ophilized benzylpenamaldic acid–buffer mixture was also redissolved in double-distilled water, and 3–20 μl was injected.

Since the refractive index response for benzylpenamaldic acid came at a retention time of 13 min, the difference between expected and actual responses for the buffer salts at retention time of 4 min was used to calculate the weight of benzylpenamaldic acid that had been injected to produce the corresponding peak from the UV detector. This information was used to construct a calibration curve of UV spectral peak areas *versus* weight of benzylpenamaldic acid. The detector response was linear over a range of 0.45–5.4 μg of benzylpenamaldic acid. A least-squares regression analysis typically gave a line of slope of 94.5 $mm^2/\mu g$, an intercept of 22 mm^2, a correlation coefficient of 0.9976, and a standard error of estimate of 27.4 mm^2.

Benzylpenilloic Acid—Aqueous solutions of benzylpenilloic acid (0.75–2.1 mg/ml) were injected in volumes ranging from 1 to 23 μl and consistently produced a pair of overlapping peaks of approximately equal size at retention times of 7 and 8 min. Florey *et al.* (12) reported that two isomers of benzylpenilloic acid are obtained from the decarboxylation of benzylpenicilloic acid, possibly accounting for the pair of peaks on the chromatograph. Because of the overlapping peaks, the total peak area of the double peak was determined using a planimeter. Linear calibration curves in the 5–27-μg range typically had a slope of 0.0021 planimeter unit/μg, an intercept of −0.0024 planimeter unit, and a correlation coefficient of 0.9950. The standard error of estimate was 0.0021 planimeter unit.

Benzylpenicilloic Acid—Benzylpenicilloic acid was eluted from the column with a retention time of 19.5 min.

CONCLUSIONS

The HPLC system is expected to have great utility in kinetic studies to monitor the occurrence of specific penicillin degradation products. For example, Fig. 1 is a chromatogram of a 0.05 *M* penicillin G potassium solution aged for 7 min and 31.5 hr at 37° in a 0.03 *M* citric acid–0.0067 *M* disodium phosphate buffer, pH 2.70. Peaks suitable for quantification are seen having retention times corresponding to penicillin G potassium, benzylpenillic acid, benzylpenamaldic acid, benzylpenilloic acid, and penicillamine. No other analytical method is available that allows the direct detection and quantification of penicillin degradation products forming during aging.

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§ Present address: Squibb Institute for Medical Research, New Brunswick, N.J.

* To whom inquiries should be directed.

Differentiating Nonaqueous Titration of Aspirin, Acetaminophen, and Salicylamide Mixtures

H. J. RHODES, J. J. DeNARDO, D. W. BODE, and M. I. BLAKE *

Abstract □ Mixtures containing aspirin, acetaminophen, and salicylamide were assayed potentiometrically by nonaqueous titration. The difference in pKa values for these weak acids was sufficient to permit successful differentiation. The titrant was tetrabutylammonium hydroxide, and the titration solvent was dimethylformamide. The procedure was applied to commercial dosage forms.

Keyphrases □ Aspirin mixtures with salicylamide or salicylamide/acetaminophen—differentiating nonaqueous titration □ Salicylamide mixtures with aspirin or aspirin/acetaminophen—differentiating nonaqueous titration □ Acetaminophen, aspirin, and salicylamide mixtures—differentiating nonaqueous titration □ Titrimetry, nonaqueous, differentiating—mixtures of aspirin, salicylamide, and acetaminophen

Aspirin, acetaminophen, and salicylamide are frequently used analgesic–antipyretics in tablet dosage forms, individually, in a variety of combinations, and often with other drugs. Suitable analytical procedures are available for estimating the individual components, but generally methods are not available for

complex mixtures of these components. Methods for the determination of acetaminophen in dosage forms, whether present as the only active ingredient or combined with other drugs, were reviewed previously (1). In that paper, a potentiometric nonaqueous titration procedure was proposed for determining acetaminophen in dosage forms. Sodium methoxide was used as the titrant and dimethylformamide was used as the titration solvent.

Procedures for analyzing mixtures containing acetaminophen and salicylamide in combination with other drugs also were reviewed (2). A differentiating nonaqueous titration method was described for this combination, using tetrabutylammonium hydroxide as the titrant and dimethylformamide as the titration solvent. Differentiating nonaqueous titration methods were reported for dosage forms containing combinations of aspirin and acetaminophen (3), acetaminophen and barbiturates (4), and aspirin and barbiturates (5). Each of these mixtures is composed of weak acids having pKa values sufficiently divergent to permit successful differentiating titrations in the appropriate nonaqueous system. Preliminary extraction and separation of the active components were not necessary, and dosage form excipients apparently did not interfere in the titration.

