(1977).
(6) W. L. Chiou, N. K. Athanikar, and S. Huang, *N. Engl. J. Med.*, **300**, 501 (1979).
(7) S. M. Huang, N. K. Athanikar, K. Sridhar, Y. C. Huang, and W. L. Chiou, "Abstracts," vol. 8, no. 2, APhA Academy of Pharmaceutical Sciences, 1980, p. 82.
(8) J. A. Thompson and F. H. Leffert, *J. Pharm. Sci.*, **69**, 707 (1980).
(9) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 562 (1974).
(10) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 48, 68, 177.
(11) D. DuBois and E. F. DuBois, *Arch. Intern. Med.*, **17**, 863 (1916).
(12) E. F. Ellis, R. Koysooko, and G. Levy, *Pediatrics*, **58**, 542 (1976).
(13) A. Rane and J. T. Wilson, *Clin. Pharmacokinet.*, **1**, 2 (1976).
(14) H. C. Shirkey, "Pediatric Clinical Pharmacology and Therapeutics in Drug Treatment," Adis Press, New York, N.Y., 1980, p. 126.

ACKNOWLEDGMENTS

Partially supported by the Council for Research and Creative Work of the University of Colorado.

The authors thank Mr. John Ivy for assistance with the serum extractions.

Application of One-Phase End-Point Change System in Two-Phase Titration to Amine Drug Analysis

MASAHIRO TSUBOUCHI *x, **HIROMI MITSUSHIO** ‡, **NAKAMICHI YAMASAKI** ‡, and **KIYOSHI MATSUOKA** ‡

Received December 2, 1980, from the *Laboratory of Chemistry, Kochi Medical School, Oko, Nankoku, Kochi 781-51, Japan, and the ‡ Department of Geology, Faculty of Science, Kochi University, Asakura, Japan. Accepted for publication March 30, 1981.

Abstract □ A titration method was developed for the determination of diphenhydramine, quinine, neostigmine, sparteine, strychnine, homatropine, atropine, physostigmine, and procaine in aqueous solution. Tetraphenylborate was used as a titrant with tetrabromophenolphthalein ethyl ester as an indicator in the presence of organic solvent. End-point detection was based on the color change of the indicator in the organic phase without movement of the indicator from one phase to the other.

Keyphrases □ Amine drugs—analysis, two-phase titration □ One-phase end-point change system—two-phase titration, amine drugs □ Two-phase titration—amine drugs, one-phase end-point change system

Titration methods permit the convenient determination of organic and inorganic ions without complex instrumentation. Solvent extraction techniques are also convenient in the titration field. Two-phase titration (ion-pair extraction–titration) using methylene blue was proposed by Weatherburn (1) in 1950. However, with this two-phase dye transfer titration method, it is generally difficult to detect the end-point because a dye color in an aqueous layer reflects to the organic layer (2). Subsequently, additional reports (2–5) described two-phase dye transfer procedures involving modifications of the methylene blue method and substitutions of various dyes for methylene blue.

Titration end-point detection, however, can be a problem with the two-phase titration method due to color reflectance between phases and differences in the color shade or hue in the two phases. These problems occur because the end-point detection in the two-phase titrations is based on the transfer of the indicator dye from one phase to the other, with end-points usually taken when color intensity is judged to be equal between the two phases. The two-phase titration has been applied only to the determination of surfactants. Mohammed and Cantwell (6) recently developed a two-phase photometric titration method for the determination of drugs and surfactants.

The present paper reports a new two-phase titration method based on the change of indicator in only one phase without movement of the indicator from one phase to the other. This one-phase end-point change system provided the two-phase titration method for the determination of amines in aqueous solution. Colorimetric and nonaqueous titrimetric methods were used for the determination of alkaloids or amines.

EXPERIMENTAL[1]

Reagents—The potassium salt of tetrabromophenolphthalein ethyl ester, dissolved in ethanol to make a 0.1% solution, was used as the indicator.

Buffer Solution—A borate–phosphate buffer was prepared by adding 2 *N* H_2SO_4 to a 0.2 *M* dibasic sodium phosphate solution containing 0.1 *M* sodium borate.

