By using receptor protection experiments, it was possible to demonstrate that D(−)-pseudoephedrine also competes for the α-adrenergic sites. However, this effect must be very weak because the effects of exogenous norepinephrine are potentiated (*i.e.*, the competition of D(−)-pseudoephedrine at α-receptors is overcome by norepinephrine, the uptake of which is being prevented in the nerve endings). Thus, D(−)-pseudoephedrine is capable of acting at the catecholamine uptake sites and the α-adrenergic sites; however, it lacks intrinsic activity at the latter site.

The pharmacological effects of L(+)-pseudoephedrine and L(+)-ephedrine are similar on the aortic strips which support the previous report that there is a small difference between the pressor effects of these L-diastereoisomers in the dog (2).

D(−)-Ephedrine is the only isomer of ephedrine which produces a marked contraction of rabbit aortic strips. In the presence of 10^{-3} *M* concentrations of L(+)-ephedrine, L(+)-pseudoephedrine, or D(−)-pseudoephedrine, the dose-response curves of D(−)-ephedrine are shifted to the right.

On the isolated rat vas deferens D(−)-pseudoephedrine did not show any intrinsic effects. However, the contractions due to tyramine and D(−)-ephedrine were markedly antagonized by 10^{-4} *M* and 10^{-3} *M* D(−)-pseudoephedrine, while the effects of norepinephrine were potentiated. In addition, D(−)-pseudoephedrine and D(−)-ephedrine also compete for the α-adrenergic sites in the reserpine pretreated animals. D(−)-Pseudoephedrine appears to act at both the catecholamine uptake site and the α-adrenergic site, but apparently lacks intrinsic effects at the latter site.

SUMMARY

In anesthetized dogs, pretreatment (30 min.) with D(−)-pseudoephedrine, 3.3 mg./Kg., can reduce or block the pressor effects of D(−)-ephedrine, L(+)-ephedrine, L(+)-pseudoephedrine, and D(+)-amphetamine. D(−)-Pseudoephedrine also promptly reduces the pressor effects, if given during the height of response. The pressor effects of norepinephrine and epinephrine appeared to be potentiated by D(−)-pseudoephedrine. Such a potentiation was best seen 45 to 60 min. after the administration of the isomer. The pressor effects due to bilateral carotid occlusion were unaffected by D(−)-pseudoephedrine.

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Differentiating Nonaqueous Titration of Salicylic Acid and Acetylsalicylic Acid Combination

By SONG-LING LIN

Synthetic mixtures of salicylic acid and acetylsalicylic acid are differentiated by potentiometric nonaqueous titration. With sodium methoxide or tetrabutylammonium hydroxide titrant and glass-calomel electrode system, dimethylformamide is found to be the best differentiating solvent. The effect of the solvent in titration and differentiation of salicylic acid and acetylsalicylic acid in water and dimethylformamide is illustrated and interpreted. Various solvent–titrant–electrode combinations are employed to explore their effects on the sensitivity of the differentiating titration. The presence of water in the titration solvent is demonstrated to be highly detrimental and undesirable on differentiation. The proposed procedure is simple, accurate, and applicable even when there is a disproportionate concentration of the components. The work suggests that by a proper combination of solvent, titrant, and electrode system, it should be possible to differentiate potentiometrically both components of acidic or basic mixtures whose dissociation constants are well below the theoretical limit of 16.

A VARIETY OF procedures (1–8) have been proposed for the analysis of acetylsalicylic acid and one of its degradation products, salicylic acid. These methods involve colorimetry, chromatography, and spectrophotometry. However, a search of the literature for titrimetric techniques applicable to differentiating salicylic acid–acetylsalicylic acid combination was not successful. It was the purpose of this study to develop such a procedure.

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Although salicylic acid and acetylsalicylic acid as individual components can be readily determined by titration in nonaqueous media, the titrimetric analysis of mixtures of these compounds has not been reported. This may be due to the fact that the difference between the pKa's, in water, of salicylic acid (2.97) and acetylsalicylic acid (3.49) (9) has been considered to be too small to accomplish the differentiation, even in nonaqueous solvents.

