 6.6 buffer solutions containing various concentrations of thiamine hydrochloride or thiamine mononitrate were assayed for thiamine dye salt concentrations. The absorbance readings were used to prepare calibration curves and to test for conformity to Beer's law. The results are shown in Fig. 3.

**Determination of Best Organic Solvent**—Using procedure *C*, various organic solvents were tested to determine the best solvent for extracting the

TABLE I—ABSORBANCE READINGS WITH INCREASING DYE CONCENTRATION[a]

| Dye Concn., moles/L. | Absorbance at 420 mμ |
|---|---|
| $2.5 \times 10^{-5}$ | 0.244 |
| $5.0 \times 10^{-5}$ | 0.272 |
| $1.0 \times 10^{-4}$ | 0.292 |
| $2.0 \times 10^{-4}$ | 0.325[b] |

[a] Each absorbance value is an average of 3 experiments. Concentration of thiamine HCl was always 4.0 mcg./ml. All individual readings were within ±3% of the mean reading. [b] Reading is high probably due to emulsion formation. This limits the maximum concentration to $1 \times 10^{-4}$ moles/L.

TABLE II—PERCENTAGE OF THIAMINE DYE SALT EXTRACTED BY VARIOUS SOLVENTS[a]

| Solvent | % Extracted |
|---|---|
| Benzene | 6.5 |
| Carbon tetrachloride | 1.3 |
| Chloroform | 95.3 |
| Methylene chloride | 80.4 |
| Petroleum ether | 2.6 |
| Toluene | 5.2 |
| Xylene | 5.2 |

[a] Each value is an average of 2 experiments. All individual readings were within ±3% of the mean reading.

TABLE III—EFFECT OF pH ON THE EXTRACTION OF THIAMINE DYE SALT[a]

| pH | Absorbance at 420 mμ, Procedure B[b] | Absorbance at 420 mμ, Procedure A[c] |
|---|---|---|
| 2.0 | 0.000 | 0.000 |
| 3.0 | 0.000 | 0.000 |
| 3.9 | 0.020 | 0.020 |
| 4.3 | 0.205 | 0.285 |
| 5.2 | 0.274 | 0.304 |
| 5.4 | 0.285 | 0.305 |
| 5.6 | 0.290 | 0.305 |
| 5.8 | 0.290 | 0.305 |
| 6.0 | 0.290 | 0.305 |
| 6.2 | 0.290 | 0.305 |
| 6.4 | 0.280 | 0.304 |
| 6.6 | 0.270 | 0.304 |
| 6.8 | 0.250 | 0.305 |
| 7.0 | 0.224 | 0.281 |
| 7.2 | 0.188 | 0.282 |
| 7.4 | 0.130 | 0.235 |
| 7.6 | 0.130 | 0.235 |
| 8.0 | 0.052 | 0 092 |
| 10.0 | 0.000 | 0.000 |

[a] Each absorbance value is an average of three experiments. All individual readings were within ±3% of the mean reading. [b] One 10.0-ml. portion of chloroform was used to extract the thiamine salt. [c] Three 10.0-ml. portions of chloroform were used to extract the thiamine dye salt.

thiamine salt. The results are presented in Table II.

**Determination of the Effect of pH**—Various solutions (pH range 2 to 10) containing 4.0 mcg./ml. of thiamine hydrochloride were prepared and assayed for thiamine dye salt according to procedures *A* and *B*. The results are shown in Table III.

**Determination of Effect of Time and Temperature on Stability of Thiamine Dye Salt**—A pH 6.6 buffer solution containing 4.0 mcg./ml. of thiamine hydrochloride was used in these studies.

Procedure *A* was used to determine the thiamine dye salt; however, after the absorbance of the chloroform layer was determined initially, it was allowed to stand for 1, 2, and 3 hr. at ambient temperature (25° ± 2°). The absorbance of the chloroform solution was measured at hourly intervals and the results are given in Table IV.

In other experiments the absorbance of the chloroform solution was determined initially, and then the solution was diluted to 3 times its initial volume with chloroform. This solution was heated in a beaker at 60° for approximately 10 to 15 min. until the volume was less than 10 ml. After cooling to room temperature, the solution was adjusted to its original volume and the absorbance of this solution measured. The result is shown in Table IV.

