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Nonaqueous Titrimetric Analysis of Aminophylline

By THOMAS MEDWICK and FREDERICK SCHIESSWOHL

A nonaqueous potentiometric titration is described by which ethylenediamine and theophylline, the components of aminophylline, are titrated differentially as a mixture of bases. Acetic acid is used as the solvent for the ethylenediamine neutralization after which acetic anhydride is added to the system to allow titration of the theophylline, a very weak base. This method was applied to the analysis of aminophylline powder, tablets, ampuls, and suppositories. When compared with the U.S.P. XVI analyses, the nonaqueous approach is simpler since no elaborate sample treatment is needed. The precision of the one titration, nonaqueous procedure is about the same as the U.S.P. XVI analyses which require two titrations to obtain the same data. An alternative approach is suggested for cases, for example, some tablets, where the nonaqueous method is not successful.

VARIOUS APPROACHES to the analysis of aminophylline, a mixture of theophylline and ethylenediamine, have been reported. In the majority, these procedures measure the theophylline which is present and (by appropriate calculation) express the result as aminophylline. Connors (1) discussed the argentometric, ultraviolet spectrophotometric, and other methods which have been used. In the U.S.P. XVI (2–5) the theophylline content of aminophylline in its various forms is determined by an argentometric titration procedure involving several preliminary steps. In aminophylline powder and ampuls only (2, 3) in addition to the theophylline analysis, an ethylenediamine assay is specified.

The xanthines, theophylline, theobromine, and caffeine possess analytically useful acid-base properties. All three xanthines have been found to be very weakly basic, pKb's (aqueous) > 13 (6), and, in addition, theophylline and theobromine are weakly acidic, pKa (aqueous) = 8.6, pKa (aqueous) = 10, respectively. Acetic anhydride has been used as a solvent component in the titration of these compounds as bases. The titrant used exclusively was acetous perchloric acid. Theophylline has been titrated in a 4:1 nitromethane-acetic anhydride solvent (7) and theobromine, dihydroxypropyltheophylline, and caffeine were titrated in mixed solvents containing acetic anhydride and nitromethane, benzene, toluene, or dioxane (8, 9). Similarly, Anastasi, Gallo, and Novacic (10) reported the satisfactory titration of caffeine in an acetic anhydride–acetic acid solvent. Recent work by Ellert, Jasinski, and Pawelczak (11) showed that, although theophylline did not titrate as a base in propionic acid, a propionic acid–propionic anhydride solvent permitted satisfactory titration as a base. Employing the acidic behavior of theophylline, McEniry (12) was able to determine the theophylline content of aminophylline by titrating a sample in dimethylformamide with sodium methoxide titrant.

Although nonaqueous titrimetric methods have been applied to theophylline determination, no work appears to have been done in regard to measuring both the theophylline and ethylenediamine by this means. Since ethylenediamine

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is a distinctly basic compound, pKb (aqueous) = 4.07 (13), it should be readily titratable. This communication deals with the selection and analytical use of a nonaqueous solvent system in which ethylenediamine and theophylline, the components of aminophylline, can be determined differentially as bases by a single titration.

EXPERIMENTAL

Pharmaceuticals, Chemicals, and Reagents Used

Aminophylline powder U.S.P., Merck and Co., Inc., Rahway, N. J.; aminophylline tablets, 100 and 200 mg., containing at least 80% anhydrous theophylline; aminophylline injection, 500 mg. in 2 ml., containing at least 80% anhydrous theophylline and benzyl alcohol, 2% v/v; aminophylline suppositories, 0.5 Gm., (cocoa-butter base with paraffin) were employed.

All chemicals were reagent grade. Acetous perchloric acid, *ca.* 0.1 *N*, was prepared according to Fritz (14) and standardized against triphenylguanidine with α-naphtholbenzein indicator. The indicator was a 0.2% w/v solution in glacial acetic acid.

Selection of a Solvent System

The properties of ethylenediamine and theophylline complicate the selection of a suitable titration medium. Although acetic acid (a valuable solvent for the titration of bases) would permit satisfactory determination of the stronger base, ethylenediamine, theophylline is too weakly basic to be determined in this solvent. Theophylline has been successfully determined in an acetic anhydride–containing solvent (7). However, if the anhydride were used here, reaction with ethylenediamine in the sample would destroy the desired basic properties of the amine unless the amine was already titrated or the titration was carried out at a low temperature (15). As a result of these considerations the following approach was developed.