Titrant—Sodium tetraphenylborate (3.422 g), dried at 80°, was dissolved in water and diluted to 1 liter to make a 0.01 *M* solution.

Amine Solution—Sample solutions of diphenhydramine, quinine, neostigmine, sparteine, strychnine, atropine, physostigmine, and procaine were prepared by dissolving their hydrochlorides, bromides, or sulfates in 0.002 *N* H_2SO_4. The solutions were standardized by the official method (7).

Titration Procedure—The proposed titration methods with a one-phase end-point change system are summarized in Table I. For example, the determination of diphenhydramine was as follows: 1–10 ml of diphenhydramine solution (0.01 or 0.005 *M*), 5 ml of borate–phosphate buffer (pH 5.5–7.5), 10 ml of ethylene dichloride, and 3–4 drops of indicator solution were placed in a 300-ml erlenmeyer flask. The mixture was titrated with 0.01 *M* tetraphenylborate solution with manual intermittent shaking to ensure equilibrium between the organic solvent and the aqueous phase.

The other amines or alkaloids in Table I were treated in the same manner as described for diphenhydramine.

RESULTS AND DISCUSSION

End-Point Color—The one-phase end-point change system is based on the hydrophobic indicator, which is able to react with ions in the aqueous phase. The tetrabromophenolphthalein ethyl ester indicator is a monoprotic acid and can form ion associates or charge transfer complexes with amines or alkaloids (8). The initial mixture of the amines

[1] All chemicals were reagent grade; Wako Pure Chemical Industries, Tokyo, Japan.

0022-3549/81/1100-1286$01.00/0

Table I—Two-Phase Titration with One-Phase End-Point Change System for Determination of Amines [a]

Amine	pH in Aqueous Phase	Organic Solvent	Color Change in Organic Phase at End-Point
Diphenhydramine	5.5–7.5	Ethylene dichloride	Violet → yellow
Quinine	4.5–6.0	Nitroethane	Violet → yellow
Berberine	5.0–9.5	Chloroform	Blue → yellow
Neostigmine	9.0–9.5	Ethylene dichloride	Greenish blue → yellow
Sparteine	5.0–9.0	Ethylene dichloride	Blue → yellow
Alkaloids [b]	5.5–6.5	Ethylene dichloride	Reddish blue → greenish yellow

[a] The indicator was tetrabromophenolphthalein ethyl ester, and the titrant was tetraphenylborate. [b] Strychnine, homatropine, atropine, physostigmine, and procaine.

or alkaloids in Table I, buffer solution, indicator, and organic solvent yields a solution with a colorless aqueous layer and a blue or violet organic phase. As the solution is titrated with tetraphenylborate, with thorough shaking after each addition of titrant, the organic solution turns green near the end-point. When 1 drop of excess is added, the organic phase turns yellow, indicating the titration end-point. The upper aqueous layer remains colorless throughout titration due to the water insolubility of the tetrabromophenolphthalein ethyl ester indicator. Amines or alkaloids cannot be determined by the two-phase dye transfer titration method with methylene blue as an indicator because the end-point is not clear.

Effect of pH on Titration—The effect of pH was studied by titrating a series of amines and alkaloids at various pH values. The titer was constant when the pH of the aqueous phase was within the range shown in Table I. When an amine (A) is titrated with a tetraphenylborate ion (B^-) solution, the $[A\text{-}H^+\cdot B^-]$ complex is formed in the organic layer. The end-point of the titration with the proposed indicator (Ind) is described by:

$$[A\text{-}H^+ \cdot Ind^-]_o + [B^-]_w + H^+ \rightarrow [A\text{-}H^+ \cdot B^-]_o + [H\text{-}Ind]_o$$

violet or blue — *yellow*

Scheme I

where o is the organic phase and w is the aqueous phase. The indicator in the $[A\text{-}H^+\cdot Ind^-]_o$ complex is substituted by the titrant ion to liberate the indicator in the nondissociated form.

