Ion Association Titration for the Determination of Local Anesthetics in Pharmaceuticals with Tetrabromophenolphthalein Ethyl Ester as an Indicator

Tadao SAKAI,[†] Norio TESHIMA, and Yasufumi TAKATORI

Department of Applied Chemistry, Aichi Institute of Technology, 1247 Yachigusa, Yakusa, Toyota 470-0392, Japan

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Introduction

Solvent extraction-spectrophotometries based on ion association formation have been established for the improvement of the sensitivity for the determination of ionic compounds such as anionic and cationic surfactants using hydrophobic dye ions. For example, anionic surfactants were determined using Methylene Blue,¹ Ethyl Violet,² Co(III)-5-Cl-PADAP³ and Malachite Green.⁴ Bromocresol Green,⁵ 2,6-dichlorophenolindophenol⁶ and tetrabromophenolphthalein ethyl ester (TBPE)^{7,8} have been used for the determination of cationic surfactants and disinfectants. These methods were developed for enhancing their extractability and removing interfering substances. Though the sensitivities of these methods were very good, the procedures were tedious and time-consuming. As a simple method, two phase titration utilizing ion associate extraction has been developed.⁹⁻¹¹ However, it was very difficult to determine an end point in two phase titration methods reported so far, because the color change at the end point was attributed to the phase transfer of the ion associate formed with an indicator; the difference of hues between an aqueous and an organic phase was not clear, and color reflection gives a serious error. Tsubouchi *et al.* reported^{12,13} that, when TBPE was used as an indicator, it was possible to detect a color change only in the organic phase because of the direct formation and extraction of ion associate with the indicator existing in the organic phase. The method has been applied to the determination of quaternary ammonium salts.^{12,13} Later, the author (T. S.) found that ion associates formed between aromatic amines and TBPE were red or red-violet.¹⁴

The present paper deals with ion association titration of local anesthetic pharmaceuticals such as procaine, dibucaine and tetracaine (Scheme 1), providing sharp end point detection and high precision as well as the possibility of differential titration of mixtures including quaternary ammonium salts and aromatic amines.

Experimental

Reagents

Sodium tetrakis(4-fluorophenyl)borate (5×10^{-4} M) standard solution was prepared by dissolving 0.0563 g of sodium tetrakis(4-fluorophenyl)borate (Dojindo Laboratories, Kumamoto) into 250 ml of distilled water.

Sodium tetraphenylborate (5×10^{-4} M) solution was prepared by dissolving 0.0428 g of sodium tetraphenylborate (Katayama Chemicals, Osaka) into 250 ml of distilled water.

Procaine hydrochloride (5×10^{-4} M) solution was prepared by dissolving 0.0376 g of procaine hydrochloride (Nacalai Tesque, Osaka) into 250 ml of water.

Scheme 1 Structures of local anesthetics.

[†] To whom correspondence should be addressed.

Table 1 Determination of local anesthetic amines

Amine compound	Added (mg/5 ml)	Found (mg/5 ml) $\pm$ SD ^a	Recovery, %
Procaine hydrochloride	0.682	0.679 $\pm$ 0.001 ^b 0.662 $\pm$ 0.008 ^c	99.6 ^b 97.1 ^c
Dibucaine hydrochloride	0.950	0.951 $\pm$ 0.004 ^b 0.948 $\pm$ 0.006 ^c	100.1 ^b 99.7 ^c
Tetracaine hydrochloride	0.752	0.742 $\pm$ 0.005 ^b 0.730 $\pm$ 0.002 ^c	98.6 ^b 97.1 ^c
Zephiramine chloride	1.010	1.046 $\pm$ 0.004 ^b 1.046 $\pm$ 0.004 ^c	99.8 ^b 103.5 ^c

a. Estimated by 3 determinations.

b. With 5×10^{-4} M tetrakis(4-fluorophenyl)borate solution.c. With 5×10^{-4} M tetraphenylborate solution.

5×10^{-4} M dibucaine hydrochloride (MW: 379.9, Wako, Osaka), tetracaine hydrochloride (MW: 300.8, Wako) and ephedrine hydrochloride (MW: 201.7, Sanwa Chemicals, Osaka) were prepared in the same manner.

Benzalkonium chloride and zephiramine chloride, 5×10^{-4} M, were prepared by dissolving a proper amount of each salt.

TBPE (2×10^{-3} M) solution was prepared by dissolving 0.140 g of potassium tetrabromophenolphthalein ethyl ester (Tokyo Kasei, Tokyo) into 100 ml of hot ethanol.

The borate buffer solution (pH 9) was prepared by 0.3 M sodium dihydrogenphosphate and 0.1 M sodium tetraborate and adjusting the pH with 1 M sodium hydroxide.

1,2-Dichloroethane (DCE) was used without any purification.

Procedure

A 1–5 ml portion of 5×10^{-4} M procaine (local anesthetics) solution, 5 ml of the borate buffer solution, 2 drops of TBPE indicator solution and 5 ml of DCE are transferred in a 100-ml Erlenmeyer flask. The mixture is titrated with 5×10^{-4} M tetrakis(4-fluorophenyl)borate solution while shaking by hand to ensure equilibrium between the organic and aqueous phases. When the equivalence point is reached, the color of the organic phase changes from red-violet to yellow. It is not necessary to make a blank titration because the color of the organic phase is yellow in the absence of local anesthetics.