**Tolerance of Assay Method to Diverse Substances**—Buffer solutions (pH 6.0 and 6.6) containing various amounts of diverse substances were prepared. The concentration of each test substance in solution was approximately 4 times that usually present in commercial products. The interference of each substance was individually determined by substituting 10.0 ml. of the test solution for 10.0 ml. of thiamine solution and carrying out the assay according to procedure *A*. Any absorbance in the final chloroform solution was indicative of interference. The results are shown in Table V.

TABLE IV—EFFECT OF TIME AND TEMPERATURE ON THE STABILITY OF THE THIAMINE DYE SALT[a]

| Time, hr. | Temp., °C. | Absorbance at 420 mμ |
|---|---|---|
| 0 | 25 | 0.307 |
| 1 | 25 | 0.306 |
| 2 | 25 | 0.306 |
| 3 | 25 | 0.306 |
| 0.25 | 60 | 0.306 |

[a] Each absorbance value is an average of two experiments. All individual readings were within ±3% of the mean reading.

TABLE V—EFFECT OF DIVERSE SUBSTANCES[a]

| Substance | Concn., mcg./ml. | Absorbance Values at 420 mμ, pH 6.0 | Absorbance Values at 420 mμ, pH 6.6 |
|---|---|---|---|
| Amaranth | 1.0 | 0.000 | 0.000 |
| Butyl-*p*-hydroxybenzoate | 0.1 | 0.000 | 0.000 |
| DL-Calcium pantothenate | 100.0 | 0.000 | 0.000 |
| Copper sulfate | 10.0 | 0.000 | 0.000 |
| Ethinyl estradiol | 1.0 | 0.000 | 0.000 |
| Ethyl alcohol[b] | 0.05[b] | 0.060[c] | 0.056[c] |
| Ferrous sulfate | 500.0 | 0.000 | 0.000 |
| Folic acid | 5.0 | 0.000 | 0.000 |
| Glycerin[b] | 0.05[b] | 0.000 | 0.000 |
| Lactose | 30.0 | 0.000 | 0.000 |
| Magnesium sulfate | 15.0 | 0.000 | 0.000 |
| Manganese sulfate | 10.0 | 0.000 | 0.000 |
| Molybdenum sulfate | 10.0 | 0.000 | 0.000 |
| Nicotinamide | 50.0 | 0.045 | 0.000 |
| Progesterone | 5.0 | 0.000 | 0.000 |
| Pyridoxine HCl | 10.0 | 0.000 | 0.000 |
| Riboflavin | 10.0 | 0.000 | 0.000 |
| Starch | 30.0 | 0.000 | 0.000 |
| Sugar | 30.0 | 0.000 | 0.000 |
| Vitamin A acetate | 50.0 | 0.000 | 0.000 |
| Vitamin $B_{12}$ | 0.02 | 0.000 | 0.000 |
| Vitamin C | 200.0 | 0.000 | 0.000 |
| Vitamin $D_2$ | 5.0 | 0.000 | 0.000 |
| Zinc sulfate | 10.0 | 0.000 | 0.000 |

[a] Each absorbance value is an average of two experiments. [b] Concentration expressed in ml./ml. for these liquids. [c] There is no interference if the alcohol present is evaporated using gentle heat before assaying.

Experiments were carried out in which pH 6.6 solutions were prepared to contain 0.0, 4.0, 6.0, and 8.0 mcg./ml. of thiamine hydrochloride or mononitrate and all of the substances listed in Table V. (Each substance was present in the concentration listed in Table V.) These solutions were tested for interference in the same manner as described above. The results are presented in Table VI.

Effect of decomposition products on the acid dye method was studied by preparing an aqueous solution containing 20 mcg./ml. of thiamine mononitrate and all the substances listed in Table V (the concentration of each substance in solution was 5 times the concentration listed in Table V). The pH of this solution was adjusted to 6.4 with sodium hydroxide (0.2 *M*). Initial assays for thiamine were run both by the USP method (2) and by the acid dye method (procedure *A* using pH 6.6 buffer). After storing this solution at 60° for 85 days, the thiamine content was again determined using the methods described above. Results are presented in Table VII.

**Reproducibility of Results**—To test the reproducibility of results, 12 assays were run by the USP method (2) and 12 by the acid dye method using procedure *A* and thiamine mononitrate (4 mcg./ml.) in a pH 6.6 buffer solution. Results are presented in Table VIII.

