Applications of Aqueous Thermometric Titration to Pharmaceutical Analysis

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Aqueous thermometric titration with standard HCl, NaOH, or $AgNO_3$ as titrant was used successfully for the analyses of aminophylline (ethylenediamine and theophylline), chlorpheniramine maleate, chlorpromazine hydrochloride, hydrochlorothiazide, and niacinamide. Most of the determinations were based on titration volumes. In cases where the curvature of the enthalpogram in the vicinity of the end point was severe, the quantitative result was derived from the temperature change due to the reaction. Exploratory studies of the applicability of simple aqueous thermometric titration, without prior separation of ingredients, to the analysis of solid and liquid dosage forms of the above compounds were conducted. With all but 1 of the solid dosage forms investigated, no interferences from inert ingredients were observed. In some of the liquid dosage form systems studied, the vehicles interfered with the titrations, while in others they did not.

THE POSSIBLE application of aqueous thermometric titration to pharmaceutical analysis was reported in a recent communication from this laboratory (1). The present paper describes the procedures and results of the aqueous thermometric titration of selected pure medicinal substances and of some preliminary studies concerning the application of thermometric titration to dosage form analysis.

The large number of systems to which thermometric titration may be applied has been the subject of several reviews.[1] The investigation described in this report was concerned primarily with simple acid-base and precipitation reactions of nitrogenous compounds. It is well known[1] that a thermometric titration will often produce a well-defined end point in a system where free energy methods such as potentiometric titration fail. Thus, in the first phase of this study, compounds whose official or other assay procedures require *nonaqueous* titration were chosen for testing the applicability of *aqueous* thermometric titration.

The second phase of this study was concerned with the assay of dosage forms. At present, "inert" binder, filler, and sealer materials, required to manufacture most solid dosage forms, often necessitate the use of separation procedures prior to the assay of the active ingredients. The problem also exists with liquid dosage forms where "inert" substances in the vehicles often interfere with the use of visual indicators and electrodes. Unfortunately, most separation procedures are tedious as well as time consuming.

While the enthalpogram for a thermometric titration of a single reacting substrate undergoing a single-step reaction is characterized (ideally) by a single "break" at the end point, the enthalpogram for a titration of 2 substrates (or a single substrate undergoing a 2-step reaction) may or may not be characterized by 2 breaks. First, the free energies of the 2 reactions must be sufficiently different so that the reactions do not occur simultaneously. Second, the enthalpies of the 2 reactions must also be sufficiently different so that the point where one reaction ceases and the second commences is discernible. One hopes then that the enthalpogram of a thermometric titration of a dosage form will be characterized by a single end point corresponding to the reaction of the active ingredient (*i.e.*, the other substances present do not react) or, if more than one substance reacts, that the thermodynamic factors are favorable, so that the enthalpogram exhibits a series of end points, the volumes between the end points corresponding to individual reactions occurring during the titration. If either hope is fulfilled, the need for prior separation of the active ingredient is eliminated.

EXPERIMENTAL

Materials.—Powdered samples of aminophylline U.S.P. (anhydrous), chlorpheniramine maleate U.S.P.,[2] chlorpromazine hydrochloride U.S.P.,[3] hydrochlorothiazide U.S.P.,[4] and niacinamide U.S.P.

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[1] See for example: Zenchelsky, S. T., *Anal. Chem.*, **32**, 289R(1960); Jordan, J., and Ewing, G. J., "Handbook of Analytical Chemistry," Meites, L., ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1963, Sect. 8, p. 3ff.; Wendlandt, W. W., "Chemical Analysis," vol. 19, Elving, P. J., and Kolthoff, I. M., eds., Interscience Publishers, Inc., New York, N. Y., 1964, Chap. 8, p. 271ff.

[2] Supplied by Schering Corp., Bloomfield, N. J.
[3] Supplied by Smith Kline & French Laboratories, Philadelphia, Pa.
[4] Supplied by Ciba Pharmaceutical Co., Inc., Summit, N. J., and Merck Sharp & Dohme Research Laboratories, West Point, Pa.

were dried according to standard procedures but not purified further. Dosage forms were purchased on the open market. Standard HCl and NaOH solutions were purchased as certified volumetric solutions. Standard $AgNO_3$ solutions were prepared from reagent grade $AgNO_3$. Only recently boiled water was used throughout. The acetone used in the hydrochlorothiazide titrations was reagent grade.

Apparatus.—The titration cell assembly, which consisted basically of a small stoppered standard Dewar flask, a 2000-ohm bead-in-glass-probe thermistor, a right-angled titrant delivery tip, and a glass stirrer with 4 quadrantal blades, is shown in Fig. 1. The thermistor and delivery tip extended to ~3 mm. above the stirrer blades. The delivery tip was positioned so that the flow of titrant was counter to the flow of the solution in the flask. The stirrer was powered with a Synchro-Tork stirring motor (General Laboratory Supply Co., Paterson, N. J.). Titrant was delivered with a 0.6 ml./min., constant-flow Menisco-matic buret (American Instrument Co., Inc., Silver Spring, Md.).

