BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 44, 1560—1562 (1971)

Spectrophotometric Determination of Benzethonium with Tetrabromophenolphthalein Ethyl Ester

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(Received August 1, 1970)

A spectrophotometric method is proposed for the determination of benzethonium. The method is based on solvent extraction of the ion-pair formed between benzethonium and colored tetrabromophenolphthalein ethyl ester, into 1,2-dichloroethane. The extract has a maximum absorbance at 615 mμ, and follows Beer's law with good precision up to 4×10^{-6} M in aqueous solution. The absorbance of the extract is constant in the pH range 7—11. Organic cations interfere.

Tetrabromophenolphthalein ethyl ester (TBPE) has been used as a pH indicator. In a previous paper,[1] this reagent was shown to be useful for the colorimetry of diphenhydramine by solvent extraction. In the course of this study, various amines or organic cations were extracted with TBPE into dichloroethane. The colors of the extracts were found to be classified into the following three categories. (1) Red-violet which is developed by the presence of diphenhydramine, *N,N*-dimethylpiperazine, papaverine, triethanolamine, pilocarpine, or eserine. (2) Blue which is extracted by the presence of benzethonium, spartein, neostigmine, tetraethylammonium, acetylcholine, or thiamine. (3) Yellow which is the same color as reagent blank for the presence of aniline, acetonitrile, 3-aminoquinoline, pyridine, *N,N*-dimethylformamide, EDTA, or NTA.

This paper deals with the determination of benzethonium with TBPE. The proposed method has a very good reproducibility and a high sensitivity. The titrimetric method[2] has been used for the determination of benzethonium which is widely used as disinfectant.

Experimental

Apparatus. Spectrophotometric measurements were carried out with a Shimadzu QR-50 spectrophotometer, with 10-mm cuvettes. An Iwaki Model KM shaker with a time switch was used for the extraction. The pH measurements were carried out with a Toa Denpa Model HM-5 pH meter.

Reagents. *Tetrabromophenolphthalein Ethyl Ester (TBPE) Solution*: Weighed amounts of tetrabromophenolphthalein

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[1] M. Tsubouchi, This Bulletin, **43**, 3164 (1970).

[2] Japanese Pharmacopoeia, VII-1, Part II, Nankodo (1965), p. 214.

ethyl ester potassium salt were dissolved in ethyl alcohol.

Standard Benzethonium Solution: A stock solution was prepared by dissolving 4.661 g of benzethonium chloride (dried at 105°C) and diluting to 1 *l* with water to make the solution 1.0×10^{-2} M. The stock solution was used to prepare the standard solution with desired concentration.

Buffer Solution: The pH 8.5 buffer was prepared by mixing 0.5 M potassium dihydrogen phosphate solution and 0.15 M sodium borate solution.

All the chemicals were of reagent grade and the water used was passed through an ion-exchange resin.

Procedure. Take 5 m*l* of benzethonium solution (less than 2×10^{-5} M), 2 m*l* of TBPE solution (10^{-3} M) and 5 m*l* of the buffer solution into a 10 -m*l* separating funnel. Dilute the mixture to 25 m*l* with water and shake the solution for 2 min with 10 m*l* of 1,2-dichloroethane. After 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 615 m*μ* using a reagent blank as a reference.

Results and Discussion

Absorption Spectra. Figure 1 shows the visible absorption spectra of benzethonium extracts with TBPE. It can be seen that the presence of benzethonium in aqueous solution leads to a considerable increase in the extraction. The absorbance maximum of the extracts is at 615 m*μ*. The same absorption spectra as in Fig. 1 were obtained for spartein, tetraethylammonium, acetylchlorine, or thiamine. The blue color in the organic layer can be attributed to the ion pair formation between the TBPE and the cations.