**Determination of Stoichiometric Balance**—Buffer solutions (pH 7.7) containing 10.0, 20.0, or 30.0 mcg./ml. of thiamine hydrochloride or thiamine mononitrate were prepared for these studies. The high pH was used to prevent the passage of any free dye into the chloroform layer. Each of the thiamine solutions was mixed with $2 \times 10^{-4}$ *M* bromothymol blue in chloroform solution according to the directions in procedure *B*. After the nonpolar and polar layers were separated and collected, the chloroform and aqueous solutions were assayed for thiamine and dye according to the methods described under *Determination of Thiamine and Dye in Aqueous and Organic Phases*. The results are presented in Table IX. The stoichiometric balance was calculated by using the following equation:

$$\frac{\text{concn. of thiamine in } CHCl_3}{\text{mol. wt. of thiamine}} : \frac{\text{concn. of dye in } CHCl_3}{\text{mol. wt. of dye}}$$

Several experiments were carried out to confirm the stoichiometric balance of 1:1. Twenty milligrams of thiamine mononitrate was added to 100 ml. of bromothymol blue in chloroform solution (12.48 mcg./ml.) in a 250-ml. separator, and the mixture was shaken intermittently over a period of 3 hr. After 3 hr. the chloroform layer exhibited a lower absorbance value (at 420 mμ) than was expected for complete salt formation. A 5-ml. quantity of distilled water was then added and the mixture was shaken vigorously for several minutes, after which time the equilibrium was established. After the two phases had separated, the aqueous solution was colorless and the chloroform solution gave an expected absorbance reading. Subsequent experiments were carried out in which 5 ml. of water was added immediately and the 3-hr. shaking period was eliminated. Similar experiments were carried out using a higher dye concentration (24.96 mcg./ml.). The results are presented in Table X.

TABLE VI—COMBINED EFFECT OF DIVERSE SUBSTANCES[a]

| Concn., Thiamine, mcg./ml. | | Absorbance at 420 mμ | |
|---|---|---|---|
| HCl | Mononitrate | Thiamine Alone | Thiamine + Added Substances |
| 0.00 | 0.00 | 0.000 | 0.000 |
| 4.00 | 0.00 | 0.305 | 0.306 |
| 6.00 | 0.00 | 0.456 | 0.457 |
| 8.00 | 0.00 | 0.608 | 0.609 |
| 0.00 | 4.00 | 0.312 | 0.313 |
| 0.00 | 8.00 | 0.622 | 0.624 |

[a] Each absorbance value is an average of 3 experiments. All individual readings were within ±3% of the mean reading. Each substance was present in the concentration listed in Table V. Procedure *A* and buffer solution of pH 6.6 were used in all the assays.

TABLE VII—EFFECT OF DECOMPOSITION PRODUCTS ON THE ACID DYE METHOD AS COMPARED WITH THE USP METHOD[a]

| | Percentage Thiamine Mononitrate Remaining After | |
|---|---|---|
| | Initial | 85 Days |
| USP method | 100.3 | 10.1 |
| Dye method | 100.4 | 9.8 |

[a] Each percentage is an average of 3 assays. All individual readings were within ±3% of the mean reading.

TABLE VIII—REPRODUCIBILITY OF RESULTS (DYE METHOD *Versus* USP METHOD) ON A SAMPLE (4.0 mcg./ml.) OF THIAMINE MONONITRATE

| Sample | Found, mcg./ml. Dye Method | Found, mcg./ml. USP Method | Deviation, mcg. Dye Method | Deviation, mcg. USP Method |
|---|---|---|---|---|
| *1* | 4.08 | 4.04 | 0.08 | 0.04 |
| *2* | 4.02 | 4.00 | 0.02 | 0.00 |
| *3* | 4.04 | 4.08 | 0.04 | 0.08 |
| *4* | 4.02 | 3.94 | 0.02 | 0.06 |
| *5* | 3.94 | 3.92 | 0.06 | 0.08 |
| *6* | 4.00 | 4.04 | 0.00 | 0.04 |
| *7* | 3.98 | 4.08 | 0.02 | 0.08 |
| *8* | 4.03 | 4.00 | 0.03 | 0.00 |
| *9* | 4.00 | 4.06 | 0.00 | 0.06 |
| *10* | 3.95 | 3.92 | 0.05 | 0.08 |
| *11* | 3.93 | 3.94 | 0.07 | 0.06 |
| *12* | 4.03 | 3.98 | 0.03 | 0.02 |
| Av. deviations | 4.00 + 0.04 | 4.00 + 0.05 | ... | . . |