The titration cell, stirring motor, and buret were enclosed in a constant-temperature air bath, maintained at 25.0 ± 0.1°. The air bath was a closed system in which air was recirculated. Cooling was achieved with copper cooling coils, in and around the air duct, through which cold water was circulated. A 200-w. (at 115 v.a.c.) heating coil in the air duct was set, with an auto-transformer, to maintain the air bath at approximately 24.5°. A 100-w. (at 115 v.a.c.) heating coil, also in the air duct, was controlled with a Micro-Set (mercury-column) thermoregulator (Precision Scientific Co., Chicago, Ill.) set at 25.0°. The voltage to the 100-w. heater was adjusted with a power rheostat to give minimum "overshoot." The exhaust fan and air entry portals were diagonally opposite each other and were equipped with deflector vents to cause turbulent flow of air in the bath. The temperature could be maintained at the desired level over extended periods. The bath was also equipped with a safety

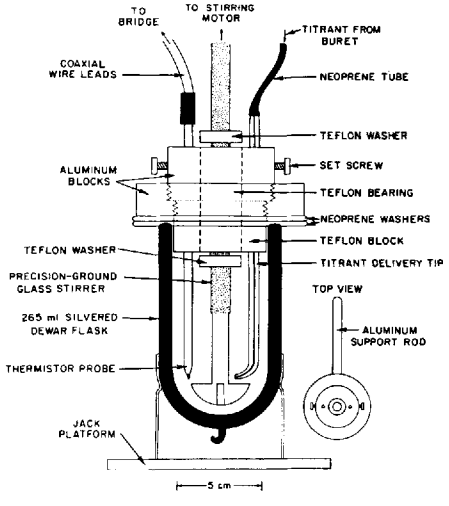

Fig. 1.—Titration cell assembly.

circuit breaker which automatically shut off the 200-w. heater in case the temperature of the bath exceeded 35°. Switches on the air bath permitted the operation of the stirrer and buret from the outside.

The thermistor was actually 1 arm of a simple bridge circuit, only slightly modified from the one described by Jordan and Alleman (2). The imbalance potential of the bridge was recorded with a 5-mv. Dynamaster recorder (The Bristol Co., Waterbury, Conn.). In cases where the magnitude of the heat change was low, the bridge signal was amplified by a factor of 5 with a solid-state variable recorder amplifier (Instruments and Communications, Inc., Wilton, Conn.).

Titration Procedure

Pure Substances.—One-liter volumetric solutions were prepared and allowed to reach thermal equilibrium, along with the standard titrants, for several hours in the air bath. The slow and low solubility of hydrochlorothiazide in water necessitated dissolving the compound in 20 ml. of acetone and slowly dispersing the acetone solution in water while stirring. The small amount of acetone (2%) appeared to have negligible effect on the titrations.

Exactly 100 ml. of solution was placed in the Dewar flask which was then positioned on the stationary titration cell head with a laboratory jack. The stirrer and bridge were turned on and the system allowed to attain thermal equilibrium. Equilibration generally required 5–10 min. and was indicated by a linear base line on the recorder. The buret was then turned on and the titration allowed to proceed until well after the end point(s) was reached.

Dosage Forms.—All dosage forms were titrated without prior separation procedures. A suitable quantity of the dosage form was dispersed, in the Dewar flask, in enough thermally equilibrated (25.0 ± 0.1°) water to make ~100 ml. In the case of hydrochlorothiazide tablets, a slurry of the powdered tablet in 5 ml. of acetone was prepared and then diluted to ~100 ml. with water. Titrations were then performed in the same manner as described for pure substances.

U.S.P. Assays

The pure substances were also analyzed by the procedures (usually nonaqueous titration) given in the U.S.P. (3).

Treatment of Data

An idealized enthalpogram for the thermometric titration of a single substrate undergoing a single reaction is shown in Fig. 2. The factors contributing to the various portions of the curve have been discussed. (See *Footnote 1.*) The section of the curve between the start of the titration and the end point is called the titration branch. The extrapolation method for obtaining the titration volume, ΔV, and the temperature rise *due to the reaction* (in arbitrary units), ΔT, is indicated on the curve.[5] For 1 titration system, chlorpromazine hydrochloride *versus* NaOH, ΔV's were also determined with

[5] Enthalpograms are usually recorded with a temperature rise reflected as an *increased* voltage. In the present investigation, this convention was used.

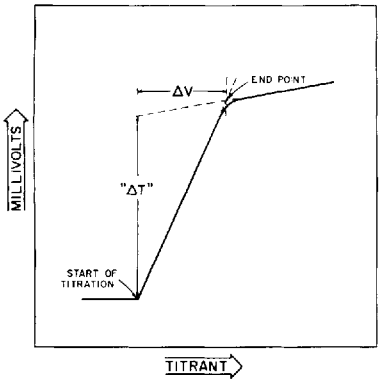

Fig. 2.—Idealized enthalpogram.

an automatic end point determination device. The procedure used has been described (4).