The present study was an extension of previous work and involved the differentiating nonaqueous titration of mixtures of aspirin (pKa 3.50), acetaminophen (pKa 9.92), and salicylamide (pKa 8.31), using tetrabutylammonium hydroxide as the titrant and dimethylformamide as the titration solvent. The procedure was applied to commercial dosage forms.

EXPERIMENTAL

Apparatus—All titrations were performed potentiometrically with a titrimeter[1] equipped with a glass–calomel electrode system or a platinum–calomel electrode system.

Reagents, Chemicals, and Dosage Forms—Acetaminophen, aspirin, and salicylamide were obtained from commercial sources and were the best quality available. Dosage forms were obtained from commercial sources. All other chemicals and solvents employed were reagent grade and were used without further purification.

Tetrabutylammonium hydroxide, 0.1 *N* in benzene–methanol, was prepared according to the method of Cundiff and Markunas (6) and was standardized potentiometrically against reference standard benzoic acid dissolved in dimethylformamide. The solution was restandardized with each series of titrations.

Titration of Individual Components—Approximately 0.5 mEq of aspirin powder, accurately weighed, was dissolved in 50 ml of dimethylformamide in a 150-ml beaker. The solution, magnetically stirred, was titrated potentiometrically with 0.1 *N* tetrabutylammonium hydroxide. Salicylamide and acetaminophen were titrated similarly as individual components.

Differentiation of Two-Component Mixtures—The differentiation of mixtures containing acetaminophen and salicylamide or aspirin and acetaminophen was reported earlier (2, 3). In the present study, mixtures of aspirin and salicylamide were prepared in the following ratios: 1:1, 1:2, and 2:1. Aliquots of these mixtures, containing at least 0.5 mEq of each component, accurately weighed, were analyzed by dissolving the aliquot in 50 ml of dimethylformamide contained in a 150-ml beaker. The solution, magnetically stirred, was titrated potentiometrically with 0.1 *N* tetrabutylammonium hydroxide.

[1] Fisher model 35.

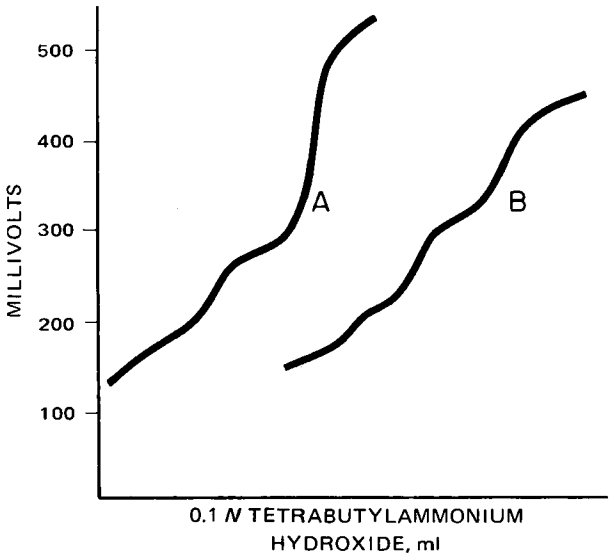

Figure 1—*Titration curves for: (A) binary mixture containing aspirin and salicylamide and (B) ternary mixture containing aspirin, salicylamide, and acetaminophen.*

The equivalence points for the differentiating titrations were determined by plotting $\Delta E/\Delta V$ *versus* V (milliliters). The volume reading where $\Delta E/\Delta V$ was a maximum was taken as an equivalence point. This procedure was not necessary for single-component systems, since the curve obtained by plotting the volume of titrant *versus* millivolt readings permitted the detection of the equivalence point by inspection. A typical titration curve (A) is shown in Fig. 1, and recovery data are presented in Table I.

Titration of Three-Component Mixtures—Mixtures of the three components were prepared so that aspirin–salicylamide–acetaminophen ratios were 1:1:1, 2:1:1, 3:1:1, and 3:2:1. Appropriate amounts of each component were accurately weighed and transferred to a 150-ml beaker. Fifty milliliters of dimethylformamide was added to the beaker, and the resulting solution was stirred magnetically. The solution was titrated potentiometrically with 0.1 *N* tetrabutylammonium hydroxide as the titrant. A typical titration curve (B) is shown in Fig. 1, and recovery data are presented in Table I.

