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Application of Thermochromism in Spectrophotometric Analysis: Selective Determination of Berberine in Pharmaceuticals by Solvent Extraction

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Received May 3, 1978, from the *Department of Chemistry, Gifu College of Dentistry, Gifu, Japan.* Accepted for publication January 19, 1979.

Abstract □ A solvent extraction and spectrophotometric method for selective determination of berberine in pharmaceuticals is proposed. Berberine forms an ion associate with tetrabromophenolphthalein ethyl ester, which is extracted into ethylene dichloride. Secondary and tertiary amines and alkaloids are coextracted with the berberine and complicate the berberine determination. The absorbance of the secondary and tertiary amines and alkaloids into ethylene dichloride, however, decreases nearly to zero when the temperature is elevated from 25 to 60°. Thus, berberine can be determined successfully in the presence of the secondary and tertiary amines and alkaloids by using thermochromism.

Keyphrases □ Berberine—analysis, solvent extraction, spectrophotometric method using thermochromism, various pharmaceuticals □ Thermochromism—analysis, berberine in various pharmaceuticals, solvent extraction, spectrophotometry □ Spectrophotometry, solvent extraction—analysis, berberine in various pharmaceuticals, using thermochromism □ Antibacterial agents—berberine, analysis in various pharmaceuticals, solvent extraction, spectrometry, using thermochromism

Bromthymol blue, bromcresol green, and bromphenol blue usually are used for the solvent extraction and spectrophotometric determination of thiamine (1), quaternary ammonium compounds (2), and quinine ethylcarbonate (3), respectively. These dyes are diprotic acids with a narrow optimum pH range and poor extractability of the ion associates into organic solvents.

Tetrabromophenolphthalein ethyl ester anion (I) is a monoprotic acid and can form ion associates (II) with quaternary ammonium cations (III) and charge transfer complexes (IV) with amines (V), which are effectively extracted into organic solvents through a wide pH range (4). Tetrabromophenolphthalein ethyl ester shows a high sensitivity for quaternary ammonium cations and amines, but the absorption spectra overlap each other. Thus, it is difficult to determine spectrophotometrically the quaternary ammonium salts and amines separately when they coexist. An attempt was made to separate the amine procaine from the quaternary ammonium salt benzethonium by solvent extraction, but it was difficult to remove the amine completely (5).

The author found, however, that the color of the charge transfer complex in ethylene dichloride reversibly disappeared with the elevation of temperature. Even at 60°, the red complex was rarely found while the absorbance of the ion associate at 610 nm was unchanged at 60°. Thus, a quaternary ammonium salt could be determined spectrophotometrically at 60° without the disturbance of amines.

This paper describes the extraction and spectrophotometric determination of berberine in pharmaceuticals after the temperature is increased to 60° to eliminate the effect of the coexisting amines.

EXPERIMENTAL

Apparatus—A double-beam spectrophotometer[1] attached to a temperature-controlled cell holder was used to measure absorbances at a constant temperature using quartz 1-cm cells with stoppers. An x–y recorder[2] was used for the spectra. Constant cell temperature was maintained by circulating constant-temperature water through the temperature-controlled cell holder by means of a temperature-controlled circulator[3]. The solvent temperature in the cell was checked by dipping a thermoelement of a thermometer[4] in the solvent. A pH meter, a shaker, and a centrifuge were also used.

Reagents—*Standard Berberine Solution*—A stock solution of 1×10^{-3} M berberine was prepared by dissolving 0.4075 g of berberine hydrochloride[5] (dried at 105°) in distilled water and diluting to 1 liter with distilled water. The stock solution was standardized by the official method (6). The solution was used after accurate dilution.

Tetrabromophenolphthalein Ethyl Ester Solution—A 2.0×10^{-3} M solution of tetrabromophenolphthalein ethyl ester was prepared by dissolving 0.1400 g of tetrabromophenolphthalein ethyl ester potassium salt[6] in ethanol to give a 100-ml solution.

Buffer Solution (pH 8.5)—The borate–phosphate buffer was prepared by adding 1 N H_2SO_4 or 1 N NaOH to the 0.3 M potassium dihydrogen phosphate containing 0.1 M sodium borate.

Procaine[7], ephedrine[8], quinine[9], papaverine[9], eserine[10], and emetine[5] were used as the hydrochloride and the sulfate. Ethylene dichloride was used as the extractant. All chemicals were reagent grade.