Pure Compounds.—Most of the analyses of the pure compounds were made by calculating the amount of substrate from the measured ΔV and the known concentration of the titrant. In 2 cases, theophylline in aminophylline *versus* NaOH and hydrochlorothiazide *versus* NaOH, the curvature in the vicinity of the end point was too severe to allow reliable extrapolation for measurement of ΔV. In these cases, quantitative determinations were made from the ΔT measurements. ΔT values from titrations of various concentrations (always 100 ml.) of the compound under consideration and of hydrochloric acid *versus* 2.0 *M* NaOH were measured. The slopes, B, of plots of ΔT *versus* molarity of substrate, M, were obtained by least-square fitting to the equation $\Delta T = BM$. The relative slope, B_H/B_C, where the subscripts H and C refer to hydrochloric acid and the compound under consideration, respectively, was evaluated. Since the B's were obtained from titrations of dilute solutions run under essentially the same conditions,[6] the relative slope should be identical to the ratio of the molar enthalpies of the reactions. Once B_H/B_C has been determined, a solution of unknown concentration of the compound under consideration can be analyzed by performing a titration of the unknown and following it with a titration of known concentration of hydrochloric acid run under identical conditions. It is easy to show that

$$M_C = M_H\,(\Delta T_C/\Delta T_H)\,(B_H/B_C) \quad \text{(Eq. 1)}$$

where the relative temperature rise, $\Delta T_C/\Delta T_H$, is determined from the enthalpograms, and M_H is the known concentration of hydrochloric acid.

Dosage Forms.—The amounts of active ingredients in the dosage forms were calculated by an absolute method and/or a standard addition method. In the absolute method, the amount of active ingredient was calculated from ΔV and the concentration of the titrant. In the standard addition method, ΔV for the titration of the dosage form was compared, in the obvious manner, to ΔV for a similar titration of the same amount of dosage form plus an accurately weighed portion of active ingredient. In the standard addition method, the purities of the added portions of active ingredients were taken to be exact.

Dosage form analyses were not carried out with ΔT measurements, because the results of the ΔT analyses of the pure compounds indicated that there are uncertainties involved in measuring relative heats without a calibration heater. In addition, there is no assurance in a dosage form titration that all reactions have ceased after the last observed end point. The later condition is necessary for the application of the back-extrapolation method for obtaining ΔT shown in Fig. 2.

RESULTS AND DISCUSSION

Pure Compounds.—Figures 3 and 4 show typical enthalpograms for the 8 titration systems investigated in detail. The 3 systems represented in Fig. 4 involve precipitate formation.

An enthalpogram for the aqueous thermometric titration of chlorpheniramine maleate *versus* HCl, corresponding to the official nonaqueous titration (3), is shown in Fig. 3, curve A. Although the end point is fairly rounded, extrapolation is not difficult. The 1 end point observed results from the protonation of the pyridine nitrogen of the chlorpheniramine; in the official nonaqueous titration, the

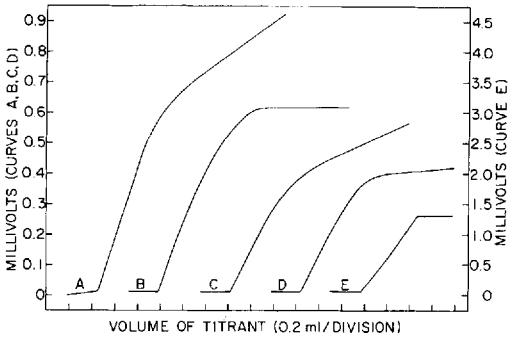

Fig. 3.—Typical enthalpograms. Key: A, 0.01 *M* chlorpheniramine maleate *vs.* 2 *M* HCl; B, 0.008 *M* aminophylline *vs.* 2 *M* NaOH; C, 0.01 *M* niacinamide *vs.* 2 *M* HCl; D, 0.0027 *M* hydrochlorothiazide *vs.* 1 *M* NaOH; E, 0.005 *M* aminophylline *vs.* 2 *M* HCl.

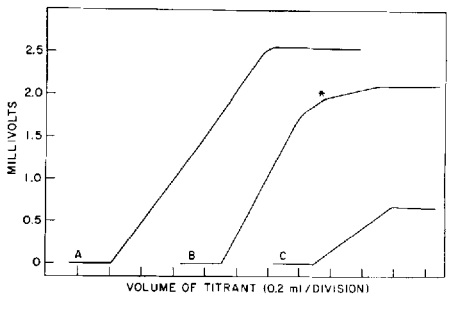

Fig. 4.—Typical enthalpograms. Key: A, 0.005 *M* aminophylline *vs.* 1 *M* $AgNO_3$; B, 0.01 *M* chlorpheniramine maleate *vs.* 2 *M* NaOH; C, 0.01 *M* chlorpromazine hydrochloride *vs.* 2 *M* NaOH.

[6] The only condition which was purposely varied was the bridge sensitivity which was set a factor of 5 more sensitive for the theophylline in aminophylline and hydrochlorothiazide titrations than for the hydrochloric acid titrations. A correction was applied to account for the nonlinearity of the sensitivity scale.

