The Determination of Berberine by Ion-pair Extraction-Titration, with Tetrabromophenolphthalein Ethyl Ester as the Indicator

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A method has been developed for the determination of berberine as a quinolizinium medicine. The method is based on the solvent extraction-titration of an ion pair. Sodium tetraphenylborate is used as the titrant, with tetrabromophenolphthalein ethyl ester as the indicator in the presence of chloroform. In the two-phase titration, the proposed procedure gives sharp end points as the organic phase changes from blue to yellow. The aqueous phase is colorless throughout the titration. From a p(berberine)-pH diagram, a theoretical titration curve is constructed. The titration curve at pH 6.5 is not too far from the ideal case.

Various reports have appeared regarding the ion-pair extraction-titration (two-phase titration) method. Methylene Blue,[1] Methyl Orange,[2] Azure A,[2] Bromophenol Blue,[3] and Neutral Red[4] have been used as indicators for the determination of anionic surfactants of quaternary ammonium salts. The ion-pair extraction-titration techniques would be convenient for use in the laboratory because, with them, there is no need for sophisticated instrumentation. End-point detection in two-phase titration is based on the movement of a dye as an indicator from one phase to the other. In general, though, it is difficult to detect the end point because a dye color in an aqueous phase or an organic phase will be reflected in the other layer.[1,2]

This paper will deal with the determination of berberine as a quinolizinium medicine. Nonaqueous titrimetric,[5] gravimetric,[6] and spectrophotometric[7-10] methods were investigated for the determination of berberine. In the two-phase titration proposed here, sodium tetraphenylborate is used as the titrant, with tetrabromophenolphthalein ethyl ester (In) as the indicator in the presence of chloroform. This procedure gives sharp end points as the organic layer changes from blue to yellow, and there is less likelihood of error. A singly charged In anion forms a 1:1 ion pair with berberine in the organic solvent.[8] The aqueous layer is colorless throughout the titration. This is because the indicator itself is not soluble in water, but gives a yellow color in chloroform. When the berberine (Bb) is titrated with a sodium tetraphenylborate (TPB) solution, the $[Bb^+ \cdot TPB^-]$ ion pair is formed in the organic phase and the reaction followed at the end point is:

$$[Bb^+ \cdot In^-]_{org} + H^+ + [TPB^-]_{aq} \longrightarrow \underset{\text{blue}}{} \quad [Bb^+ \cdot TPB^-]_{org} + [In]_{org} \quad \underset{\text{yellow}}{}$$

Experimental

Reagents. Tetrabromophenolphthalein ethyl ester potassium salt was dissolved in ethanol to make the solution 0.1%. A proper quantity of berberine chloride was dissolved in the distilled water. Standardization was done spectrophotometrically with a potassium dichromate solution.[7] The phosphate buffer solution (pH 6.5) was prepared from a 0.3 M disodium hydrogenphosphate solution with several drops of dilute sulfuric acid.

Procedure. The berberine solution (1—10 ml of 0.001 M), 5 ml of the phosphate buffer solution, 10 ml of chloroform, and 2—3 drops of a tetrabromophenolphthalein ethyl ester solution were placed in a 200-ml Erlenmeyer flask. The mixture was titrated with a 0.002 M sodium tetraphenylborate solution, with intermittent shaking by hand, to ensure an equilibrium between the organic solvent and the aqueous phase. The organic phase changes from greenish blue to yellow at the end point.

Results and Discussion

When the mixture of the berberine solution, the buffer solution, chloroform, and the indicator solution is shaken as has been described above, the aqueous phase is colorless and the organic phase is greenish blue. Figure 1 shows the visible absorption spectra of the indicator in chloroform. Near the end point of the titration, the organic phase starts to turn green. When one drop excess of the tetraphenylborate solution is added, the organic phase acquires a distinct yellow color, while the aqueous phase is still colorless. This is due to the fact that the indicator forms an organophilic ion pair with berberine. Therefore, some reflection of the color in the aqueous phase does not interfere with the end-point detection.

The effect of the pH on the proposed method was studied by titrating a series of berberine solutions buffered at various pH values. The results are summarized in Fig. 2. When the berberine solution is titrated with sodium dodecylbenzenesulfonate solution, quan-

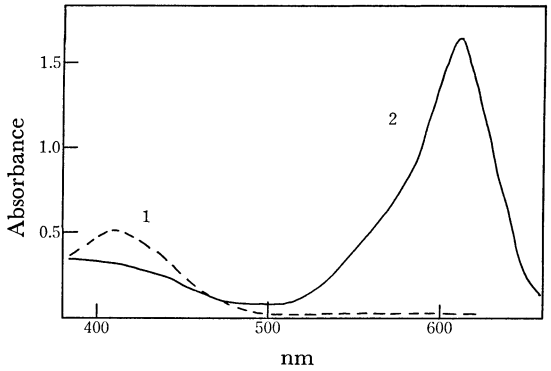

Fig. 1. Absorption spectra of chloroform phase. 1: Extract with 2×10^{-5} M indicator, 2: extract with 2×10^{-5} M indicator and 2×10^{-5} M berberine. Reference: water.

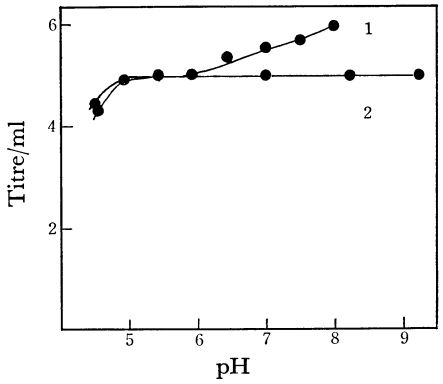

Fig. 2. Effect of pH on titration. 1: Titration with 0.002 M laurylbenzenesulfonate, 2: titration with 0.002 M tetraphenylborate, berberine, 10 ml of 0.001 M.

titative titrations are obtained in the pH range of 5—6. Larger amounts of buffer solution had no influence on the titration, but when less than 1 ml was added, the phase separation was poor.