Theoretical expressions for the titration of a mixture of two monoprotic acids can be treated as in the titration involving a single diprotic acid. Auerbach and Smolczyk (10) have shown theoretically that no inflection occurs at the first equivalence point in the potentiometric titration curve of polyfunctional acids in aqueous solvents unless the ratio of the first to the second dissociation constant is greater than 16. It is difficult, in fact, to locate the inflection accurately even at a ratio of 100. Kolthoff and Stenger (11) state that, in water, an accurate and satisfactory differentiation of acids or bases can be obtained only with a pKa (H_2O) difference of approximately 4.0. When titration is performed in nonaqueous solvents, differentiation is possible even with a pKa (H_2O) difference of about or below 2.0. Many dibasic acids which give only a single inflection in water give two sharp inflections in nonaqueous solvents. For example, the two acidic groups of succinic acid (pK_1 of 4.2 and pK_2 of 5.6) were differentiated in acetone (12). In this investigation, the resolution of acid mixtures with a pKa (H_2O) difference of only 0.52 [or with Ka (H_2O) difference of only 3] is successfully accomplished by selecting the proper combinations of titrant, solvent, and electrode system.

In this investigation, differentiating nonaqueous titration was performed with a Fisher automatic titrimeter. Various solvent–titrant–electrode combinations were employed to explore their effects on the sensitivity of differentiation. The feasibility of resolution and characteristics of titration curves are markedly different from one solvent–titrant–electrode system to another. The presence of water in the titration solvent is shown to be highly detrimental and undesirable on differentiation. The degree of water tolerance with sodium methoxide titrant is threefold as resistant as with tetrabutylammonium hydroxide titrant.

With sodium methoxide or tetrabutylammonium hydroxide in benzene–methanol as titrant and a glass–calomel electrode pair as the electrode system, dimethylformamide was found to be the best differentiating solvent. The suppression of the sharpness of the inflections for both compounds in differentiating titration as compared with the titration of individual components is realized. Acetylsalicylic acid offers sharper inflection than salicylic acid in differentiating titration, although the latter component demonstrates better inflection than its acetylated derivative when titrated individually. The proposed procedure is simple, accurate, and applicable even when there is a disproportionate concentration of the components. Preliminary treatment of the sample is unnecessary, and tedious separation and extraction techniques are obviated.

The work suggests that, by a proper combination of solvent, titrant, and electrode system, it would be possible to titrate and differentiate potentiometrically both components of an acidic and basic mixture whose dissociation constants are well below the theoretical limit of 16 proposed by Auerbach and Smolczyk (10). The findings obtained will serve as guidelines for future work on the differentiating nonaqueous titration of acetylsalicylic acid–salicylic acid–acetic acid mixtures.

EXPERIMENTAL

Apparatus—Titrations were performed potentiometrically with a Fisher automatic titrimeter, model 36, equipped with a glass electrode (Beckman No. 40498) and a sleeve-type calomel electrode (Beckman No. 40463). For this study, the calomel electrode was modified by replacing the aqueous saturated potassium chloride solution in the bridge with a saturated solution of potassium chloride in methanol. Other electrodes evaluated were platinum electrode (Fisher 13-639-102) and antimony electrode (Fisher 9-313-212). A 25-ml. automatic buret, graduated to 0.05 ml., with a titrant storage container, was used to prevent any contamination of the titrant.

Reagents and Solutions—Acetylsalicylic acid U.S.P. (Mallinckrodt) was dried at 60° for 4 hr. and was used without further purification. Analysis by the official U.S.P. assay procedure indicated a purity of better than 99.9%. This meets the U.S.P. requirements for the allowable salicylic acid content (<0.1%). The salicylic acid used was U.S.P. grade. Analysis by U.S.P. method showed a purity of 99.8%. A 0.1 *N* solution of sodium methoxide in benzene–methanol (10:1) was prepared and standardized potentiometrically against a reference standard benzoic acid dissolved in dimethylformamide. A 0.1 *N* solution of tetrabutylammonium hydroxide in benzene–methanol (10:1) was prepared by the silver oxide method and standardized potentiometrically against a reference standard benzoic acid dissolved in dimethylformamide. Both titrants were protected from the atmosphere by an Ascarite guard tube. Dimethylformamide was purified by distillation (b.p. 153°). Other chemicals and solvents used in this study were reagent grade and used as received without further purification.