The basic equilibria for the titration of diphenhydramine can be represented by:

$$D + B \rightleftharpoons [DB]$$

Scheme IIa

$$D + Ind \rightleftharpoons [DInd]$$

Scheme IIb

where D is the diphenhydramine. (For simplicity, charges are omitted.) The corresponding equilibrium constants are:

$$K_{DB} = [DB]/([D][B])$$

Scheme IIIa

$$K_{DInd} = [DInd]/([D][Ind])$$

Scheme IIIb

The effective stability constants (K') at room temperature can be obtained by determining [DB] and [DInd] in the organic phase using spectroscopic absorption measurements at various pH values (9). A simple log K'–pH diagram is given in Fig. 1 for the titration. Line 1 represents pD or $-\log[D]$ at two equivalents of tetraphenylborate where $[B]_w = [BD]_o$. Line 2 corresponds to the pD or $-\log[D]$ values at which $[DInd]_o = [Ind]_o$. From the log K'–pH diagram, a theoretical titration curve is constructed for the 0.01 M tetraphenylborate titration of 10 ml of 0.01 M diphenhydramine at pH 6.5 (Fig. 2). The shaded areas indicate the region of the color change of tetrabromophenolphthalein ethyl ester indicator, with C corresponding to the point of 50% color change. A maximum color change is obtained by a minimum increment of titrant at this point. The fractional color change from 0.1 to 0.9 covers ~2 pD units. The theoretical titration curve at pH 6.5 was in good agreement with experimental values.

Other Variables and Foreign Substances—Larger amounts of buffer solution had no influence on titration, but amounts of <0.5 ml caused problems in phase separation. Initial volume fluctuations of the aqueous layer (5–25 ml) and the organic layer (7–15 ml) had no influence on the determination of the titration end-point. The effect of water-immiscible solvents on titration were tested: butyl acetate, benzene, carbon tetrachloride, chlorobenzene, chloroform, ethylene dichloride, n-hexane, isopentyl alcohol, methyl isobutyl ketone, nitrobenzene, nitroethane, and toluene. Of these solvents, the best ones for the titration are summarized in Table I.

The effect of several salts on the titration of 10 ml of 0.01 M diphenhydramine solution indicated that the following ions did not interfere at the 0.01 M level: Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, K^+, NO_3^-, SO_4^{2-}, CO_3^{2-}, Br^-, I^-, Cl^-, acetate, citrate, and tannate. Amines such as thiamine and papaverine caused positive errors. Lauryl sulfate and mercury II interfered.

Precision and Analysis of Practical Samples—The reproducibility of the proposed method with 0.005 M titrant solution was estimated from