Results and Discussion

Experimental conditions for ion association formation of local anesthetics with TBPE

The color of the ion associate between procaine and TBPE was red-violet, having its maximum absorption at 580 nm. The ion associate of dibucaine was red and its maximum absorption wavelength was 555 nm. The ion associate of tetracaine was also red, the maximum absorption being at 570 nm. The maximum wavelengths of the ion associates of all quaternary ammonium ions with TBPE were at 610 nm. The optimum pHs for the extraction of the ion associates were 8–9 for procaine, 6–9 for dibucaine and 7–9 for tetracaine. Therefore, the extraction was done at pH 9.

Detection of equivalence point

When two drops of TBPE were added to amine compounds in the aqueous phase and the container was shaken by hand, the aqueous phase became colorless, and a red or red violet color resulted in the DCE phase. As an amine sample was titrated

Table 2 Analytical results of local anesthetic drug in commercial samples

Sample	Labeled	Found ^a	R.S.D., %	Recovery, %
Procaine hydrochloride	1%	1.02%	0.6	102
Dibucaine hydrochloride	0.3%	0.3%	1.0	100
Tetracaine hydrochloride	20 mg/ampoule	19.6 mg/ampoule	0.9	98

5×10^{-4} M tetraphenylborate solution was used.

a. Mean values of 3 determinations.

with the tetrakis(4-fluorophenyl)borate solution under shaking, the protonated amine formed the colorless ion associate (titrant- R_3NH^+), and the ion associate formed was extracted into the organic phase. When the equivalence point was reached, the organic layer showed a dull light green color. When a drop of excess titrant was added, the color of organic phase changed to yellow. The chemical reactions for the ion association titration are as follows:

Initial stage:

Reaction with titrant before equivalence point:

Equivalence point:

Determination of local anesthetic amines using tetrakis(4-fluorophenyl)borate and tetraphenylborate

According to the Procedure, procaine hydrochloride, dibucaine hydrochloride and tetracaine hydrochloride solutions were titrated with the 5×10^{-4} M tetrakis(4-fluorophenyl)borate solution and the 5×10^{-4} M tetraphenylborate solution. Table 1 shows the results. The recoveries were 97.1–103.5% and the standard deviations were very small. Zephiramine chloride, a typical quaternary ammonium salt, was also titrated in the same manner. In this case, the blue color of Zephiramine-TBPE associate turned yellow at the equivalence point. The recovery was good (almost 100%) similarly to the amine associates.

Application to pharmaceuticals

Amine compounds in commercial samples were determined by the proposed method. A sample solution in an ampoule was diluted to the appropriate concentration. The results are shown in Table 2.

Possibility of simultaneous determination of amine and quaternary ammonium salt by differential titration

Sakai *et al.*¹⁵ reported that the ion association ability of quaternary ammonium salts (R_4N^+) and aliphatic amines (R_3N) with TBPE was different because quaternary ammonium salts formed a deprotonated ion associate $R_4N^+ \cdot TBPE^-$, whereas aliphatic amines formed a protonated charge transfer complex of $R_3N \cdot H \cdot TBPE$. Accordingly, the author (T. S.) predicted that,

Table 3 Determination of the mixture of benzalkonium chloride (BZ) and ephedrine hydrochloride (EP) by the proposed titration

Combined concentration/M BZ:EP	End point/ml	RSD, % ^a	Recovery, %
1 × 10 ⁻⁴	1.01	1.2	101
:2 × 10 ⁻⁴	2.08	1.4	104
1 × 10 ⁻⁴	1.03	1.9	103
:1 × 10 ⁻⁴	1.01	1.0	101
1 × 10 ⁻⁴	1.02	0.6	102
:0.5 × 10 ⁻⁴	0.52	3.3	104
5 × 10 ⁻⁴	5.02	0.3	100
:1 × 10 ⁻³	10.6	0.2	106
5 × 10 ⁻⁴	5.0	0.5	100
:5 × 10 ⁻⁴	5.17	1.5	103
5 × 10 ⁻⁴	4.99	0.4	99
:2.5 × 10 ⁻⁴	2.65	1.3	106

A 5 ml of the mixture of BZ and EP was taken in an Erlenmeyer flask.

5 × 10⁻⁴ M sodium tetraphenylborate was used as a titrant.

a. RSD was estimated for 3 determinations.

when the hydrophobic tetraphenylborate (TPB⁻) is added to the mixture of quaternary ammonium salts and amines, the ion association formation would occur in the order of R₄N⁺-TPB⁻ and R₃NH⁺-TPB⁻. If the assumption is correct, the differential titration among quaternary ammonium salts and amines can be possible. The mixtures of benzalkonium chloride and ephedrine hydrochloride combined in several proportions were titrated. The results are shown in Table 3. The recoveries and relative standard deviations obtained by the proposed titration method were good and small.

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