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Spectrophotometric Determination of Quinine, Emethine and Ephedrine in Pharmaceutical Preparations with Tetrabromophenolphthalein Ethyl Ester by Solvent Extraction

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A singly charged tetrabromophenolphthalein ethyl ester (TBPE) anion was found to be extracted with alkaloids as 1: 1 complexes in 1,2-dichloroethane. Of various solvents, TBPE-1,2-dichloroethane system gave a red color for alkaloids, while in the absence of alkaloids, an organic phase shows a pale yellow color. In this way, the spectrophotometric methods were investigated for the determination of a small amount of the alkaloids, such as quinine, emethine and ephedrine, by solvent extraction. Quinine is determined by measuring the absorbance of the extracts over a range from $0.5—5\times10^{-6}$ M (0.20—1.98 μg/ml) at 555 nm. Emethine is determined from 2×10^{-6} M to 1×10^{-5} M (1.1—5.5 μg/ml) at 570 nm and ephedrine, from 1×10^{-6} M to 1×10^{-5} M (0.20—2.0 μg/ml) at 555 nm. From the conductivity measurements, it is concluded that red species in the extract is considered to be a kind of charge transfer complexes.

Introduction

Most colorimetric methods for the determination of amines or quaternary ammonium salts have been reported by using diprotic acids such as bromothymol blue (BTB),[2-4] bromocresol green (BCG)[5] or bromophenol blue (BPB).[6] These are usually achieved by forming an ion-pair between an organic cation and a dye anion. However, in the case of such diprotic acids, the acidity gave complicated effect on the extraction because the stepwise dissociation occurred in the aqueous phase. On the other hand, a singly charged tetrabromophenolphthalein ethyl ester (TBPE) was extracted with various amines, alkaloids and quaternary ammonium salts as 1: 1 ion-pairs or molecular addition complexes over a wide pH range into 1, 2-dichloroethane as shown in our previous papers.[7,8] In the previous studies, the colors of the extracts were found to be classified into the following three categories. (1) Blue extracts which are observed in the presence of the quaternary ammonium salts or alkaloids such as benzethonium, berberine, sparteine and N-methylatropine (2) red extracts which are developed in the presence of alkaloids such as quinine, emethine and ephedrine (3) yellow extracts which are of the same color as the reagent blank even in the presence of aniline, dimethylamine and guanine. On the other hand, Davis[9] has found the same phenomena in benzene solution.

This paper deals mainly with the determination of alkaloids, such as quinine, emethine and ephedrine. In addition, the discussion has been done on the different extraction behavior between blue and red colored extracts. Hexanitrodiphenylamine[10] was used as a reagent

1) Location: *a) Takano 1851, Hozumi-cho, Gifu; b) Koyama 1-1, Tottori-shi.*
2) V.D. Gupta and D.E. Cadwallader, *J. Pharm. Sci.*, **57**, 112 (1968).
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for the spectrophotometric determination of quaternary ammonium salts or alkaloids. Nonaqueous titrimetric[11] and spectrophotometric[6] methods were investigated for the determination of quinine. Titrimetric[12] method was studied for emethine. Gravimetric,[13] spectrophotometric[14] and titrimetric[15] methods have been developed for the determination of ephedrine. However, the proposed method with TBPE is more suitable in sensitivity, selectivity and small dependence on the pH on the extraction as compared with known methods for the determination of these alkaloids.

Experimental

Apparatus——(1) The spectrophotometric measurements were made with a Hitachi Model 124 spectrophotometer with 10 mm cells. (2) The pH measurements were made with a Hitachi-Horiba Model M-5 pH meter. (3) An Iwaki Model KM shaker was used for the extraction. (4) A Toa Denpa Model CM-2A electrical conduct meter was used for the measurements of electrical conductivity.

Reagents——Weighed amount of tetrabromophenolphthalein ethyl ester potassium salt (mol. wt. 700.1) was dissolved in ethyl alcohol.