TABLE I.—THERMOMETRIC TITRATIONS OF PURE COMPOUNDS

Compd.	Thermometric Titration Analysis: Concn., 100 ml.	Titrant	Titrations, No.	% Found	U.S.P. Analysis: Titrations, No.	% Found
Chlorpheniramine maleate	0.01 *M*	2.0 *M* HCl	6[a]	99.36 ± 0.30	5	99.64 ± 0.18
	0.01 *M*	2.0 *M* NaOH	5	97.81 ± 0.57[b]		
				99.80 ± 0.23[c]		
Niacinamide	0.01 *M*	2.0 *M* HCl	6	98.62 ± 0.37	5	99.03 ± 0.14
Chlorpromazine hydrochloride	0.01 *M*	2.0 *M* NaOH	6	98.58 ± 0.31	5	99.89 ± 0.20
	0.01 *M*	2.0 *M* NaOH	5[a]	100.15 ± 0.70[d]		
	0.01 *M*	1.0 *M* NaOH	5	99.69 ± 0.33[e]		
Hydrochlorothiazide	0.0027 *M*	1.0 *M* NaOH	5	99.67 ± 0.20[c]	5	100.76 ± 0.29
Aminophylline	0.005 *M*					
Ethylenediamine[f]		2.0 *M* HCl	5	98.90 ± 0.25	5	100.58 ± 0.11
Theophylline[f]		1.0 *M* $AgNO_3$	5	99.93 ± 0.80	5	99.25 ± 0.40

[a] One titration eliminated by Chauvenet's criterion. [b] Based on first end point. [c] Based on second end point. [d] Automatic end point determination apparatus; automatic buret; lag time taken as 0.050 min. (See *Reference 4.*) [e] Automatic end point determination apparatus; manual buret; lag time taken as 0.050 min. (See *Reference 4.*) [f] Percentages based on theoretical content in anhydrous aminophylline (2 moles theophylline:1 mole ethylenediamine).

bimaleate ion is titrated along with the chlorpheniramine.

An enthalpogram for the titration of chlorpheniramine maleate *versus* NaOH is shown in Fig. 4, curve B. Two true end points appear, the first corresponding to the neutralization of the bimaleate ion and the second to the neutralization of the protonated amine moiety of the chlorpheniramine ion. The false "end point" indicated by the asterisk has no stoichiometric relationship and is due to the delayed precipitation of the chlorpheniramine base.

Aqueous titration of niacinamide *versus* HCl, corresponding to the official nonaqueous titration (3), resulted in an enthalpogram (Fig. 3, curve C) with a rounded end point. Extrapolation to the end point is, however, not difficult.

Aqueous thermometric titration of chlorpromazine hydrochloride *versus* HCl, corresponding to the official nonaqueous titration (3), failed to exhibit an end point since the phenothiazine nitrogen is not sufficiently basic to become protonated in aqueous solution. Neutralization of the protonated amine nitrogen with base, however, resulted in an enthalpogram with an extremely sharp end point (Fig. 4, curve C).

The official assay procedure for hydrochlorothiazide requires titration in *n*-butylamine with sodium methoxide in benzene as titrant (3). Aqueous thermometric titration with NaOH involves 2 equivalents of base, since there are 2 sulfonamide groups in hydrochlorothiazide. Midway in the titration branch of the enthalpogram (Fig. 3, curve D), there is a slight change in slope, corresponding to the first end point. The change is not sharp enough to permit precise determination of this end point. The second end point is quite rounded so that extrapolation to either end point is subject to large errors. Thus, for the hydrochlorothiazide determinations, ΔT rather than ΔV analyses were carried out.

Aminophylline is a mixture of 2 moles of theophylline to 1 mole of ethylenediamine. The official assay (3) involves separate procedures for the 2 components. Ethylenediamine is analyzed by aqueous titration with HCl, and theophylline is determined by precipitation with $AgNO_3$ and titration of the excess silver ion with NH_4SCN. Medwick and Schiesswohl (5) have developed a nonaqueous potentiometric analysis for both components which uses acetous perchloric acid as titrant and an acetic acid–acetic anhydride solvent.

Three aqueous thermometric titration systems for aminophylline analysis were investigated. Titration with HCl resulted in an enthalpogram (Fig. 3, curve E) indicating reaction with ethylenediamine only. The change in slope midway in the titration branch is the end point for the protonation of 1 of the amine groups, but this point is difficult to determine precisely. The second end point, however, is extremely sharp.

Aqueous thermometric titration of aminophylline *versus* base is feasible due to the acidic nature of theophylline. The resulting enthalpogram (Fig. 3, curve B) is extremely curved throughout the titration branch so that only ΔT analysis can be performed precisely.

Direct titration of the theophylline in aminophylline *versus* $AgNO_3$ resulted in an enthalpogram (Fig. 4, curve A) with a well-defined end point. There is a very slight change of slope approximately midway in the titration branch which is probably due to initial supersaturation followed by precipitation (similar to the false "end point" in the chlorpheniramine maleate *versus* NaOH system). Keily and Hume have observed similar changes in slope in nonaqueous thermometric titrations involving precipitations (6). The best stoichiometric end point for the aminophylline *versus* $AgNO_3$ titrations resulted from taking an average of the end points obtained by extrapolating segments of the titration branch on both sides of the slight change in slope. However, because the change in slope is so slight, little error (~1%) is introduced if only the final straight portion of the titration branch is used to locate the end point.