[1] Hitachi model 556.
[2] Hitachi model 057.
[3] Komatsu-Yamato model CTE-240.
[4] Anritsu model HP-4F.
[5] Nakarai Kagaku Yakuhin Co., Kyoto, Japan.
[6] Tokyo Kasei Kogyo Co., Tokyo, Japan.
[7] Daiichi Seiyaku Co., Tokyo, Japan.
[8] Sanwa Kagaku Co., Nagoya, Japan.
[9] Katayama Kagaku Kogyo Co., Osaka, Japan.
[10] E. Merck, Darmstadt, West Germany.

0022-3549/79/0700-0875$01.00/0

Table I—Effect of Foreign Substances on Berberine Determination [a]

Substance	Mole Ratio	Berberine Recovery, % 25°	60°
Glucose	1:1000	100	—
Lactose	1:1000	98	—
Ammonium sulfate	1:1000	101	—
Calcium chloride	1:1000	100	—
Sodium carbonate	1:1000	98	—
Sodium chloride	1:1000	102	—
Sodium nitrate	1:1000	101	—
Sodium acetate	1:1000	101	—
Sodium citrate	1:1000	100	—
Sodium salicylate	1:1000	99	—
Starch, 0.4%	—	101	—
Thiamine	1:1	102	—
Triethanolamine	1:500	105	105
Caffeine	1:100	101	—
Benzethonium	1:1	185	185
Neostigmine	1:0.1	111	111
Diphenhydramine	1:0.3	107	102
	1:0.5	112	104
Procaine	1:2	129	102
	1:4	159	106
Emetine	1:1	113	102
	1:2	130	106
Papaverine	1:10	105	98
	1:20	114	101
Pilocarpine	1:30	106	101
Eserine	1:1	111	100
	1:2	126	102
Chlorophenylamine	1:0.3	115	104
	1:0.5	128	106

[a] The berberine taken was 1×10^{-6} *M*, the wavelength was 610 nm, and the pH was 8.5.

Recommended Procedure—Berberine solution, 0.5–5 ml (2.5×10^{-5} *M*), 2 ml of tetrabromophenolphthalein ethyl ester (2×10^{-3} *M*), and 5 ml of borate–phosphate buffer (pH 8.5) were pipetted into a 100-ml separator. The mixture was diluted with water to 50 ml and shaken with 10 ml of ethylene dichloride for 5 min mechanically.

The organic layer was transferred into a stoppered test tube and centrifuged to remove water droplets. The absorbance (610 nm) of the organic phase was measured at 25° in the temperature-controlled cell holder against the reagent blank. The temperature was raised to 60° with the cell remaining in the holder, and the absorbance of the same phase was measured.

The calibration curves were straight, starting at the origin, over 0.25–2.5 $\times 10^{-6}$ *M* berberine aqueous solution. The molar absorptivities at 25 and 60° in ethylene dichloride were 9.80×10^4 and 9.24×10^4 mole^{-1} cm^{-1} liter, respectively. The precision was checked by 10 measurements, and the variation coefficient was ±1% at each temperature.

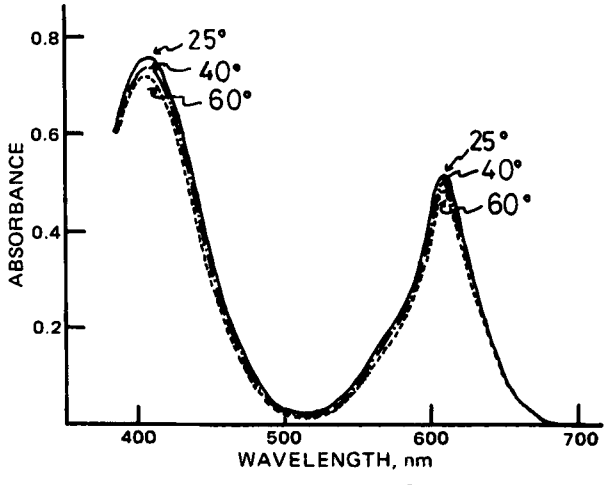

Figure 1—*Absorption spectra of 6.4 × 10^{-6} M tetrabromophenolphthalein ethyl ester–1 × 10^{-6} M berberine at different temperatures and pH 8.5. The shaking time was 5 min, and the reference was water.*

Table II—Berberine Analysis in Practical Samples

Sample	Amount Manifested, mg	Amount Found, mg 25°	60°
1[a]	75	81	76
2[b]	50	53.5	51
3[c]	100	105	101

[a] Also contained thiamine hydrochloride (5 mg), diphenhydramine hydrochloride (10 mg), and methylephedrine hydrochloride (15 mg). [b] Also contained diphenhydramine hydrochloride (10 mg), papaverine hydrochloride (5 mg), and thiamine hydrochloride (5 mg). [c] Also contained chlorpheniramine maleate (15 mg), thiamine hydrochloride (10 mg), and papaverine hydrochloride (20 mg).