A standard quinine, emethine or ephedrine solution was prepared by dissolving the weighed amounts of quinine hydrochloride (dried at 105°), emethine hydrochloride (dried at 105°) or ephedrine hydrochloride (dried at 105°) in water. The pH 8.5 and 9.5 buffer solutions were prepared by mixing a 0.4 M potassium dihydrogen phosphate solution containing 0.08 M sodium borate and a 3 N sodium hydroxide solution. All the chemicals were of reagent grade, and distilled water was used.

Recommended Procedure——Quinine: Take 0.5—5 ml of a standard quinine solution (5×10^{-5} M), 2 ml of a TBPE solution (4×10^{-3} M), and 5 ml of the buffer solution (pH 8.5) into a 100 ml separatory funnel. Dilute the mixture to 50 ml with water and shake the solution for 3 min with 10 ml of 1,2-dichloroethane. After the separation of the two layers, run off the extract into a glass tube through a filter paper to remove droplets of water. Measure the absorbance of the extract at 555 nm, using a reagent blank or water as a reference.

Emethine: Take 1—5 ml of a standard solution (5×10^{-5} M), 2 ml of a TBPE solution (4×10^{-3} M), and 10 ml of the buffer solution (pH 8.5) into a 100 ml separatory funnel. Dilute the mixture to 25 ml with water. Treat the mixture in the same manner as described above. Measure the absorbance at 570 nm.

Ephedrine: Take 1—10 ml of a standard ephedrine solution (5×10^{-5} M), 4 ml of a TBPE solution (4×10^{-3} M), and 10 ml of the buffer solution (pH 9.5) into a 100 ml separatory funnel. Dilute the mixture to 50 ml with water. Treat the mixture in the same manner as described above. Measure the absorbance at 555 nm.

A linear relationship between the absorbance of the extract and the concentration of an aqueous solution was observed for the following concentration range, $0.5—5\times10^{-6}$ M (0.20—1.98 μg/ml) for quinine, $2—10\times10^{-6}$ M (1.1—5.5 μg/ml) for emethine and $1—10\times10^{-6}$ M (0.20—2.0 μg/ml) for ephedrine in aqueous solution. The absorbance for 2×10^{-6} M quinine is 0.470 at 555 nm, for 4×10^{-6} M emethine, 0.440 at 570 nm, for 5×10^{-6} M ephedrine, 0.415 at 555 nm. The standard deviation of the proposed method with TBPE was estimated from the results of ten sample solutions; quinine 2×10^{-6} M, 1%; emethine 4×10^{-6} M, 1.3%; ephedrine 5×10^{-6} M, 2%.

Results and Discussion

Absorption Spectra

Fig. 1 shows the visible absorption spectra of quinine, emethine or ephedrine extracts with TBPE. It can be seen that the presence of quinine, emethine or ephedrine in aqueous solution leads to a considerable increase in the extraction of TBPE. In the absence of these alkaloids, a red color is observed. The absorption maximum for quinine is at 555 nm (Curve 1), emethine at 570 nm (Curve 2) or ephedrine at 555 nm (Curve 3). However, in the presence of quaternary ammonium salts, a blue color is observed. The change in color may be attribut-

11) "The Japanese Pharmacopoeia," VIII-1, Hirokawa Publishing Co., Tokyo, 1971, p. c-364.
12) "The Japanese Pharmacopoeia," VIII-1, Hirokawa Publishing Co., Tokyo, 1971, p. c-351.
13) Welsh, *J. Amer. Pharm. Assoc.*, **41**, 545 (1952).
14) N.L. Allport and N.R. Jones, *Quart. J. Pharm. Pharmacol.*, **15**, 238 (1942) [*Chem. Abstr.*, **37**, 5192[7] (1943)].
15) "The Japanese Pharmacopoeia," VIII-1, Hirokawa Publishing Co., Tokyo, 1971, p. c-349.