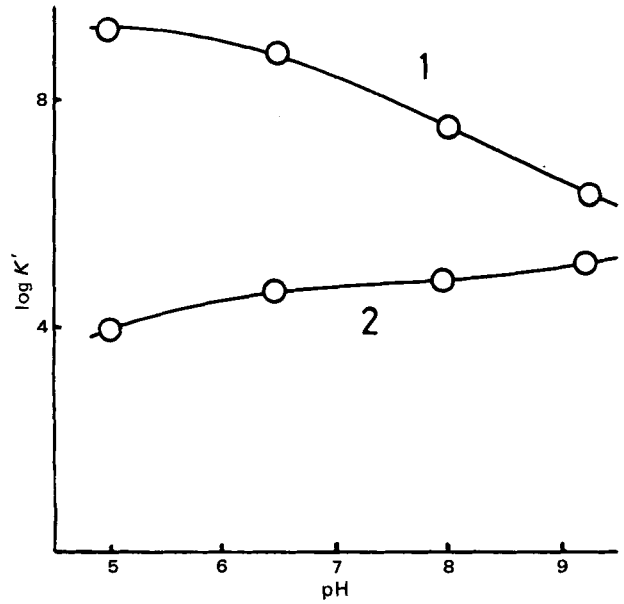

Figure 1—*Log K′–pH diagram. Key: 1, log K'_{DB}; and 2, log K'_{DInd}.*

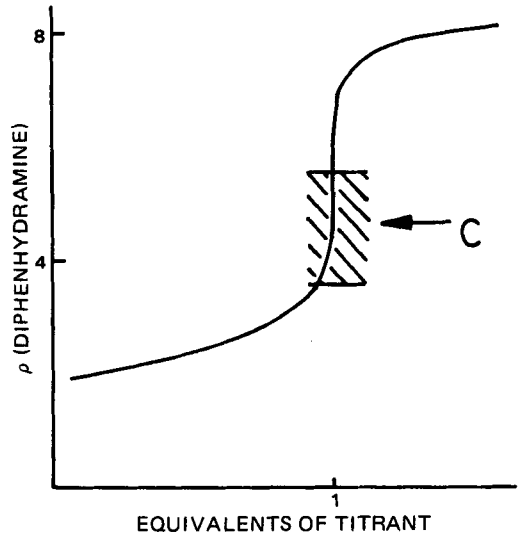

Figure 2—*Theoretical titration curve at pH 6.5; 10 ml of 0.01* M *diphenhydramine is titrated with 0.01* M *tetraphenylborate.*

the results of 10 sample solutions (5 ml), each with a sparteine concentration of 0.005 *M*. The mean titer was 5.02 ml, with a standard deviation of 0.05 ml. The relative error was ~2% when 5 ml of diphenhydramine solution was titrated according to the procedure.

Commercial products containing diphenhydramine (injection, 10 mg/ml; tablet, 90 mg/g; and ointment, 10 mg/g) were analyzed according to the proposed method and a spectrophotometric method (8). The results were 9.97, 91.0, and 9.90 mg of diphenhydramine/ml in the injection, tablet, and ointment, respectively, by the proposed method and 9.95, 90.7, and 9.87 mg/g, respectively, by the spectrophotometric method. Tablet samples were dissolved in dilute sulfuric acid. The solution was filtered with a glass filter. Ointment samples were dispersed with 15 ml of ether in a separator. The contents were extracted three times with 10-ml portions of 0.1 *N* H_2SO_4. The extracts were neutralized slightly with 0.1 *N* NaOH solution.

REFERENCES

(1) A. S. Weatherburn, *J. Am. Oil Chem. Soc.*, **28**, 233 (1950).

(2) L. K. Wang and P. J. Panzardi, *Anal. Chem.*, **47**, 1472 (1975).

(3) M. E. Turney and D. W. Cannell, *J. Am. Oil Chem. Soc.*, **42**, 544 (1965).

(4) J. T. Gross, *Analyst*, **90**, 315 (1965).

(5) Y. Kasai, Y. Ueda, W. Yano, and W. Kimura, *Nippon Kagaku Kaishi*, **1972**, 2394.

(6) H. Y. Mohammed and F. F. Cantwell, *Anal. Chem.*, **52**, 553 (1980).

(7) "The Japanese Pharmacopoeia," 9th ed., Hirokawa Publishing, Tokyo, Japan, 1976.

(8) M. Tsubouchi, *J. Pharm. Sci.*, **60**, 943 (1971).

(9) M. Tsubouchi, *Bull. Chem. Soc. Jpn.*, **43**, 3164 (1970).

Measurement of Pseudoephedrine Hydrochloride Dissolution Using Chloride-Ion Electrode

S. T. CHEN, R. C. THOMPSON, and R. I. POUST ˣ

Received October 24, 1980, from the *Pharmaceutical Research and Development Laboratories, Burroughs Wellcome Co., Greenville, NC 27834* Accepted for publication April 17, 1981.

Abstract □ Experiments were performed to determine the suitability of using a chloride-ion electrode for the measurement of pseudoephedrine hydrochloride dissolution from commercially available compressed tablets. Dissolution experiments were carried out in 500 ml of distilled water using the USP paddle method at 100 rpm. Both chloride ion and pseudoephedrine (UV spectrophotometry) were measured at six different sampling times. Percent dissolved *versus* time values were linearized on a log-normal probability basis. The slopes of individual lines obtained from the chloride and pseudoephedrine measurements were compared using a Student *t* test and did not differ significantly ($t = 0.415$, $df = 5$, $p > 0.05$). In addition to providing an efficient, inexpensive, and simple method for measuring pseudoephedrine hydrochloride dissolution rates, the chloride-ion electrode could be used in the measurement of dissolution rates for a wide variety of drugs available as hydrochloride salts.

