viscosity. When an additive was added, it would penetrate the framework of the structure and weaken or disrupt the building units. It is expected that the macromolecules are joined by weak cohesive forces and not by strong bonds. Once the structural elements were affected, the system became unstable and the viscosity began to decrease. When the volume of additive present was increased, the gel-like character of the product gradually became less apparent and finally it was converted to a system of very low viscosity.

The initial rise in viscosity could not be attributed to solubilization of the additive by the surfactant to cause a swelling of the gel-like product, because most of the cetrimide molecules would have interacted with salicylic acid already, the latter being present in sufficient quantities to saturate practically the system. In support of this, it was observed that heptane and hexadecane, which are water insoluble, and amyl acetate, which is slightly water soluble, did not produce a rise in viscosity initially, although they could be solubilized. In addition, pyridine and liquefied phenol, both of which are miscible with water and could not be solubilized, also caused an initial increase in viscosity. Apparently the additives that increased the viscosity when first added were not able to disrupt the links at once, but they instead probably tended to attract the macromolecules closer together and thereby made the system more viscous. However, this situation did not remain for long; as soon as more of the additive was present, the bridges were unable to prevent a rupture of the framework and instability of the system was produced.

The decrease in viscosity after the initial increase was also associated with the change of the nature of the system, from a gel-like product to a cloudy dispersion or emulsion containing droplets of the immiscible additive. Generally the viscosity commenced to fall when the additive present had already exceeded its solubility or miscibility limits. However, this was not always the case. Pyridine and liquefied phenol, which did not produce cloudiness in the system, showed the same rise and fall in viscosity.

The findings of this investigation showed that two effects are possible when additives, many of which are simple solvents, are added to the system. One is the effect of immediate reduction in viscosity with a small volume of the additive; the other is an initial increase with very small amounts of the additive, followed by a decrease when a larger volume is included. These results will be of value in determining the usefulness of the gel-like product when incorporated in suspension formulation.

REFERENCES

(1) L. S. C. Wan, *J. Pharm. Sci.*, **55**, 1395(1966).
(2) *Ibid.*, **56**, 743(1967).
(3) *Ibid.*, **57**, 1903(1968).

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Spectrophotometric Determination of Organic Cations by Solvent Extraction with Tetrabromophenolphthalein Ethyl Ester

MASAHIRO TSUBOUCHI

Abstract □ Spectrophotometric methods are proposed for the determination of organic cations such as neostigmine, benzethonium, tetraethylammonium, sparteine, and diphenhydramine. The methods are based on solvent extraction into dichloroethane of the ion-pairs or addition compounds formed between colored tetrabromophenolphthalein ethyl ester and the cations. Calibration graphs were linear in the range 8.0×10^{-7}–4.0×10^{-6} *M* for neostigmine, benzethonium, tetraethylammonium, and sparteine, and in the range 2.0×10^{-6}–1.0×10^{-5} *M* for diphenhydramine in aqueous solution. Optimum conditions for the extraction and the composition of the extracted species were also investigated.

Keyphrases □ Organic cations—spectrophotometric determination by solvent extraction with tetrabromophenolphthalein ethyl ester □ Tetrabromophenolphthalein ethyl ester, solvent extraction—spectrophotometric determination of organic cations □ Spectrophotometric determination—organic cations–tetrabromophenolphthalein ethyl ester, solvent extraction

In general, acid dye is known to react with amine or quaternary ammonium salt to form a colored compound (1, 2). Bromthymol blue (3), bromcresol green (4), or bromphenol blue (5) was used for the determination of thiamine, quaternary compound, or quinine.

Tetrabromophenolphthalein ethyl ester has been used as a pH indicator. Various amines and organic cations were extracted with tetrabromophenolphthalein ethyl ester into 1,2-dichloroethane. During this study, the colors of the extracts were found to be classified into three categories: (*a*) red-violet, which is developed by the presence of diphenhydramine, pilocarpine, eserine, *N,N*-dimethylpiperazine, papaverine, or triethanolamine; (*b*) blue, which is extracted by the presence of neostigmine, benzethonium, sparteine, tetraethylammonium, acetylcholine, or thiamine; and (*c*) yellow, which is the same color as the reagent blank for the presence of aniline, 3-aminoquinoline, *N,N*-dimethylformamide, ethylenediaminetetraacetic acid, or nitrilotriacetic acid.