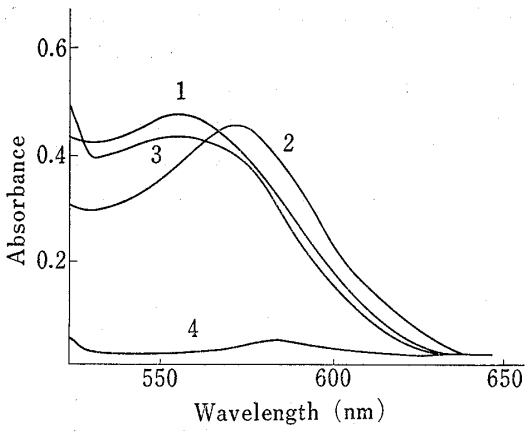

Fig. 1. Absorption Spectra

1: extract with 2×10^{-6}M quinine, TBPE: 1.6×10^{-4}M, pH: 8.5
2: extract with 4×10^{-6}M emethine, TBPE: 3.2×10^{-4}M, pH: 8.5
3: extract with 5×10^{-6}M ephedrine, TBPE: 3.2×10^{-4}M, pH: 9.5
4: reagent blank, reference: water

ed to the formation of the molecular addition complex or ion-pair between the TBPE and alkaloids or quaternary ammonium salts, respectively.

Effect of pH

The effect of pH on the extraction was studied by extracting alkaloids with TBPE from a series of aqueous solution buffered at various pH values. As shown in Fig. 2, the absorbances of the extracts were constant when the pH of the aqueous phase lay within the range of 8—10 for quinine and emethine, and 9—10.5 for ephedrine. The pH range on constant extraction of quaternary ammonium salts with TBPE was more wide. Still more, the pH range using the other monoacidic dyes was investigated on the extraction of benzethonium. The optimum pH ranges of the constant and maximum extraction are found to be 8—10 and 7—11 for 2,5-dinitrophenol-methyl isobutyl ketone and 2,6-dichlorophenolindophenol-1,2-dichloroethane system, respectively. However, these monoacidic dyes are not favorable for alkaloids on poor sensitivity. On the other hand, a pH dependence was observed for the determination of alkaloids with dibasic acid dye such as bromophenol blue. Therefore, TBPE-1,2-dichloroethane system is most available for the determination of alkaloids.

Other Variables

It was found that the concentration of TBPE should be maintained at more than 25-fold molar excess over quinine, emethine or ephedrine to obtain a maximum and constant extraction. Excess amounts (2—10 ml) of the buffer solution used in the procedure had not appreciable influence on the absorbance of the extract. When the addition of the buffer solution was less than 2 ml, a good separation of the two layers was not done. Full color development with TBPE took about 1 min of shaking. Continued shaking up to 1 min produced no further change in absorbance. The color intensity of 1,2-dichloroethane extracts remained constant for 1 hour.

Composition of the Extracted Species

In order to clarify the composition of the extracted species, continuous variation plots were made at 555 and 590 nm for quinine, 570 nm for emethine and 555 nm for ephedrine. The results reveal that a 1:1 compound is formed between TBPE and quinine, emethine or ephedrine in the 1,2-dichloroethane layer. Fig. 3 shows the continuous variation plots of TBPE-quinine and BCG-quinine. It is clearly suggested that the monoprotic acid dye, TBPE, forms a 1:1 compound and dibasic acid dyes such as BCG form a 1:2 ion-pair in 1,2-dichloroethane.

Effect of Foreign Substances

Table I shows the effect of foreign substances on the quinine, emethine or ephedrine. The determination was not interfered by the presence of amounts of the additive materials; chloride, glucose, bromide and starch, while a small amount of alkaloids, such as papaverine and caffeine or quaternary ammonium, such as benzethonium and tetraethylammonium gave a strong interference.