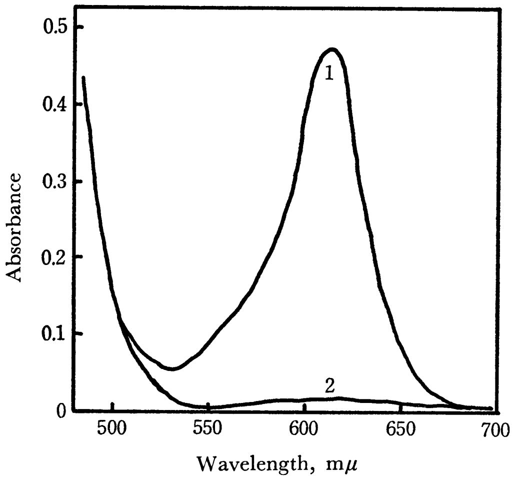

Fig. 1. Absorption spectra.
1: Spectrum of organic phase extracted from the aqueous solution (25 m*l*) containing 2×10^{-6} M of benzethonium, 8×10^{-5} M of TBPE and 5 m*l* of pH 8.5 buffer solution.
2: Spectrum of organic phase extracted from the aqueous solution (25 m*l*) containing the same component as that of curve 1 but benzethonium is absent.
Reference: Water

Effect of pH. The effect of pH on extraction was studied by extracting benzethonium from a series of aqueous solutions buffered at various pH values. As shown in Fig. 2, the absorbance of the extract was constant when the pH of the aqueous phase was in the range 7—11.

The extreme pH dependence was observed for the determination of quinine[3] or thiamine[4] with bromophenol blue or bromothymol blue. Similarly, no constant absorbance in any pH range could be obtained, when bromophenol blue or bromocresol green was used as extracting agent for benzethonium.

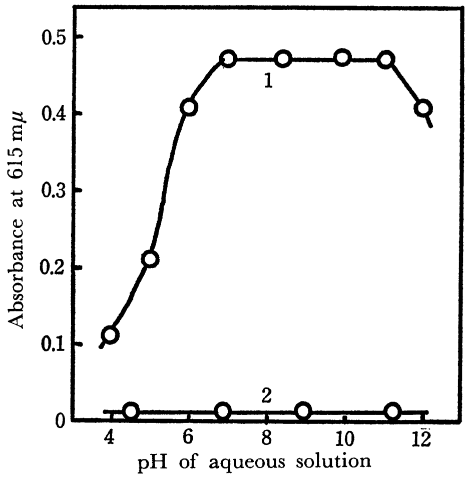

Fig. 2. Effect of pH.
1: Extract with 2×10^{-6} M benzethonium
2: Extract without benzethonium
Reference: Water

Effect of Reagents. The influence of TBPE concentration on extraction is illustrated in Fig. 3. It is apparent that the concentration of TBPE should be maintained greater than 10-fold molar excess over benzethonium to obtain a constant extraction. Excess amounts (2—10 m*l*) of the buffer solution used in the procedure had no influence on the absorbance of the

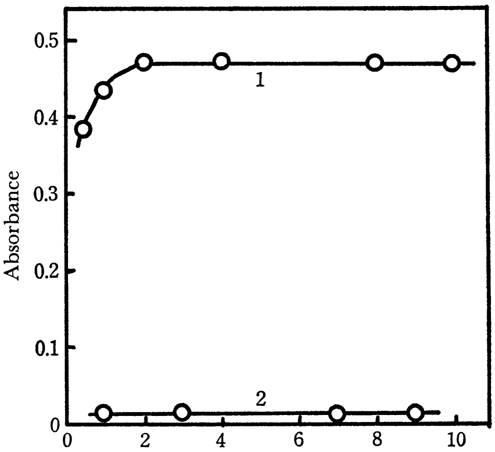

Fig. 3. Effect of the amount of TBPE.
1: Extract with 2×10^{-6} M benzethonium
2: Extract without benzethonium
Reference: Water

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[3] M. Tatsuzawa, S. Nakayama, and A. Okawara, *Bunseki Kagaku*, **19**, 761 (1970).

[4] V. D. Gupta and D. E. Cadwallader, *J. Pharm. Sci.*, **57**, 112 (1968).

extract. When the addition of the buffer solution was less than 2 m*l*, no effective separation of the two layers was observed.

Solvent for Extraction. The behavior of various solvents in the extraction was studied. Solvents were found to be classified into the following three categories. (1) Those with which the presence of benzethonium leads to a considerable increase in the extraction of blue TBPE: *e.g.* chloroform, 1,2-dichloroethane. (2) Those which do not extract the blue TBPE even in the presence of benzethonium: *e.g.* monochlorobenzene, toluene, *n*-hexane, cyclohexane, carbon tetrachloride. (3) Those with which the blue TBPE is extractable even without benzethonium: *e.g.* methyl isobutyl ketone, nitromethane, nitrobenzene, butyl acetate, ethyl acetate, ether, isoamylalcohol. 1,2-Dichloroethane was found to be most suitable for the extraction of a TBPE-benzethonium system.