Keyphrases □ Pseudoephedrine hydrochloride—measurement of dissolution using chloride-ion electrode □ Chloride-ion electrode—measurement of pseudoephedrine hydrochloride dissolution □ Dissolution—pseudoephedrine hydrochloride, measurement using chloride-ion electrode

There has been a significant increase in the use of dissolution rate testing for the development of new dosage forms as a quality assurance tool and as a predictor of bioavailability in instances where successful *in vivo–in vitro* correlations have been established. Recent activity has centered on the establishment of automated procedures and the optimization of existing methodology with emphasis on the USP rotating-basket and USP paddle methods (1). One interesting aspect has been the automation of analytical measurements, mainly through the use of flow-through cells in conjunction with various spectrophotometers (2–7). Another analytical procedure that lends itself to automation is the use of a specific ion electrode to measure directly the drug's counterion in the dissolution flask.

BACKGROUND

A survey of the recent literature revealed limited use of selective ion electrodes for measurement of tablet or capsule dissolution rates. Mason *et al.* (8) described the use of a sodium-ion selective electrode for the measurement of dissolution rates of specially prepared sodium salicylate tablets. They found agreement between sodium-ion concentrations measured by the selective ion electrode and atomic absorption spectrophotometry. Sodium-ion concentrations agreed with salicylate concentrations measured spectrophotometrically. The method was later extended (9) to measure dissolution rates of commercial tablets containing warfarin sodium, butabarbital sodium, and sodium bicarbonate. Data obtained by selective ion electrode and spectrophotometric measurements were in agreement when both methods were utilized.

Thomas (10) used a potassium-ion specific electrode to study the release rate of potassium from several brands of slow-release potassium chloride tablets. Other investigators (11), using similar methodology, also studied the dissolution release pattern of sustained-release potassium chloride tablets. This report presents the results of similar studies using anion measurements; *i.e.*, a chloride-ion electrode was used to measure the dissolution rate of commercial pseudoephedrine hydrochloride tablets.

EXPERIMENTAL

Materials—Pseudoephedrine hydrochloride tablets[1] (60 mg/tablet) and pseudoephedrine hydrochloride[2] were used as received. An ionic strength adjuster (5 *M* $NaNO_3$) was prepared by dissolving 42.5 g of sodium nitrate[3] in 100 ml of distilled water.

Chloride-Ion Measurements—Chloride ions were detected by a chloride-ion electrode[4] together with a double-junction electrode[5]. The electrodes were connected to an analyzer[6], which displayed direct millivolt readings of the measured potentials.

A calibration curve was constructed for pseudoephedrine hydrochloride. Standard solutions of 0.01–1.00 mg/ml were prepared. For each standard solution, 100 ml of solution and 2 ml of ionic strength adjuster were poured into a 150-ml glass beaker and thoroughly stirred. The millivolt reading was recorded once the reading stabilized. These values were then linearized by plotting them on a linear scale as a function of the logarithm of the chloride concentration.

UV Spectrophotometric Measurements—A Beer's law curve was constructed for pseudoephedrine hydrochloride in distilled water. The

[1] Sudafed tablets, batches 7L2089 and 7L2087, Burroughs Wellcome Co., Research Triangle Park, N.C.

[2] Batch P5705-4G, Burroughs Wellcome Co., Research Triangle Park, N.C.

[3] ACS reagent, Fisher Scientific, Pittsburgh, Pa.

[4] Model 94-17A, Orion Research, Cambridge, Mass.

[5] Model 90-02, Orion Research, Cambridge, Mass.

[6] Microprocessor Ionlyzer/901, Orion Research, Cambridge, Mass.

0022-3549/81/1100-1288$01.00/0