Analysis of Dosage Forms Containing Three-Component Mixture—Twenty tablets from each of two commercial products

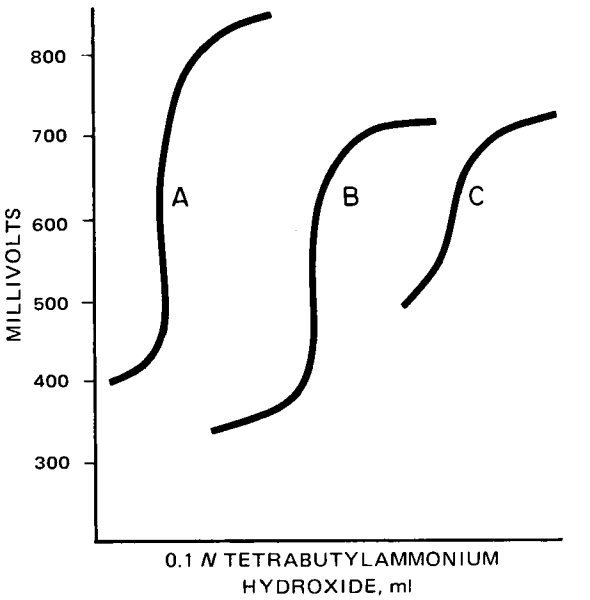

Figure 2—*Typical titration curves: (A) aspirin, (B) salicylamide, and (C) acetaminophen.*

Table I—Analytical Data

Sample	Ratio of Components	Recovery, %		
		Aspirin	Salicylamide	Acetaminophen
Aspirin		99.4 ± 0.6		
Salicylamide			100.9 ± 0.6	
Acetaminophen				101.2 ± 0.4
Aspirin–salicylamide	1:1	95.4 ± 0.4	100.4 ± 1.0	
	2:1	99.7 ± 1.0	95.7 ± 1.1	
	1:2	96.3 ± 5.0	100.3 ± 0.7	
Aspirin–salicylamide–acetaminophen	1:1:1	97.3 ± 1.5	102.3 ± 1.3	100.1 ± 1.1
	2:1:1	95.4 ± 1.9	97.8 ± 1.2	99.3 ± 0.4
	3:1:1	97.8 ± 0.3	97.8 ± 0.4	98.9 ± 2.7
	3:2:1	97.1 ± 0.2	100.6 ± 2.8	96.1 ± 1.1
Tablet dosage form				
A	3:2:1.5	98.5 ± 1.3	96.6 ± 1.7	97.9 ± 2.0
B	3:2:1.5	98.9 ± 0.8	101.5 ± 2.3	100.1 ± 2.0

were accurately weighed and then reduced to a fine powder in a mortar. An aliquot of the powder mass, accurately weighed, was transferred to a 150-ml beaker; then 50 ml of dimethylformamide was added, and the mixture was magnetically stirred for about 10 min. The resulting suspension was filtered through filter paper, which had been wetted with dimethylformamide. The beaker was rinsed with two 10-ml portions of dimethylformamide, which were then passed through the filter. The filtrate containing the active components of the dosage form was titrated potentiometrically as previously described.

RESULTS AND DISCUSSION

Mixtures of weak acids are readily analyzable by differentiating nonaqueous titration if the pKa values of the individual acids are sufficiently divergent and a suitable titrant, titration solvent, and electrode system are selected. This procedure is applicable to pharmaceutical dosage forms if excipients and other active ingredients do not interfere.

Typical two-component weak acid mixtures related to the systems reported in the present study were already mentioned. Other examples of two-component weak acid mixtures that have been successfully titrated differentially include benzoic acid and salicylic acid (6) as synthetic mixtures and in ointment dosage forms, cyclamates and saccharin (7) in liquid dosage forms, and *p*-aminosalicylic acid and its decomposition product *m*-aminophenol (8). The present study reports the analysis of mixtures containing aspirin and salicylamide and three-component mixtures containing acetaminophen in addition to aspirin and salicylamide.

Typical titration curves for the individual components are shown in Fig. 2. Curve A, Fig. 1, is a typical differentiating curve for mixtures containing equimolar amounts of aspirin and salicylamide. The first inflection is for aspirin, and the second end-point is for salicylamide. Curve B, Fig. 1, depicts a typical titration curve for the three-component system in which the components are present in about equimolar concentrations. The third inflection of the curve corresponds to acetaminophen.

Analysis data for the individual components, for the components of the binary and ternary synthetic mixtures, and for some typical tablet dosage forms are listed in Table I. The presence of other components and excipients apparently did not interfere in the titration. Although a preliminary extraction of the active components was performed, separation of the individual compounds was not necessary.

Two electrode systems were used in this study: the glass–calomel and the platinum–calomel pairs. The latter appeared to give sharper end-points, although both systems proved applicable.

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[x] To whom inquiries should be directed.