**Physical Properties of the Salts**—A 300-ml. quantity of pH 6.6 buffer solution containing 100 mcg./ml. of thiamine hydrochloride or mononitrate was vigorously skaken with 300 ml. of dye in chloroform solution ($1 \times 10^{-4}$ *M*) in a 1,000-ml. separator for 1 min. The phases were allowed to separate and the chloroform solution containing the thiamine dye salt was collected in a 600-ml. beaker, and evaporated to a small volume (3 to 5 ml.) on a hot plate (60°). The remaining chloroform was allowed to evaporate at ambient temperature and the salt was dried in a desiccator. The melting points of the salts were determined using a Thomas-Hoover capillary melting point apparatus.

## DISCUSSION AND CONCLUSIONS

**Optimum Conditions for Assay**—The results show that a method based on the reaction between bromothymol blue and thiamine (procedure *A*) in a pH 6.6 buffer solution can be used for the analysis

TABLE IX—DISTRIBUTION OF THIAMINE AND DYE BETWEEN CHLOROFORM AND WATER (pH 7.7) FOR THE DETERMINATION OF STOICHIOMETRIC BALANCE[a]

| Total Thiamine Salt Concn., mcg./ml. | Concn., mcg./ml. of Thiamine Salt: In Water | Thiamine Salt: In Chloroform | Bromothymol Blue: In Water | Bromothymol Blue: In Chloroform | *M* Ratio of Thiamine to Dye |
|---|---|---|---|---|---|
| | | | Hydrochloride | | |
| 10.0 | 4.71 | 5.29 | 114.70 | 10.10 | 1.00:1.03 |
| 20.0 | 9.43 | 10.57 | 104.58 | 20.22 | 1.00:1.03 |
| 30.0 | 14.15 | 15.85 | 94.48 | 30.32 | 1.00:1.03 |
| | | | Mononitrate | | |
| 10.0 | 4.50 | 5.50 | 114.35 | 10.45[b] | 1.00:1.00 |
| 20.0 | 9.25 | 10.75 | 104.25 | 20.55 | 1.00:1.00 |
| 30.0 | 13.95 | 16.05 | 94.28 | 30.52 | 1.00:1.00 |

[a] Each value is an average of 3 experiments. All individual readings were within ±3% of the mean reading. [b] Absorbance value for this concentration of thiamine dye salt in chloroform was 0.398.

TABLE X—CONFIRMATION OF STOICHIOMETRIC BALANCE[a]

| Dye Concn., mcg./ml. | Absorbance at 420 mμ: Expected[b] | Found |
|---|---|---|
| 12.48 | 0.475 | 0.472 |
| 24.96 | 0.950 | 0.944 |

[a] Each absorbance value is an average of 3 experiments All individual readings were within ±3% of the mean reading. [b] From Table IX (*Footnote b*) we find that 10.45 mcg./ml. of dye in chloroform gives an absorbance value of 0.398. Assuming a stoichiometric balance of 1:1, a dye concentration of 12.48 mcg./ml. in chloroform will be expected to give an absorbance value of 0.475.

of thiamine in pharmaceutical preparations (Tables III and V) and the best dye concentration is $1 \times 10^{-4}$ moles/L. (Table I). The optimum pH value of 6.6 is not in agreement with a 7.4 value reported by Schill (6).

Beer's law is followed within the range of 4 to 10 mcg./ml. of thiamine (Fig. 3) at 420 mμ (Fig. 2). The best solvent for extraction is chloroform (Table II) and the thiamine dye salt is very stable in this solvent (Table IV). The acid dye method is at least as accurate and precise as the USP method. The reproducibility of results based on 12 samples (4 mcg./ml.) was 4.00 ± 0.04 *versus* 4.00 ± 0.05 by the method (Table VIII).

**Nature of Thiamine Dye Salt**—The nature of the reaction between bromothymol blue and thiamine seems to be a simple acid–base reaction. The stoichiometric balance of the reaction is 1:1 (Tables IX and X). The thiamine salts formed with bromothymol blue have high molecular weights (925.2 for thiamine monochloride dye salt and 915.28 for thiamine mononitrate dye salt) as compared with thiamine hydrochloride (337.28) and thiamine mononitrate (327.36); hence they are more soluble in organic solvents. The melting points of thiamine dye salts were 255–258° (with decomposition). These high melting points indicate that ion–ion pairs are salts and not loose complexes. Additional work is being carried out to establish the composition of these salts.