Differentiating Titration of Salicylic Acid and Acetylsalicylic Acid—Approximately 0.50 meq. of salicylic acid and 0.50 meq. of acetylsalicylic acid, accurately weighed, were dissolved in 50 ml. of solvent or solvent mixture in a 150-ml. beaker covered with a rubber plate having holes for the passage of the electrodes and the buret tip. The solution, under constant stirring, was titrated potentiometrically with 0.1 *N* sodium methoxide solution or 0.1 *N* tetrabutylammonium hydroxide solution, using a modified calomel and glass electrode system. A blank titration was also performed. Titration curves were obtained by plotting the potential reading, *E*, in millivolts (mv.) *versus* volume (ml.) of titrant. The exact end point was determined by plotting $(\Delta E/\Delta \bar{V})$ *versus* ml. The titration solvents evaluated in this study included dimethylformamide, pyridine, nitromethane, nitrobenzene, acetone, methyl ethyl ketone, methyl isobutyl ketone, methanol, ethanol, isopropanol, and *tert*-butylalcohol. Prior to carrying out the differentiating titration of salicylic acid and acetylsalicylic acid in each solvent, the individual compounds were titrated separately.

Electrode Systems—The six electrode combinations employed were glass–calomel, platinum–calomel, antimony–calomel, antimony–platinum, glass–antimony, and glass–platinum. The titration solvents used in the evaluation of these electrode systems included dimethylformamide, nitromethane, acetonitrile, and methyl isobutyl ketone. The concentration of the acids, volume of solvent, and titrants were the same as above.

Variation of Salicylic Acid-to-Acetylsalicylic Acid Ratio—A series of differentiating titrations was performed in which the milliequivalent ratio of salicylic acid to acetylsalicylic acid was varied from 1 to 1 to greater than 10 to 1. Amounts of salicylic acid and acetylsalicylic acid calculated to give the desired milliequivalent ratio of components were accurately weighed and dissolved in 50 ml. of dimethylformamide. The solution was titrated potentiometrically with 0.1 *N* sodium methoxide solution or 0.1 *N* tetrabutylammonium hydroxide solution using the glass–calomel electrode system.

Effect of Varying Water Concentration in Titration Solvent on Differentiating Titration—Accurately weighed quantities of salicylic acid and acetylsalicylic acid were dissolved in 50 ml. of water–dimethylformamide solvent mixture composed of varying amounts of water. The solution was titrated potentiometrically with 0.1 *N* sodium methoxide solution or 0.1 *N* tetrabutylammonium hydroxide solution, using the glass–calomel electrode pair.