After the aminophylline sample is dissolved in glacial acetic acid, it is potentiometrically titrated with acetous perchloric acid in the presence of an indicator which shows the completion of the ethylenediamine neutralization without the necessity of a preliminary potential (mv.) *versus* titrant added (ml.) plot. A small additional volume of titrant is added, followed by a volume of acetic anhydride. The titration is continued in this new medium until complete. This basic procedure is varied as needed in cases of specific dosage form analysis.

Procedures

The potentiometric titrations were conducted using a Beckman model N pH meter equipped with a glass electrode (Beckman No. 41263) and a sleeve-type calomel electrode (Beckman No. 40463) in which the aqueous saturated potassium chloride solution was replaced with 0.2 *M* acetous lithium chloride. A 10-ml. teflon stopcock Koch microburet was employed.

Aminophylline Powder.—About 0.135 Gm. of aminophylline is accurately weighed and dissolved in 30 ml. of glacial acetic acid in a beaker. After six drops of α-naphtholbenzein indicator solution are added, the solution is potentiometrically titrated with *ca.* 0.1 *N* acetous perchloric acid using the electrode system previously described. When the solution exhibits a green tinge, the volume is recorded and a total of 2 ml. of the titrant is added in two increments. Ten milliliters of acetic anhydride is introduced into the solution; the titration is continued until complete. A plot of the data, mv. observed *versus* ml. of titrant, is constructed and the end points graphically determined (16).

Aminophylline Tablets.—A sample of 20 tablets is accurately weighed, ground to a fine powder, and a sample of the powder equivalent to *ca.* 0.135 Gm. of aminophylline is then accurately weighed into a beaker. Thirty milliliters of glacial acetic acid are added and the analysis is carried out as described above under *Aminophylline Powder* by following the procedure from "After six drops of α-naphtholbenzein . . ."

Aminophylline Injection.—A volume of the injection equivalent to 500 mg. of aminophylline is accurately measured and transferred into a 100-ml. volumetric flask. Acetic acid is used to bring the solution to the mark. A sample of exactly

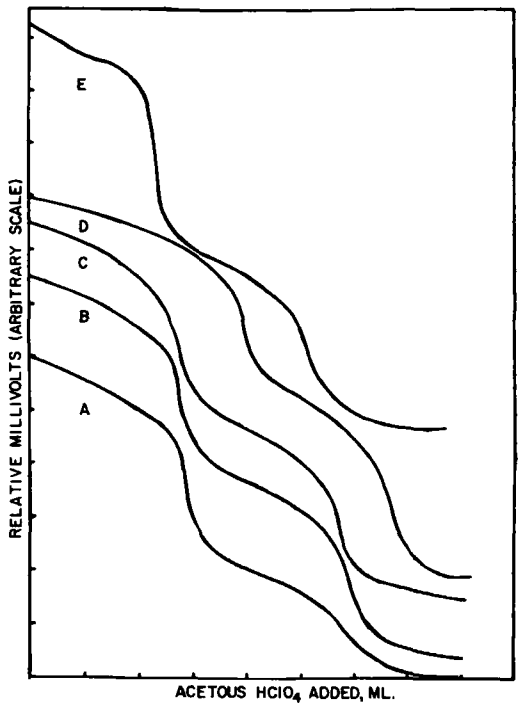

Fig. 1.—Each ordinate division is equivalent to 100 mv. Each abscissa division is equivalent to 2 ml. Curve A–Titration of aminophylline powder in glacial acetic acid. Curve B–Titration of aminophylline powder in glacial acetic acid with the addition of acetic anhydride after the first inflection point. Curve C–Titration of a sample of 200-mg. aminophylline tablets in glacial acetic acid with the addition of acetic anhydride after the first inflection point. Curve D–Titration of a sample of aminophylline injection 500 mg./2 ml. in glacial acetic acid with the addition of acetic anhydride after the first inflection point. Curve E–Titration of a sample of 0.5-Gm. aminophylline suppositories in chloroform-acetic acid with the addition of acetic anhydride after the first inflection point.