The quantitative results of the aqueous thermometric titrations (ΔV analyses) of the 5 compounds under investigation are given in Table I.[7] Included in the table are the results of the official assay procedures. The values obtained from the thermometric analyses are generally slightly lower but still in good agreement with the results of the U.S.P. analyses. The poor results obtained from the first end point in the chlorpheniramine maleate *versus* NaOH thermometric titrations can be ascribed to the difficulty in extrapolating the small segment

[7] All appended error limits in this paper are average deviations unless specified.

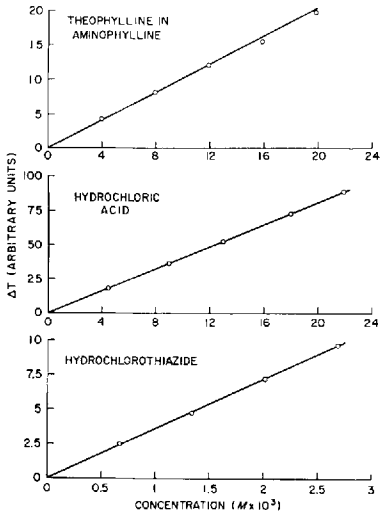

Fig. 5.—Plots of temperature rise *vs.* concentration of substrate for titrations with 2 *M* NaOH. The solid lines represent the least-square fits to $\Delta T = BM$, where the points were weighted as (average deviation)$^{-2}$. The average deviations of the points generally fall within the circles.

between the first end point and the false "end point." The reasonableness of the results of the hydrochlorothiazide *versus* NaOH thermometric titrations must be taken to be fortuitous because of the previously discussed uncertainty in the location of the end point.

Plots of ΔT *versus* concentration for aminophylline *versus* NaOH, HCl *versus* NaOH, and hydrochlorothiazide *versus* NaOH titrations are shown in Fig. 5. The lines drawn through the points were determined by least-square fitting to $\Delta T = BM$ with each point weighted as (average deviation)$^{-2}$. The relative slopes were found to be $B_{HCl}/B_{theophylline} = 4.004 \pm 0.048$ and $B_{HCl}/B_{hydrochlorothiazide} = 1.132 \pm 0.008$, where the appended error limits are estimated standard deviations. These results indicate that the use of Eq. 1 to determine theophylline or hydrochlorothiazide concentrations from thermometric titrations run concurrently with HCl *versus* NaOH titrations should be accurate to ~1%.

Approximately 6 months after the determinations of $B_{HCl}/B_{theophylline}$ and $B_{HCl}/B_{hydrochlorothiazide}$ were made, the ΔT procedure was tested by performing titrations with an entirely new apparatus (bridge, recorder, titration cell, etc.) and calculating the concentrations with Eq. 1. Such analyses yielded for theophylline (0.005 *M* aminophylline; 3 titrations) 94.97 ± 0.36% recovery, as compared to the U.S.P. analysis of 98.60 ± 0.17%. Similar titrations for hydrochlorothiazide (0.0017 *M;* 3 titrations) yielded 95.31 ± 0.44% recovery, as compared to the U.S.P. analysis of 100.76 ± 0.29%. (The U.S.P. analysis result is probably high, due to the broad color change of the indicator.) The poor agreements cast some doubt on the absolute values of the relative slopes.

The proposed method of using an external standard to determine heat changes suffers several disadvantages, especially when it is attempted to translate results from one apparatus to another. Bridge sensitivity nonlinearities are difficult to determine accurately, and they vary, depending on the bridge design, with the age and voltage of the batteries. In addition, it has been found that bridge response changes when switches are opened and closed again. Furthermore, factors such as differences in heat capacities due to differences in composition and volume (it is difficult to dry the titration cell completely between titrations) and distortions of the enthalpograms due to the dilution effect of the titrant can introduce errors. A more reliable method is one in which a calibrated heater immersed in the solution is used as an *internal* heat standard. This method would eliminate most of the possible sources of error discussed above, since the composition and volume of the solution could not change and the bridge need not be turned off or adjusted between calibration and titration. The effects of dilution by the titrant would still be a problem, but these effects should be small if the titrant is always much more concentrated than the solution to be titrated.

Solid Dosage Forms.—Thermometric titration of niacinamide tablets *versus* HCl, chlorpromazine hydrochloride sustained - release capsules *versus* NaOH, hydrochlorothiazide tablets *versus* NaOH, and aminophylline tablets *versus* NaOH resulted in enthalpograms which were almost identical to the enthalpograms obtained from the corresponding titrations of the pure substances. Thus, the inert materials in the dosage forms investigated do not undergo simple acid–base reactions and therefore do not interfere with the titrations of the active ingredients. Thermometric titration of aminophylline tablets *versus* $AgNO_3$ produced an enthalpogram which differed from that of the corresponding titration of pure aminophylline only in that the slight change in slope in the titration branch did not appear. The insoluble materials in the tablets probably act as "seeds" to prevent supersaturation. Enthalpograms for the titration of aminophylline tablets *versus* HCl are shown in Fig. 6. The curves are similar to that of the corresponding titration of pure aminophylline, and the end point is very sharp. The only difference is the fact that in the titration of tablets, the first end point does not appear (curve B) or appears as a slight change in slope (curve A) of opposite direction to that observed in the titration of pure aminophylline. The reason for this difference need not be of concern in strict analytical applications in which the second end point is used.