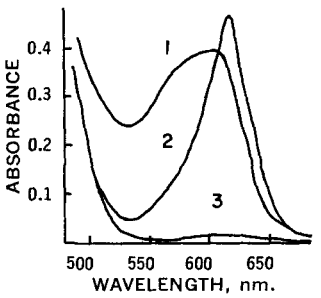

Figure 1—*Absorption spectra. Key: 1, extract with 5.0 × 10⁻⁶ M diphenhydramine; 2, extract with 2.0 × 10⁻⁶ M neostigmine; and 3, extract without neostigmine and diphenhydramine. Reference = water.*

This paper deals mainly with the determination of neostigmine with tetrabromophenolphthalein ethyl ester based on such a phenomenon; the results are then summarized for the determination of diphenhydramine, benzethonium, sparteine, or tetraethylammonium, which are on the market as medicines or disinfectants. The proposed methods have a very good reproducibility and a high sensitivity.

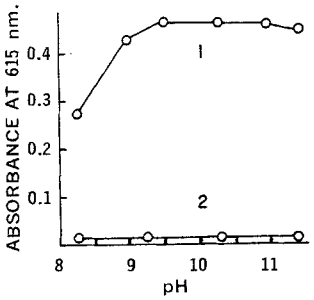

Figure 2—*Effect of pH. Key: 1, 2.0 × 10^{-6} M neostigmine; and 2, reagent blank. Reference = water.*

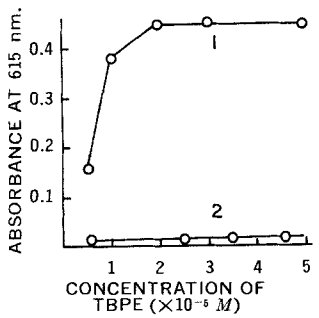

Figure 3—*Effect of concentration of tetrabromophenolphthalein ethyl ester (TBPE). Key: 1, 2.0 × 10^{-6} M neostigmine; and 2, reagent blank. Reference = water.*

The titrimetric method (6) has been used for the determination of neostigmine, which is widely used as a stimulant for parasympathetic nerves.

EXPERIMENTAL

Apparatus—The spectrophotometric measurements were made with a Shimazu model QR-50 spectrophotometer, with 10-mm. cells. An Iwaki model KM shaker with a time switch was used for the extraction. The pH measurements were made with a Toa Denpa model HM-5 pH meter.

Reagents—*Standard Solution*—A stock solution of 1.0 × 10^{-2} *M* neostigmine was prepared by dissolving 3.032 g. of neostigmine bromide[1] (dried at 105°) and diluting to 1 l. with water. The stock solution was used to prepare the standard solution with the desired concentration.

A standard solution of diphenhydramine[1], benzethonium[1], sparteine[1], or tetraethylammonium[1] was prepared by dissolving their chlorides, bromides, or sulfates in water.

Tetrabromophenolphthalein Ethyl Ester Solution—A 1.0 × 10^{-3} *M* solution of tetrabromophenolphthalein ethyl ester was prepared by dissolving 0.700 g. of tetrabromophenolphthalein ethyl ester potassium salt[2] to a volume of 1 l. with ethyl alcohol.

Buffer Solution—The borate–phosphate buffer was prepared by adding 3 *N* sulfuric acid or 3 *N* sodium hydroxide solution to the 0.3 *M* potassium dihydrogen phosphate solution containing 0.1 *M* sodium borate.

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

Recommended Procedures—*Neostigmine*—Pipet 5 ml. of neostigmine solution (less than 2.0 × 10^{-5} *M*), 2 ml. of tetrabromophenolphthalein ethyl ester solution (1 × 10^{-3} *M*), and 5 ml. of borate–phosphate buffer solution (pH 9.5–11.0) into a separator. Dilute the mixture to 25 ml. with water, and shake the solution for 2 min. with 10 ml. 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 nm., using a reagent blank as a reference.