Analysis of Practical Samples

Commercial samples were analyzed according to the proposed method. Dissolve a sample (tablet or injection type) in water. Centrifuge the solution and dilute the supernatant solution

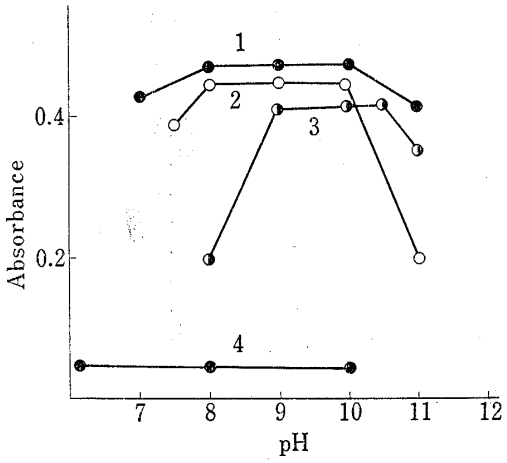

Fig. 2. Effect of pH on Extraction

1: extract with 2×10^{-6}M quinine, TBPE: 1.6×10^{-4}M, wavelength: 555 nm, Reference: water
2: extract with 4×10^{-6}M emethine, TBPE: 3.2×10^{-4}M, wavelength: 570 nm, reference: water
3: extract with 5×10^{-6}M ephedrine, TBPE: 3.2×10^{-4}M, wavelength: 555 nm, reference: water
4: reagent blank, wavelength: 555 nm, reference: water

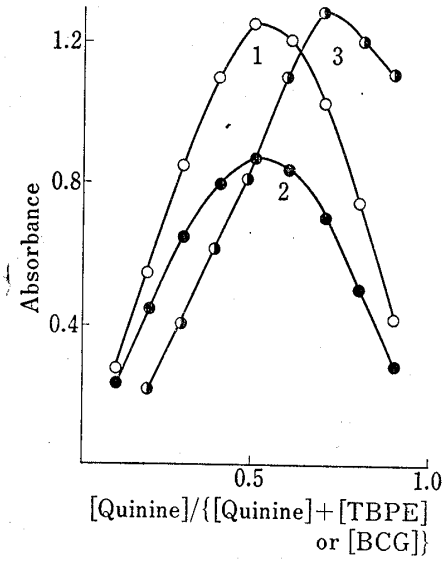

Fig. 3. Continuous Variation Plots of Quinine to TBPE or BCG

1: total concentration: [quinine+TBPE]=2×10^{-5}M, reference: reagent blank, wavelength: 555 nm
2: total concentration: [quinine+TBPE]=2×10^{-5}M, wavelength: 590 nm
3: total concentration: [quinine+BCG]=7×10^{-4}M, reference: reagent blank, wavelength: 630 nm

Table I. Effect of Foreign Substances

Substances	Mole ratio	Recovery (%)		
		Quinine	Emethine	Ephedrine
Sodium chloride	5000	100	100	100
Potassium bromide	5000	100	99	98
Ammonium sulfate	5000	113	125	123
Magnesium chloride	5000	102	98	—
Sodium acetate	5000	103	102	102
Sodium carbonate	5000	101	98	101
Sodium citrate	5000	100	101	102
Phenol	2000	100	100	100
Caffeine	100	100	95	105
Benzethonium	0.1	110	—	113
Tetraethylammonium	0.1	—	115	—
Papaverine	1	105	108	111
Glucose	5000	98	101	99
Thiamine	5	104	109	99
Triethanolamine	20	100	104	101
Starch	0.02	101	98	100

quinine: 2×10^{-6}M, emethine: 4×10^{-6}M, ephedrine: 5×10^{-6}M

with water in the case of tablets. Treat the solution in the same manner as the proposed method procedure. Table II shows that the analytical values obtained by the calibration curve method were essentially the same as those by the standard addition method. Further, on examination of the titrimetric[15] method and this method on ephedrine injection, the result was 0.197M by the titrimetric method and 0.191M by the proposed method. Accordingly, the proposed method has proved applicable to the determination of alkaloids in pharmaceutical preparations.