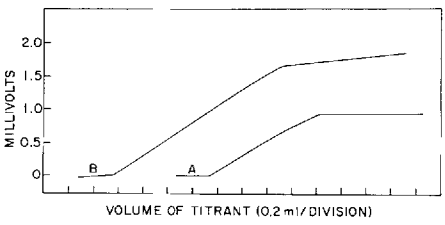

Fig. 6.—Enthalpograms for titrations of aminophylline tablets (100 mg.). Key: A, 2 tablets *vs.* 1 *M* HCl; B, 2 tablets plus 100 mg. aminophylline *vs.* 1 *M* HCl.

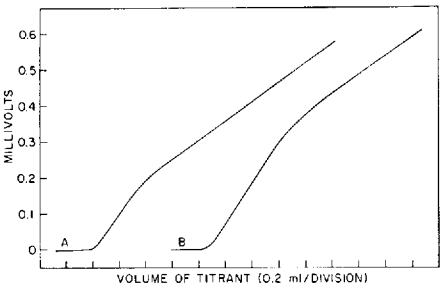

Fig. 7.—Enthalpograms for titrations of chlorpheniramine maleate tablets (4 mg.). Key: A, 30 tablets *vs.* 1 *M* HCl; B, 30 tablets plus 100 mg. chlorpheniramine maleate *vs.* 1 *M* HCl.

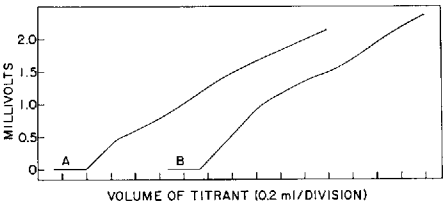

Fig. 8.—Enthalpograms for titrations of chlorpheniramine maleate tablets (4 mg.). Key: A, 30 tablets *vs.* 1 *M* NaOH; B, 30 tablets plus 100 mg. chlorpheniramine maleate *vs.* 1 *M* NaOH.

Enthalpograms for the titration of chlorpheniramine maleate tablets *versus* HCl and of a corresponding standard addition titration are shown in Fig. 7. The roundness at the start of the titration must be due to the reaction of some ingredient in the tablet with the acid. This reaction, however, is not "clean cut," as evidenced by the fact that the region of roundness expanded when additional chlorpheniramine maleate was added prior to the titration. The authors have found no reliable method of determining the initiation of the chlorpheniramine reaction itself. Estimations of ΔV did not yield quantitative results for either the absolute method or the standard addition method.

Titration of chlorpheniramine maleate tablets *versus* NaOH produced an enthalpogram quite different from the curve obtained in the corresponding titration of pure chlorpheniramine maleate. Figure 8 shows typical curves for the titrations of tablets alone and of tablets plus added chlorpheniramine maleate. The high final slopes indicate that some reaction was occurring even after ~2 ml. of 1.0 *M* NaOH had been added (~3 times the amount needed to completely neutralize 120 mg. of chlorpheniramine maleate). None of the breaks is easy to pick out as an end point. Attempts to obtain quantitative results from the first break with both the absolute and standard addition methods were unsuccessful.

The results of the thermometric titrations of the solid dosage forms studied are given in Table II. Although the curvature in the vicinity of the aminophylline *versus* NaOH and hydrochlorothiazide *versus* NaOH end points did not allow accurate extrapolation for absolute analyses, the standard addition method was feasible since errors tend to cancel if the curves are always extrapolated in the same manner. For the hydrochlorothiazide standard additional analyses, "end points" were obtained by extrapolating the initial portions of the titration branches and the final segments of the curves to their intersections; *i.e.*, the fact that there are actually 2 end points was ignored.

Since this phase of the investigation was exploratory in nature, detailed evaluations of precision and accuracy were not attempted. The over-all results are quite favorable and indicate that thermometric titrations of many solid dosage forms without prior separation of the active ingredients are feasible. The rather large error limits encountered with some systems may be due to the fact that individual dosage forms were titrated. The differences between the results of the absolute and standard addition methods may also be due, at least in part, to the use of individual dosage forms as well as to the assumption of 100% purity of the added portion of active ingredient. The latter may be particularly important in the titrations involving aminophylline, which is not always homogeneous.

The most interesting results of these exploratory studies is the fact that, with the exception of the titrations of chlorpheniramine maleate tablets, even the simple acid–base titrations of the solid dosage forms indicated negligible interference due to inert ingredients.