Other Variables. Full color development required about 1 min shaking. Continued shaking up to 5 min produced no further change in absorbance. The color intensity of dichloroethane extracts remains constant for 1 hr. Fluctuation of room temperature (16—27°C) gave no measurable effect on absorbance.

Calibration and Precision. The system followed Beer's law up to 4×10^{-6} M of benzethonium in aqueous layer, with molar absorptivity of 9.00×10^{4} mol^{-1} cm^{-1} liter at 615 mμ.

The reproducibility of the proposed method was estimated from the results of ten sample solutions, each with a final benzethonium concentration of 2.0×10^{-6} M. The mean absorbance was 0.450 with a standard deviation of 0.005 absorbance unit.

Percentage of Extraction and Composition of the Colored Species. A sample (25 m*l*) containing 4.0×10^{-6} M of benzethonium was extracted with 10 m*l* of dichloroethane at pH 8.5. The amounts of benzethonium in the aqueous layer and organic layer were then determined according to the proposed method. The percentage of extraction was about 5% in the absence and 99% in the presence of TBPE.

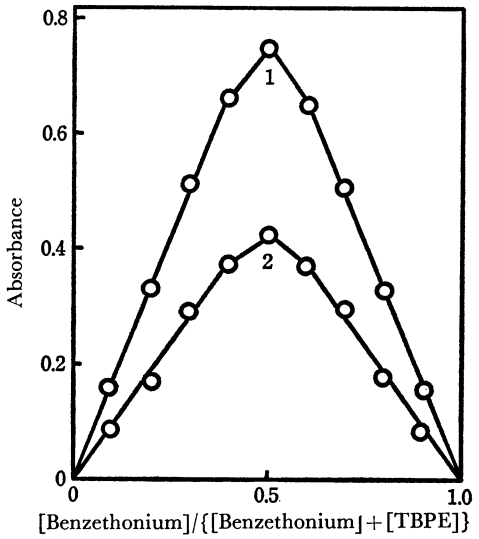

Fig. 4. Continuous variation curves.
Extraction was done from the aqueous solutions in which the total concentration of benzethonium plus TBPE was 8×10^{-6} M.
Reference: Reagent blank extracted from an aqueous solution without benzethonium
1: 615 mμ, 2: 590 mμ

Figure 4 shows the continuous variation curve for benzethonium. It might be suggested that a 1 : 1 ion-pair compound is formed in the dichloroethane phase between TBPE and benzethonium.

These results are characteristic of benzethonium as compared with diphenhydramine1) in the extraction system.

Effect of Diverse Substances. Table 1 shows the effect of diverse substances on the determination of benzethonium. Organic cations interfere.

Table 1. Effect of diverse substances

Added extraneous substance	Ratio of extraneous substance over benzethonium, mol/mol	Error expressed by % recovery
Ammonium sulfate	3000	101
Calcium chloride	3000	100
Sodium carbonate	3000	100
Sodium chloride	3000	100
Sodium nitrate	3000	100
Potassium bromide	3000	100
Antipyrine	40	109
Benzyl alcohol	30000	100
Caffeine	30	111
o-Cresol	30000	100
Ethyl alcohol	30000	100
Glucose	30000	100
Lactose	30000	100
Nicotinamide	100	110
Papaverine	10	108
Phenol	30000	99
Sodium acetate	30000	100
Sodium citrate	30000	100
Sodium salicylate	30000	99
Sodium tannate	30000	100
Spartein	0.1	110
Tetraethylammonium bromide	0.1	110
Triethylamine	70	104
Starch (0.4%)	—	100

Benzethonium taken: 2×10^{-6} M

Analysis of Practical Samples. Waste Water: The method was applied to a waste water containing benzethonium which had been used as a disinfectant solution for cupboard and plate basket. The sample solution was diluted or concentrated, and then analyzed by the proposed method or titrimetric method.2) The result was 3.97×10^{-4} M of benzethonium by the proposed method, and 3.95×10^{-4} M by the titrimetric method.

Pharmaceutical Preparation: A commercially available disinfectant solution obtained was treated in the same manner as the waste water. The result was 0.220 M of benzethonium by the proposed method, and 0.218 M by the titrimetric method.

The author expresses appreciation to Professor Y. Yamamoto of Hiroshima University for valuable advice.