Table II. Analysis of Commercial Samples

Substances	Type		Obtained value (mg/l) Method[a]	Method[b]
Quinine	tablet	A	37.4	37.5
		B	36.5	36.3
Emethine	injection	A	38.7	38.7
		B	41.0	41.2
Ephedrine	injection	A	39.4	39.4
		B	38.3	38.0
	tablet	A	24.1	24.4
		B	26.2	26.0

a) by the calibration curve fitting method
b) by the standard addition method

Table III. Colors of the Extracts

Solvent for extraction	Dielectric constant	Blank	Berberine	Sparteine	Ephedrine
Nitrobenzene	34.38	blue	blue	blue	blue
Isoamyl alcohol	14.70	blue	blue	blue	blue
Methyl isobutyl ketone	13.11	blue	blue	blue	blue
1,2-Dichloroethane	10.36	yellow	blue	blue	red
Chlorobenzene	5.62	yellow	blue	blue	red
Chloroform	4.81	yellow	blue	blue	red
Toluene	2.38	yellow	blue	red	red
Benzene	2.28	yellow	blue	red	red
Carbon tetrachloride	2.24	yellow	blue	red	red
Cyclohexane	2.02	colorless in any case			
n-Hexane	1.89				

Solvents and Colors of the Extracts

Table III shows the colors of the extract by various solvents. TBPE is extractable into nitrobenzene, isoamyl alcohol and methyl isobutyl ketone even without alkaloids, amines or quaternary ammonium salts. In the case of some solvents with higher dielectric constant, blue colors appeared in the blank extract which is considered to be the dissociated species of TBPE. On the other hand, in other solvents, the reagent blank shows faint yellow because of poor extractability of the TBPE molecule. However, the color of the extract is blue in all these solvents by the presence of quaternary ammonium salts such as benzethonium and berberine.[16] By the presence of alkaloids such as quinine, emethine and ephedrine, the red color is developed in the solvents of the low dielectric constant, such as 1,2-dichloroethane, chlorobenzene, chloroform, toluene, benzene and carbon tetrachloride. This may be attributed to the formation of a charge transfer complex by transition of the electron through the hydrogen bridge between the nitrogen of the base and oxygen of the dye. Furthermore, the molecular structure of the cation may change this color extracts; for example, in the case of berberine and benzethonium, the cationic charge is buried or sterically hindered, while in the case of alkaloids, the change is exposed to the molecular surface which is favorable for ionic association. In the case of sparteine,[17] the molecular structure is intermediate between the above extreme cases, thus showing blue color in the solvents of higher dielectric constant and red one in the non-polar solvents as seen in Table III. *n*-Hexane, cyclohexane do not

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17) M. Tsubouchi, *Nippon Kagaku Zasshi* (Japan), **91**, 1061 (1970).

extract the dye even in the presence of alkaloids, amines and quaternary ammonium. And then, in order to investigate the state of the colored species in 1,2-dichloroethane, the electrical conductivity was measured for TBPE-berberine and TBPE-ephedrine extracts. The results are summarized in Table IV. It shows that TBPE-berberine species is in the ionized state and TBPE-ephedrine species is in the molecular state in 1,2-dichloroethane. Berberine is extracted into the organic phase as a blue dissociated ion-pair with the dye anion. And then, a reddish charge transfer complex may be formed between the ephedrine and the dye in the organic phase. Therefore, the extracted species may be formed as $[\text{berberine}]^{+}\cdot[\text{TBPE}]^{-}$ and $[\text{ephedrine}]\cdot H\cdot[\text{TBPE}]$. When other monoprotic acids such as deep red, 2,6-dichlorophenolindophenol, 2,5-dinitrophenol and resazurine were used, the color showed that the associated ion-pair did not exist.

Table IV. Electric Conductivity of Extracts

Aqueous phase (50 ml) containing	Color of extracts	Electric conductivity of 1,2-dichloroethane extracts (μv/cm)
10^{-4}M Berberine or ephedrine only	colorless	0.0
10^{-4}M TBPE only	yellow	0.0
10^{-4}M Berberine and 10^{-4}M TBPE	blue	3.9
10^{-4}M Ephedrine and 10^{-4}M TBPE	red	0.0

Extract with 40 ml of 1,2-dichloroethane was filtered with a filter paper to remove droplets of water, and the conductivity was measured at 25°.

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