25 ml. is transferred to a beaker which contains 5 ml. of glacial acetic acid. The analysis is carried out as described above under *Aminophylline Powder* by following the procedure from "After six drops of α-naphtholbenzein ..." being certain to note the following conditions.

When the injection taken for analysis contains 500 mg. in 2 ml., then 10 ml. of acetic anhydride is used as directed under *Aminophylline Powder*. However, when the injection to be analyzed contains 500 mg. in 20 ml., then 40 ml. of acetic anhydride is added, the remainder of the procedure continuing unchanged.

Aminophylline Suppositories.—A small beaker and glass rod are tared after which five suppositories are placed into the beaker and accurately weighed. The beaker is heated until the suppositories are melted; the melt is allowed to cool without mixing. When solid, an amount of sample equivalent to about 0.5 Gm. of aminophylline is weighed and placed into a beaker. Chloroform, 60 ml., and acetic acid, 40 ml., are added alternately in small quantities and mixed with the sample until solution is completed. The sample solution is transferred quantitatively to a 100-ml. volumetric flask and brought to volume with any chloroform and acetic acid remaining. Chloroform–acetic acid, 3:2 v/v, may be used, if needed, to complete the dilution. A sample of exactly 25 ml. is transferred to a beaker containing 15 ml. of glacial acetic acid. The analysis is carried out as described above under *Aminophylline Powder* by following the procedure from "After six drops of α-naphtholbenzein ..."

U.S.P. Analyses.—Each sample was analyzed by the official U.S.P. XVI methods (2–5).

Calculation for the Nonaqueous Procedures.—

$$\text{Ethylenediamine, \%} = \frac{\text{ml. (first end point)} \times N_{HClO_4} \times 0.03005}{\text{Gm. aminophylline in sample}}$$

$$\text{Theophylline, \%} = \frac{\text{ml. (second end point} - \text{first end point)} \times N_{HClO_4} \times 0.1802}{\text{Gm. aminophylline in sample}}$$

$$\text{Aminophylline dihydrate, \%} = \frac{\text{ml. (second end point)} \times N_{HClO_4} \times 0.1141}{\text{Gm. aminophylline dihydrate in sample}}$$

RESULTS AND DISCUSSION

The titrimetric behavior of aminophylline as a mixture of two bases was observed as expected according to the aqueous pKb values. Although the pKb difference (*ca.* 10 units) is sufficient to permit the bases to be differentiated in glacial acetic acid solvent (Fig. 1, Curve A), it is noted that only the first inflection, representing the neutralization of the stronger base, ethylenediamine, is analytically useful. The second inflection point, assignable to theophylline neutralization, is not satisfactory.

Acetic anhydride has been found to be a valuable solvent for the titration of very weak bases (15, 17). More than simply producing an anhydrous medium by reacting with water (a weak base), this ability has been related to the presence in acetic anhydride of a species more acidic than the solvated proton in acetous perchloric acid (18). Figure 1, curve B, represents the titrimetric behavior of a sample of aminophylline powder in acetic acid solution to which acetic anhydride was added after the first (ethylenediamine) neutralization was completed. It is noted that the character of the second or theophylline neutralization is improved. Both inflections are now analytically suitable.

The nature of Fig. 1, curves B, C, D, and E indicates that satisfactory titrimetric behavior was experienced when aminophylline analyses were carried out on the powder, tablets, injection, and suppositories, respectively. Graphical determination of the end point was possible without difficulty.

The analytical results are summarized in Table I. Each of the samples were analyzed by the official analytical procedures as well as by the nonaqueous titration technique. All of the aminophylline monographs (except for the powder) require the argentometric result (theophylline determination) to be expressed as aminophylline dihydrate. Where possible, the nonaqueous data are presented in the same form as the official analytical results. The nonaqueous calculation is made using the total volume of acid consumed, *i.e.*, the volume needed to neutralize both the ethylenediamine and theophylline. Because of the differential nature of the nonaqueous titration, the percentage of ethylenediamine is calculated from the first end point volume obtained from the same curve.