**Effect of Diverse Substances**—No interference in the analytical procedure was observed from other vitamins, minerals, hormones (ethinyl estradiol and progesterone), and excipients except for a slight interference from ethyl alcohol and nicotinamide (Tables V and VI). Lack of interference was sxpected, since none of these substances is as etrong a base as is thiamine.

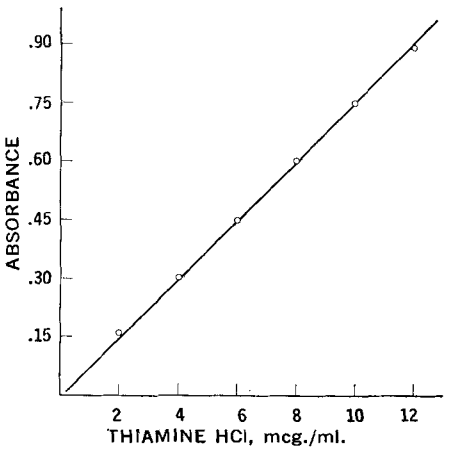


*Fig. 3—Standard curve for thiamine hydrochloride. A similar curve was obtained with thiamine mononitrate.*

The interference from ethyl alcohol may be due to increase in the polarity of the nonaqueous phase which results in the occurrence of more dissociated dye in this phase. A slight interference from nicotinamide at pH 6.0 and no interference at pH 6.6 was not studied further.

Interference from the decomposition products of thiamine and other vitamins is also negligible (Table VII). Apparently none of the products of decompositon behaves as a strong base. This being the case, it may be possible to ascertain the degradation products. If, for example, it is assumed that thiamine was oxidized on storage at 60°, then thiochrome, the decomposition product, is formed, which means the amino group of the pyrimidine ring, which is probably responsible for the formation of thiamine dye salt, is lost.

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Keyphrases

Thiamine
Fluorophotometry–analysis
Thiamine–bromothymol blue salt formation
Thiamine–bromothymol blue salt physical properties
Stoichiometric balance
Bromothymol blue isosbestic point

# Shortcomings in Pharmacokinetic Analysis by Conceiving the Body to Exhibit Properties of a Single Compartment

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**In the past, pharmacokinetic assessment of drug absorption, metabolism, and excretion usually have been based on the concept of the body behaving as a single compartment. After rapid i.v. injection, provided that blood samples are taken sufficiently soon after injection, at least a bi-exponential curve is obtained. The initial portion of this curve, the so-called rapid distribution phase, has been ignored without proof, and it has been assumed that the single-compartment concept does not introduce large errors into the subsequent calculations. On the basis of first principles, at least one additional peripheral compartment must exist for virtually any compound introduced into the body. Such a model is physiologically compatible with distribution of the drug throughout the body under perfusion and diffusion forces. The two-compartmental open-system model is discussed in respect to the error introduced into the usual absorption rate and elimination rate calculations and on the estimation of the volume of distribution of various drugs.**

PHARMACOKINETIC STUDIES are usually undertaken to attempt to define, as accurately as possible, the rates of absorption, metabolism, and excretion of a drug and its metabolites. The analyses of data, obtained after administration of a drug to man or animals, have most commonly been based on the presumption that it is adequate to consider the body as exhibiting the properties of a single compartment. Since a model is only conceived to serve the purposes of the scientist, it need not be any more complex (nor should it be any less simplified) than required to serve this function. However, it is scientifically unsound to continue to accept a model because it is often used; instead it should be questioned until there is sufficient evidence to support its adequacy for the purposes for which it was designed. Surprisingly, within the knowledge of the authors, there appears to have been no critical tests of the validity of the single-compartmental model. This paper is the first of a series which will attempt to examine this model and point to some of the limitations and incorrect reasoning that it imposes on these analyses.

It is the purpose of this paper to emphasize that a central and at least one peripheral compartment appear essential to describe adequately the distribution of the drug in the body. Not only is such a model sufficiently realistic when viewed on a physiological basis, but it is mathematically more acceptable than the one-compartmental model.

## DISCUSSION

**Physiological Aspects of the Mammillary Model**—Compounds are distributed throughout the body by the blood and vascular network acting as a carrier system. One conceives of a model which represents drug distribution and elimination to be made up of a central compartment with interchanging connections with one or more peripheral compartments. Such a model has been called a mammillary model (1). While blood is a major

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