As in the pH titration of acids and bases or in the pM titration of the metal ion and ethylenediaminetetraacetic acid,[11] berberine titration by the extraction may be generally represented by p(berberine) titration curves. The basic equilibria are given by:

$$(Bb)_w + (TPB)_w \rightleftharpoons (Bb\cdot TPB)_o$$

$$(Bb)_w + (In)_o \rightleftharpoons (Bb\cdot In)_o$$

For simplicity, the charges are omitted. The symbols are: Bb, berberinium ion; TPB, tetraphenylborate ion; In, indicator; o, in the organic phase; w, in the aqueous phase. The corresponding equilibrium constants are:

$$K_{Bb\cdot TPB} = [Bb\cdot TPB]_o/([Bb]_w \times [TPB]_w)$$

$$K_{Bb\cdot In} = [Bb\cdot In]_o/([Bb]_w \times [In]_o)$$

The effective stability constants (12—17 °C) were obtained from absorption measurements with berberine-tetraphenylborate extracts and berberine-indicator extracts at 440 nm[7] and 610 nm[8] respectively. A simple pBb-pH diagram is given in Fig. 3 for the titration. When 50% of TPB is present in free-ion form and 50% in Bb–TPB ion-pair form, the pBb is equal to the logarithm of the effective stability constant (K') of the Bb–TPB ion pair:

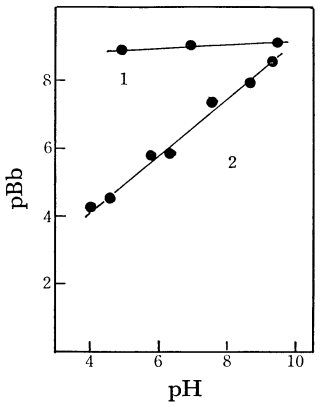

Fig. 3. p(berberine)-pH diagram. 1: $\log K'_{Bb\cdot TPB}$, 2: $\log K'_{Bb\cdot In}$.

$$\log K'_{Bb\cdot TPB} = \log 1/[Bb]_w = pBb$$

Line 2 in Fig. 3 corresponds to the pBb value at which 50% of In is present in free-dye form and 50% in Bb–In ion-pair form:

$$\log K'_{Bb\cdot In} = \log 1/[Bb]_w = pBb$$

From the pBb-pH diagram, a theoretical titration curve is constructed for the 0.002 M TPB titration of 10 ml of 0.001 M Bb at the pH value of 6.5 as is shown in Fig. 4. The pBb at the 50%-color-change point, C, is equal to the logarithm of the effective stability constant of the Bb–In ion pair. A maximum color change is obtained by a minimum increment titrant at this point. The fractional color change from 0.1 to 0.9 covers about 2 pBb units. The shaded areas indicate the region of the color change of the indicator. The titration curve at pH 6.5 is not too far from the ideal one.

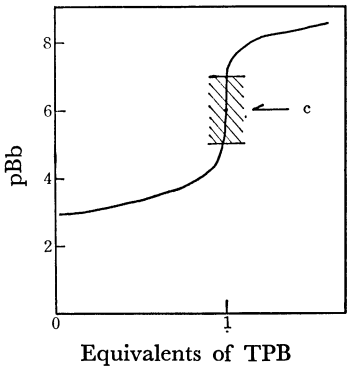

Fig. 4. Titration curve at pH 6.5. 10 ml of 0.001 M berberine is titrated with 0.002 M tetraphenylborate.

Ten identical samples, each with 10 ml of a 0.001 M berberine solution, were titrated with a 0.002 M tetraphenylborate solution according to the procedure. The mean titre was 5.00 ml, with a standard deviation of 0.04 ml.

Various water-immiscible solvents, such as nitrobenzene, isopentyl methyl ketone, butyl acetate, isoamyl alcohol, toluene, 1,2-dichloroethane, chloroform, benzene, carbon tetrachloride, and hexane, were tested. Of these, chloroform was the best solvent for the titration of berberine. The initial volume fluctuations of the aqueous phase (5—20 ml) and the organic phase (7—15 ml) did not have any measurable effect on the determination of the end point.

The effects of other ions on the titration process were studied for 10 ml of the 0.001 M berberine solution. The following ions did not interfere at the 0.01 M level: Na^+, Ca^{2+}, Mg^{2+}, K^+, NH_4^+, NO_3^-, SO_4^{2-}, Br^-, Cl^-, I^-, acetate, carbonate, citrate, and tannate. Amines such as thiamine, papaverine, and diphenhydramine caused positive errors; the maximum permissible amount was the 10^{-4} M level. Dodecyl sulfate, quaternary ammonium, and mercury(II) ions interfered.

Sample of medicine containing berberine chloride (40 mg), tannic acid (40 mg), aluminium 2-hydroxy-3-naphthoate (100 mg), pectin (50 mg), and silicon powder (5 mg) were dissolved in water. The solutions

were filtered and analyzed according to the proposed method and a spectrophotometric method.[7] The mean result of six samples was 39.0 mg of berberine chloride, with a standard deviation of 0.3 mg, while the result was 38.2 mg of berberine chloride by the spectrophotometric method. The proposed indicator has proved applicable to the two-phase titration of anionic surfactants with quaternary ammonium salts.

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