Benzethonium, Tetraethylammonium, and Sparteine—Pipet 5 ml. of benzethonium, tetraethylammonium, or sparteine solution (less than 2.0 × 10^{-5} *M* for each solution), 2 ml. of tetrabromophenolphthalein ethyl ester solution (1 × 10^{-3} *M*), and 5 ml. of borate–phosphate buffer solution (pH 7.0–11.0 for the determination of benzethonium or sparteine, pH 9.5–10.5 for the determination of tetraethylammonium) into a separator. Treat the mixture in the same manner as described for neostigmine. Measure the absorbance at 615 nm.

Diphenhydramine—Pipet 5 ml. of diphenhydramine solution (less than 5.0 × 10^{-5} *M*), 5 ml. of tetrabromophenolphthalein ethyl ester solution (1 × 10^{-3} *M*), and 5 ml. of borate–phosphate buffer solution (pH 7.0–9.0) into a separator. Treat the mixture in the same way as for neostigmine, and measure the absorbance at 594 nm.

[1] Purchased from Tokyo Kasei Kogyo Co., Toshima, Kita-ku, Tokyo, Japan.

[2] Purchased from Wako Pure Chemical Industries, Doshu, Higashi-ku, Osaka, Japan.

Table I—Effect of Foreign Substances on the Determination of Neostigmine

Substance	Mole Ratio	Recovery of Neostigmine, %[a]
Ammonium sulfate	50000	101.0
Calcium chloride	50000	99.5
Sodium carbonate	50000	100.0
Sodium chloride	50000	100.0
Sodium nitrate	50000	100.0
Potassium bromide	50000	100.0
Aminopyrine	50	103.0
Antipyrine	40	106.0
Benzyl alcohol	50000	100.0
Caffeine	30	113.0
o-Cresol	50000	98.5
Ethyl alcohol	50000	99.7
Glucose	50000	100.0
Lactose	50000	100.0
Nicotinamide	100	107.5
Papaverine	20	105.0
Phenacetin	10	104.2
Phenol	50000	98.0
Sodium acetate	50000	100.0
Sodium citrate	50000	100.0
Sodium salicylate	50000	100.0
Sodium tannate	50000	98.5
Vitamin B_1	10	109.7
Vitamin C	50000	100.0
Starch	0.5%	100.0

[a] Neostigmine taken: 2.0 × 10^{-6} *M* as neostigmine bromide.

RESULTS AND DISCUSSION

Absorption Spectra—Figure 1 shows the visible absorption spectra of neostigmine or diphenhydramine extracts with tetrabromophenolphthalein ethyl ester. The absorbance maximum of the extracts was at 615 nm. for neostigmine and 594 nm. for diphenhydramine. The same absorption spectra as for neostigmine were obtained for sparteine, benzethonium, or tetraethylammonium extracts. The blue or red-violet color in the organic layer may be attributed to the effect of ion-pair formation or association between those substances and tetrabromophenolphthalein ethyl ester.

Effect of pH—The effect of pH on the extraction was studied by extracting the neostigmine 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 lay within the range 9.5–11.0.

An extreme pH dependence was observed for the determination of quinine (5) or thiamine (4) with bromphenol blue or bromthymol blue. Similarly, the constant absorbance in any pH range could not be obtained when bromphenol blue or bromcresol green was

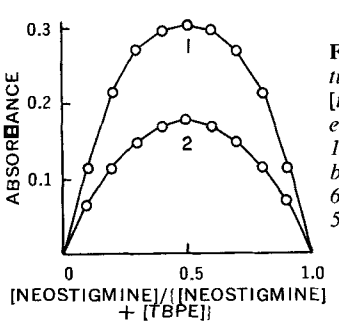

Figure 4—*Continuous variation curves. [Neostigmine] + [tetrabromophenolphthalein ethyl ester (TBPE)] = 8.0 × 10^{-6} M. Reference = reagent blank. Key: 1, measured at 615 nm.; and 2, measured at 590 nm.*

Table II—Analysis of Practical Samples

Analysis for	Sample	Titrimetric Method	Proposed Method
Neostigmine	Injection	0.367 mg./ml.	0.368 mg./ml.
	Tablet	3.69 mg./g.	3.66 mg./g.
	Powder	3.65 mg./g.	3.66 mg./g.
Benzethonium	Disinfectant solution	0.218 *M*	0.220 *M*
Sparteine	Injection	27.7 mg./ml.	27.6 mg./ml.
Tetraethyl-ammonium	Injection	61.6 mg./ml.	61.3 mg./ml.
Diphenhydramine	Ointment	9.90 mg./g.	9.87 mg./g.

used as the extractant for neostigmine. The extreme dependence of pH may be attributed to the hydrophilic radical such as sulfone or carboxylic acid in the dye molecule. The hydrophilic radicals may cause the extraction with the dye to be difficult.