It is noted that the ethylenediamine content of the

TABLE I.—SUMMARY OF AMINOPHYLLINE ANALYSES[a]

	Nonaqueous			U.S.P. XVI[b]		
	n[d]	Ethylenediamine, %	Xanthine, %	n[d]	Ethylenediamine, %	Xanthine, %
Aminophylline powder U.S.P.	8	12.9 ± 0.2	83.2 ± 1.2 (as theophylline)	3	13.1 ± 0.1 (12.8–14.1)	82.5 ± 0.8 (78 to 83.5) (as theophylline)
Aminophylline tablets, 200 mg.	4	12.6 ± 0.1	98.8 ± 0.1 (as aminophylline dihydrate)	4	...[c]	102.0 ± 0.6 (93–107) (as aminophylline dihydrate)
Aminophylline injection, 500 mg./2 ml.	3	19.4 ± 0.1	81.3 ± 0.4 (as theophylline)	3	19.0 ± 0.1 (18.8–20.1)	102.1 ± 1.3 (93–107) (as aminophylline dihydrate)
Aminophylline suppositories, 500 mg.	4	12.0 ± 0.2	101.7 ± 0.9 (as aminophylline dihydrate)	4	...[c]	102.9 ± 0.2 (90–110) (as aminophylline dihydrate)

[a] Values are means and their standard deviation. [b] Values in parentheses indicate acceptable U.S.P. range. [c] No official analysis specified in this case. [d] Number of determinations is n.

injection is higher than in the other dosage forms when determined by either method. The U.S.P. provides for the addition of excess ethylenediamine to assure a clear solution (3). Thus, when the nonaqueous analysis is carried out, the total volume of acid consumed does not represent aminophylline only but includes the additional ethylenediamine. The calculation reported in Table I, in this instance, is made from the volume of acid used to neutralize the theophylline present and is expressed as percentage of theophylline. The percentage of ethylenediamine is reported also.

When the procedure for the analysis of aminophylline injection is examined, it is noted that the amount of acetic anhydride to be added is variable. Since injections of different concentration are available, the amount of acetic anhydride added must be altered to react with all the water introduced in sampling the aqueous injection solution. In addition to creating a water-free solution, enough excess anhydride remains to be consistent with the 15–20% (by volume) anhydride–acetic acid composition employed in the other dosage form procedures.

Inspection of the data presented in Table I indicate reasonable agreement between the nonaqueous and official methods. The largest deviation exists in the case of the 200-mg. tablets where the nonaqueous value is about 3% lower than the official result. The precision of both methods is found to be about 0.1 to 0.2% for ethylenediamine and about 0.7% for the xanthine (as theophylline or aminophylline dihydrate).

This nonaqueous method was not applicable in the case of some tablets. Although satisfactory results were achieved with 200-mg. tablets, very high results were noted in the analysis of 100-mg. tablets. From the appearance of the titration curves, it was apparent that some tablet constituent other than ethylenediamine and theophylline was acting as a base and contributing to the high results. This behavior was not unexpected and was attributed to the presence of sodium sulfate in the formulation. The interference of various tablet components in acetic acid–nonaqueous titrimetric procedures has been reported (19, 20) and should be anticipated.

Since the nonaqueous method was not successful in the analysis of some tablets, an alternative means of analysis was sought. The official approach to the measurement of ethylenediamine in aminophylline powder and injection employs titration of a sample in water to a methyl orange end point. This simple aqueous titration was found to work well for the determination of ethylenediamine in aminophylline tablets. However, in the present work, better end point detection was achieved by the use of bromcresol green indicator (21). Thus, when the nonaqueous method is unsuccessful, the argentometric or ultraviolet spectrophotometric methods for theophylline determination (1) may be combined with the aqueous titration of ethylenediamine for complete aminophylline analysis.

The nonaqueous procedure offers certain advantages when compared with the official analytical approach. The nonaqueous method is capable of measuring both components of aminophylline in a single titration without elaborate sample treatment. There are fewer reagents needed and the time-consuming heating, cooling, and filtration of the official argentometric assay are eliminated. The simplified procedure is of particular note in the case of the official suppository analysis which involves liquid-liquid extraction as well.

SUMMARY

A time saving, nonaqueous titrimetric procedure has been developed whereby the components of aminophylline, ethylenediamine, and theophylline are titrated differentially as bases during a single titration.

The precision of the described method is similar to the official analysis which requires elaborate sample treatment as well as two titrations to obtain the same data.

The nonaqueous method was not successful in the analysis of certain aminophylline tablets because of the presence of basic tablet components. An alternative approach has been suggested.

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