Liquid Dosage Forms.—The general objective of this phase of the investigation was to determine if, without detailed knowledge of the composition of a

TABLE II.—THERMOMETRIC TITRATIONS OF SOLID DOSAGE FORMS

Dosage Form	Dosage Units Titrated, No.	Determinations, No.	Titrant	Amt. Added[a] in S.A. Method, mg.	% Labeled Amt. Absolute	% Labeled Amt. Std. Addition
Niacinamide tablets, 100 mg.	1	3	1.0 *M* HCl	100	107.33 ± 0.31	111.25 ± 0.69
Chlorpromazine hydrochloride sustained-release capsules, 200 mg.	1	2	1.0 *M* NaOH	200	103.51 ± 0.93	105.22 ± 1.45
Hydrochlorothiazide tablets, 50 mg.	1	2	1.0 *M* NaOH	50	[b]	98.76 ± 0.96
Aminophylline tablets, 100 mg.						
Ethylenediamine[c]	1, 2	2	1.0 *M* HCl	100[d]	92.48 ± 0.22	92.29 ± 3.16
Theophylline[c]	1	4	1.0 *M* NaOH	100[d]	[b]	109.92 ± 6.78
Theophylline[c]	1	2	0.5 *M* $AgNO_3$	100[d]	96.56 ± 0.55	101.63 ± 0.48

[a] Purity of added active ingredient taken to be exact. [b] Extreme curvature in vicinity of end point(s) precluded direct analysis. [c] Percentages based on theoretical content in anhydrous aminophylline (2 moles theophylline:1 mole ethylenediamine). [d] Aminophylline.

TABLE III.—THERMOMETRIC TITRATIONS OF LIQUID DOSAGE FORMS

Dosage Form	Titrated, ml.	Determinations, No.	Titrant	Aminophylline Added[a] in S.A. Method, mg.	% Labeled Amt. Absolute	% Labeled Amt. Std. Addition
Aminophylline elixir[b]						
Ethylenediamine[c]	25	2	1.0 *M* HCl	100	94.00 ± 0.07	102.63 ± 0.32
Theophylline[c]	10	1	1.0 *M* NaOH	100	[d]	97.16
Theophylline[c]	10	2	0.5 *M* $AgNO_3$	100	103.88 ± 0.85	102.77 ± 0.40
Aminophylline compound syrup[e]						
Theophylline[c]	10	2	0.5 *M* $AgNO_3$	50	81.33 ± 0.99	82.71 ± 0.36
KI				...	89.87 ± 0.76[f]	...
Compound elixir[g]						
KI	10, 15	2	0.5 *M* $AgNO_3$	...	98.62 ± 0.15	...

[a] Purity of added aminophylline taken to be exact. [b] 12.5 mg. aminophylline and 3.125 mg. diphenhydramine/ml. [c] Percentages based on theoretical content in anhydrous aminophylline (2 moles theophylline:1 mole ethylenediamine). [d] Extreme curvature in vicinity of end point precluded direct analysis. [e] 10 mg. aminophylline, 2.5 mg. diphenhydramine, 7.5 mg. KI, and 2 mg. $CHCl_3$/ml. [f] Average of 4 titrations: 2 of 10 ml. syrup and 2 of 10 ml. syrup plus 50 mg. added aminophylline. [g] 10 mg. KI, 3 mg. theophylline, 0.8 mg. ephedrine sulfate, 0.4 mg. phenobarbital, and 0.167 mg. isoproterenol hydrochloride/ml.

liquid dosage form, one could correlate end points of enthalpograms with the reactions of individual active ingredients. Only a few systems were examined and only sparse quantitative data were obtained.

Titrations of a chlorpheniramine maleate syrup (0.5 mg./ml.) *versus* NaOH and *versus* HCl produced enthalpograms exhibiting single end points which did not correlate with the chlorpheniramine maleate content. When additional chlorpheniramine maleate was added to the syrup prior to the titrations, the titration volumes increased, but no correlation with the original chlorpheniramine maleate content could be made by standard addition analysis. The high final slopes of these enthalpograms indicated that reactions were still occurring even after several times the stoichiometric amounts of titrant had been added.

Results similar to those obtained with the chlorpheniramine maleate syrup titrations were obtained with titrations of a syrup containing 10 mg. aminophylline, 2.5 mg. diphenhydramine, 7.5 mg. KI, and 2 mg. $CHCl_3$ per ml. (this syrup will be referred to subsequently as "aminophylline compound syrup") *versus* NaOH and titrations of a chlorpromazine hydrochloride syrup (2 mg./ml.) *versus* NaOH. In the latter titration system, the volume to the end point did not increase upon the addition of excess chlorpromazine hydrochloride. Titration of aminophylline compound syrup *versus* HCl produced an enthalpogram, shown in Fig. 9, curve A, which does not exhibit any real evidence of reaction.

Thus, in all of the above liquid dosage form titration systems, there is interference from other (inert and/or active) ingredients. In the absence of detailed information as to the compositions of the liquids, any discussion of the natures of the interferences would be highly speculative.

On the other hand, titrations of an elixir containing 12.5 mg. aminophylline and 3.125 mg. diphenhydramine per ml. (subsequently referred to as "aminophylline elixir") *versus* HCl, NaOH, or $AgNO_3$ produced enthalpograms almost identical to those obtained in the corresponding titrations of pure aminophylline. (The end points in the HCl titrations were slightly more rounded than in pure aminophylline *versus* HCl titrations.) Quantitative results obtained from the titrations of aminophylline elixir are given in Table III.