Effect of Reagent Concentration—The influence of tetrabromophenolphthalein ethyl ester concentration on the extraction is illustrated in Fig. 3. It is apparent that the concentration of tetrabromophenolphthalein ethyl ester should be maintained at more than 10-fold molar excess over neostigmine to obtain a constant extraction.

Excess amounts of borate–phosphate buffer solution had no influence on the absorbance of the extract. When the addition of the buffer solution was less than 2 ml. in the aqueous layer, a good separation of the two layers was not observed.

Solvent for Extraction—The behavior of various solvents in the extraction was studied. The solvents could be classified into three categories: (*a*) those with which the presence of neostigmine led to a considerable increase in the extraction of blue tetrabromophenolphthalein ethyl ester, *e.g.*, 1,2-dichloroethane and chloroform; (*b*) those which do not extract the blue dye even in the presence of neostigmine, *e.g.*, carbon tetrachloride, cyclohexane, *n*-hexane, monochlorobenzene, and toluene; and (*c*) those with which the blue dye is extractable even without neostigmine, *e.g.*, butyl acetate, ether, ethyl acetate, isoamyl alcohol, nitrobenzene, nitromethane, and methyl isobutyl ketone.

1,2-Dichloroethane was found to be most suitable for the extraction of a neostigmine–tetrabromophenolphthalein ethyl ester system.

Other Variables—Full color development took about 1 min. of shaking. Continued shaking up to 5 min. produced no further change in absorbance. The color intensity of dichloroethane extracts remained constant for 1 hr. Normal room temperature fluctuations (16–25°) were without measurable effect in absorbance.

Calibration and Precision—The system followed Beer's law up to 4.0×10^{-6} *M* of neostigmine in aqueous layer, with a molar absorptivity of 2.25×10^{5} moles^{-1} cm.$^{-1}$ l. at 615 nm.

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

Extraction Rate and Composition of Colored Species—A sample (25 ml.) containing 4.0×10^{-6} *M* of neostigmine was extracted with 10 ml. of dichloroethane at pH 10.0. Then the amounts of neostigmine in the aqueous layer and organic layer were determined according to the proposed method. The extraction rate was about 2% in the absence of tetrabromophenolphthalein ethyl ester and 95% in its presence.

Figure 4 shows the continuous variation curve for neostigmine. It may be suggested that a 1:1 ion-pair compound is formed in the dichloroethane phase between tetrabromophenolphthalein ethy ester and neostigmine.

Effect of Foreign Substances—Table I shows the effect of foreign substances on the determination of neostigmine. Chloride, glucose, lactose, bromide, and starch, which are apt to exist in pharmaceutical preparations with neostigmine, do not interfere.

Analysis of Practical Samples—Commercial samples as medicine or disinfectant obtained from a drugstore were analyzed according to the proposed method and titrimetric method (6–10).

Injection and Disinfectant Solution—Dilute a sample with water and treat the solution in the same manner as the recommended procedure.

Powder and Tablet—Dissolve a sample in dilute sulfuric acid. Filter the solution with a glass filter and dilute the filtrate with water. Treat the solution in the same manner as the recommended procedure.

Ointment—Place a sample in a separator and add 15 ml. of ether. Extract the contents three times with 30-ml. portions of 0.03 *N* sulfuric acid. Combine the extracts and dilute with water. Treat the solution in the same manner as the recommended procedure.

The results obtained are summarized in Table II.

REFERENCES

(1) M. E. Auerbach, *Anal. Chem.*, **15,** 492(1943).

(2) P. B. Marshall and E. W. Rogero, *Biochem. J.*, **39,** 258(1945).

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

(4) H. M. N. H. Irving and J. J. Markham, *Anal. Chem. Acta*, **39,** 7(1967).

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

(6) "Japanese Pharmacopoeia," VII-1, part II, Nankodo. 19 p. 628.

(7) *Ibid.*, p. 214

(8) *Ibid.*, p. 594.

(9) *Ibid.*, p. 625.

(10) *Ibid.*, p. 1383.

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