RESULTS AND DISCUSSION

Extraction Mechanism with Tetrabromophenolphthalein Ethyl Ester—Tetrabromophenolphthalein ethyl ester anion (I) reacted with quaternary ammonium salts such as berberine, tetraethylammonium, benzethonium (7), and sparteine to form blue-color ion associates, which were extracted into ethylene dichloride. Secondary and tertiary amines such as ephedrine, quinine (4), papaverine, procaine (5), and emetine also reacted with I to form reddish complexes, which were extracted into ethylene dichloride. The ratio of I to the compounds of both the blue ion associates and the reddish complexes in ethylene dichloride was 1:1, as determined by the continuous variation method.

The electric conductivities of the dye molecule (VI), of the ion associate with berberine, and of the complex with procaine in ethylene dichloride were measured. The solutions containing 10^{-4} *M* VI and its charge transfer complex with procaine did not show conductivity when measured at various temperatures from 20 to 50°. The solutions containing 10^{-4} *M* of the ion associate with berberine showed some small conductivity (micro-ohms per centimeter): 20°, 6.14; 30°, 6.78; 40°, 7.29; and 50°, 8.0.

Accordingly, the following extraction scheme is presented. The subscripts *o* and *w* refer to the organic and aqueous phases, respectively.

$$\underset{\text{blue}}{I_w} + H_w^+ \rightleftarrows \underset{\text{yellow } (\lambda_{max} = 410 \text{ nm})}{VI_0}$$

Scheme I—Extraction of tetrabromophenolphthalein ethyl ester

$$I_w + III_w \rightleftarrows \underset{\text{blue}}{I\text{-}III_0(II)} \rightleftarrows \underset{\text{blue } (\lambda_{max} = 610 \text{ nm})}{I_0 + III_0}$$

Scheme II—Extraction of ion associate

$$I_w + H_w^+ + V_w \rightleftarrows \underset{\text{red } (\lambda_{max} = 545 - 585 \text{ nm})}{I\text{-}H\text{-}V_0(IV_0)}$$

Scheme III—Extraction of charge transfer complex

Thermochromism of Ion Associate and Charge Transfer Complex with I—The absorption spectra of the I–berberine ion associate and the

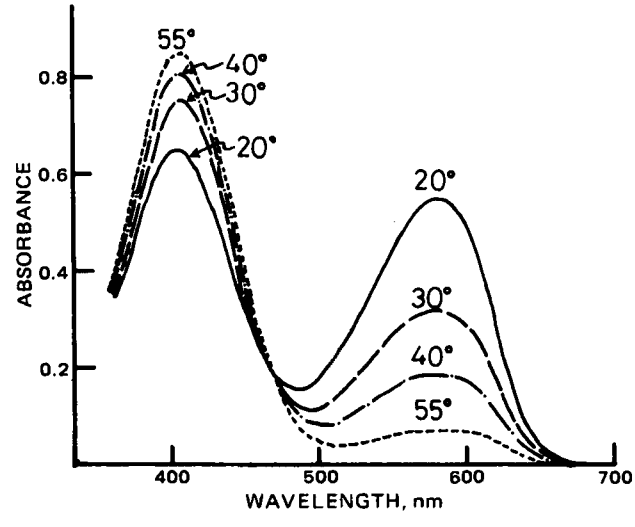

Figure 2—*Changes in absorption spectra of the charge transfer complex by thermochromism. The amine was 2.5 × 10^{-5} M procaine with 1.28 × 10^{-5} M tetrabromophenolphthalein ethyl ester at pH 8.5. The reference was water.*