Figure 9, curve B, represents the titration of aminophylline compound syrup (content given previously) *versus* $AgNO_3$. The first end point is fairly sharp while the second is broadly rounded. Titrations with added active ingredients indicated that the first end point corresponds to iodide precipitation and the second end point to theophylline precipitation. Quantitative results from a few titrations are given in Table III. The reason for the low per cent recoveries obtained may be the fact that a sample of syrup from a very old, previously opened bottle whose history was unknown was used for the analyses.

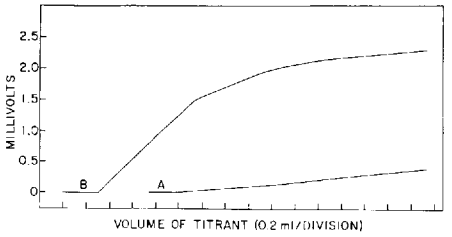

Fig. 9.—Enthalpograms for titrations of aminophylline compound syrup (10 mg. aminophylline, 2.5 mg. diphenhydramine, 7.5 mg. KI, and 2 mg. $CHCl_3$/ml.). Key: A, 25 ml. syrup *vs.* 1 *M* HCl; B, 10 ml. syrup *vs.* 0.5 *M* $AgNO_3$.

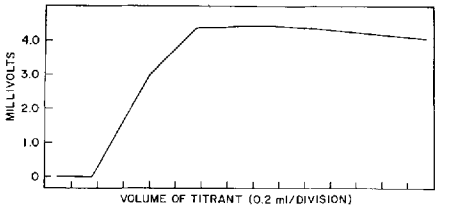

Fig. 10.—Enthalpogram for titration of 15 ml· compound elixir (10 mg. KI, 3 mg. theophylline, 0.8 mg. ephedrine sulfate, 0.4 mg. phenobarbital, and 0.167 mg. isoproterenol hydrochloride/ml.) *vs.* 2 *M* $AgNO_3$.

The last liquid dosage form studied was an elixir containing 10 mg. KI, 3 mg. theophylline, 0.8 mg. ephedrine sulfate, 0.4 mg. phenobarbital, and 0.167 mg. isoproterenol hydrochloride per ml. (This elixir will be referred to subsequently as "compound elixir".) Since several, if not all, of the active ingredients may precipitate silver ion, a complex enthalpogram for titration with $AgNO_3$ would be expected. The actual enthalpogram for such a titration is shown in Fig. 10. Titrations with added KI indicated that the first end point corresponds to iodide precipitation. The volume between the first and second end point is quantitatively in excess of the amount necessary for reaction with all of the other active ingredients combined. When KCl was added to the elixir prior to the titration, this volume increased without introducing any new breaks in the curve. This behavior indicates that the reaction that occurred between the first and second end points was precipitation of excess chloride ion present in the elixir. There is some indication from the enthalpogram that reactions occurred after the second end point, but the breaks are not sharp enough to pick out additional end points. The shape of the curve beyond the second end point was somewhat altered when titrations with added aminophylline were performed, but again no additional end points could be distinguished. Table III includes the results of KI determinations from the first end point in the compound elixir *versus* $AgNO_3$ titrations.

It appears from the rather sparse data presented in this section that simple thermometric titrations of liquid dosage forms are subject to far more interferences than are titrations of solid dosage forms. Some liquid dosage forms may be titrated successfully with nonspecific titrants without prior separation of ingredients. The applicability of such titrations to particular dosage forms must be determined on an individual basis. A more elegant approach, although one requiring knowledge of the composition of the vehicle, would be the use of more selective titrants.

SUMMARY

Aqueous thermometric titrations of chlorpheniramine maleate *versus* HCl or NaOH, niacinamide *versus* HCl, chlorpromazine hydrochloride *versus* NaOH, hydrochlorothiazide *versus* NaOH, ethylenediamine in aminophylline *versus* HCl, and theophylline in aminophylline *versus* NaOH or $AgNO_3$ yielded results with precision and accuracy sufficiently good to propose the methods as alternatives to the official assay procedures (generally nonaqueous titrations). In cases where the end points are extremely rounded (theophylline in aminophylline *versus* NaOH and hydrochlorothiazide *versus* NaOH), the usual volume determinations are not reliable but recourse to temperature-rise determinations is possible. The temperature-rise method used in this investigation employed titrations of HCl *versus* NaOH as heat references. Caution must be exercised if the relative heats determined on one apparatus are used for analyses performed with another apparatus. The use of a calibrated heater is suggested as a more reliable method.

Exploratory studies of aqueous thermometric titrations of solid dosage forms of the compounds listed above were conducted. These titrations employed the same titrants used for the analyses of the corresponding pure compounds. In all except the titrations of chlorpheniramine maleate tablets, negligible interference due to inert ingredients was observed. It appears that successful thermometric titrations of many solid dosage forms can be performed, either by direct volume analysis or by standard addition analysis, without prior separation of active ingredients.

Aqueous thermometric titrations of a few liquid dosage forms were attempted. While in some of the systems studied successful titrations without prior separation of ingredients could not be performed, in other systems no interference from the vehicle was observed. In some titrations of liquid dosage forms with $AgNO_3$, several end points were observed, some of which could be correlated quantitatively with specific ingredients in the mixtures.

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