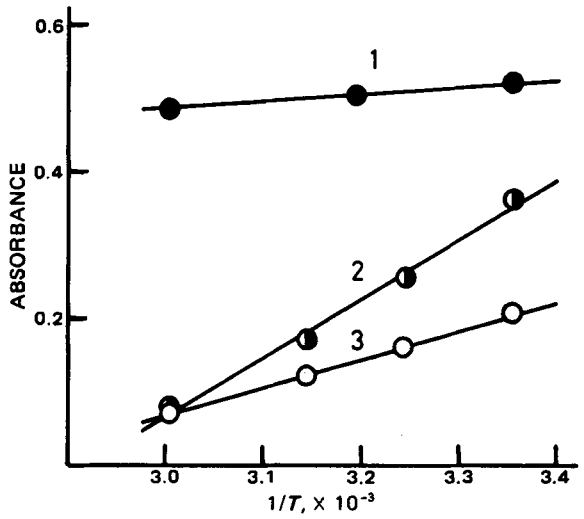

Figure 3—*Effect of temperature on tetrabromophenolphthalein ethyl ester–berberine and amines. Key: 1, 1 × 10⁻⁶ M berberine with 8 × 10⁻⁵ M tetrabromophenolphthalein ethyl ester at 610 nm; 2, 2.0 × 10⁻⁵ M papaverine with 1.6 × 10⁻⁴ M tetrabromophenolphthalein ethyl ester at 572 nm; and 3, 1.0 × 10⁻⁶ M procaine with 1.6 × 10⁻⁴ M tetrabromophenolphthalein ethyl ester. The reference was water.*

VI–procaine complex in ethylene dichloride at various temperatures are shown in Figs. 1 and 2, respectively. The absorption spectrum of the I–berberine ion associate was scarcely affected by the temperature change. The slight decrease in absorbance with a rise in temperature depended on the expansion of ethylene dichloride.

On the other hand, the VI–procaine complex absorption spectrum in ethylene dichloride was greatly influenced by temperature. When the temperature was elevated, the absorbance at 585 nm decreased and the absorbance at 410 nm increased. The isosbestic point in this absorption spectrum was 465 nm.

Thus, Scheme IV is presented.

$$\underset{\text{red}}{IV_0} \underset{\text{cool}}{\overset{\text{warm}}{\rightleftharpoons}} \underset{\text{yellow}}{VI_0} + \underset{\text{colorless}}{V_0}$$

Scheme IV

The absorbances of the I–berberine ion associate at 610 nm, of the VI–procaine complex at 585 nm, and of the VI–papaverine complex at 572 nm were plotted against $1/T$(T = absolute temperature) (Fig. 3). The absorbance change of the I–berberine ion associate is attributed to solvent expansion, and the absorbance decreases of the VI–papaverine and the VI–procaine complexes with increased temperature are due to complex dissociation according to Scheme IV. At 60°, the absorbances of the VI–procaine complex at 585 nm and of the VI–papaverine complex at 572 nm were almost the same as the reagent blank.

When berberine (1×10^{-6} M) was extracted into ethylene dichloride with I, the absorbance at 610 nm was 0.520 at 25° and 0.484 at 60°, with water as a reference. When berberine (1×10^{-6} M) and eserine (1×10^{-6} M) coexisted, the absorbance at 610 nm was 0.577 at 25° and 0.485 at 60°. When the eserine concentration was doubled, the absorbance was 0.655 at 25° and 0.493 at 60°. Therefore, the berberine concentration can be determined successfully at 60° without hindrance from coexisting amines. The other amines such as emetine, papaverine, procaine, and pilocarpine were tested, with the same results as eserine.

Effect of Foreign Substances—Various foreign substances were added to a solution containing 1×10^{-6} M berberine, and their influences were examined by the recommended procedure (Table I). Glucose, lactose, and inorganic ions such as ammonium, calcium, sodium, chloride, and sulfate did not interfere with the determination of berberine. Quaternary ammonium salts such as benzethonium and neostigmine severely affected the determination, with positive errors even at 60°. Amines such as emetine, papaverine, pilocarpine, and eserine interfered with the determination at 25° but not at 60°.

Analysis of Practical Samples—Commercial samples were analyzed according to the proposed method (Table II). Of the coexisting substances, diphenhydramine, methylephedrine, and chlorophenylamine gave some interference at 25°; at the elevated temperature, their influences were suppressed by thermochromism. As a result, this thermochromism method is more selective, sensitive, and accurate than the official methods of determining berberine in pharmaceuticals.

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ACKNOWLEDGMENTS

The author thanks Professor K. Toei of Okayama University and Professor Y. Yamamoto of Hiroshima University for their valuable advice.