RESULTS AND DISCUSSION

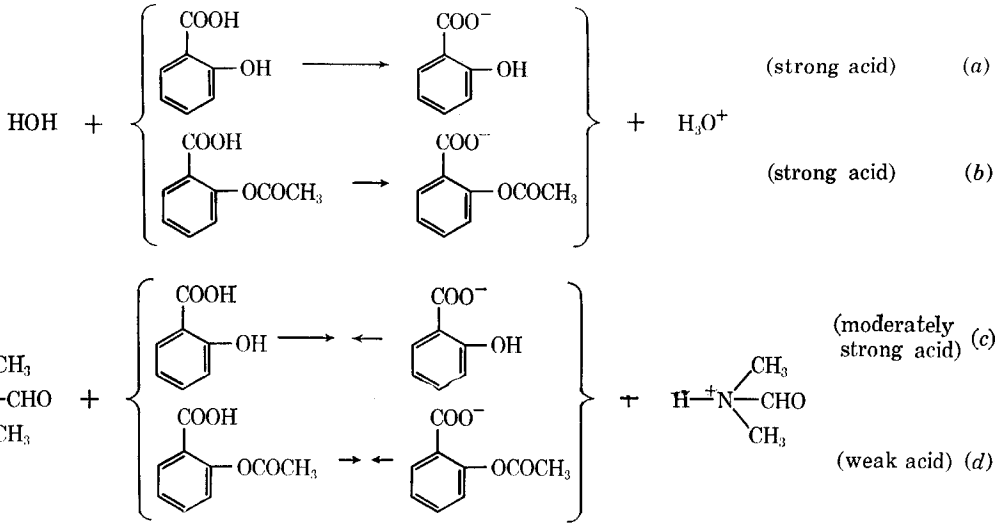
An ideal solvent for the satisfactory differentiation of an acid mixture should be sufficiently weak in basicity to permit the titration of the stronger acidic component and sufficiently weak in acidity to permit the titration of the weaker acidic component. From a practical standpoint, the differentiating quality of the solvent can be realized from the characteristics of the potentiometric titration curves obtained. A well-defined inflection will be observed for the potentiometric titration of the stronger acid if the solvent is a good differentiating solvent. The better the differentiating ability of the solvent, the greater the inflection will be. In preliminary experiments a series of solvents and combinations of solvents were employed as titration solvents to test their differentiating ability. Titrations were effected potentiometrically with 0.1 *N* sodium methoxide or 0.1 *N* tetrabutylammonium hydroxide using a glass–modified calomel electrode system. Of the solvent systems tested, dimethylformamide was found to yield the most satisfactory end points in the differentiating titration of the salicylic acid–acetylsalicylic acid mixture, despite the fact that dimethylformamide has been reported to be of only limited use for the titration of acids and their mixtures because of strong acid–solvent interaction (13). The autoprotolysis constant for this solvent, as determined from potentiometric measurements, was found to be 10^{-18} (14).

When an electrically neutral acid (HA) is dissolved in the solvent (SH), species with various charges are formed upon the ionization of the solvent:

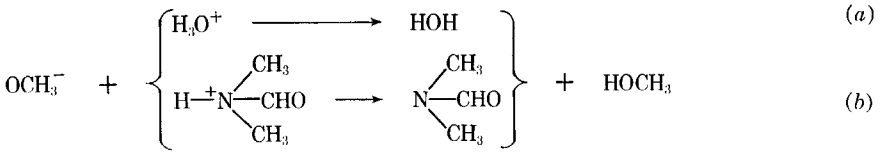
$$SH + HA \rightleftarrows A^- + SH_2^+ \qquad \text{(Eq. 1)}$$

The reaction is not solvent-independent since reactants and products differ significantly in their charge densities, and therefore, in their ability to accept hydrogen bonds or form ion pairs in the solvent. The effect of the solvent in titration and differentiation of salicylic acid and acetylsalicylic acid in water and dimethylformamide is illustrated in Schemes I–III.

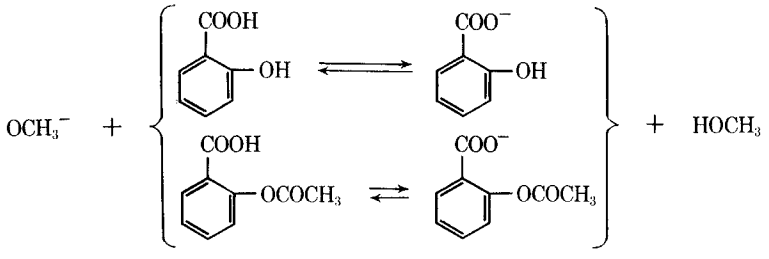
It was observed that salicylic acid and acetylsalicylic acid, alone or in combination, are readily dissolved in dimethylformamide but slowly dissolved in water. This may be explained by the important role of the nature of the solvent molecule on the strength of the solute–solvent interactions. The nature of the solution also varies with the solvent. Ions and molecules have a tendency to achieve the lowest possible energy state, and the formation of certain structure in the solution process is one way of attaining this. The greater the solvation, the lower the resulting energy of the solute, and its stability in solution is increased. This is achieved when a charged species is surrounded by suitably oriented dipoles of a solvent of high dielectric constant. In water, both anions and cations are well solvated and thus shielded from each other because of van der Waal forces and other intermolecular attractions. Anions, because of their great solvation energies, are well stabilized in solution, and the forward reactions of Scheme I (*a* and *b*), are greatly favored. In other words, since the anion is much more densely charged than the electrically neutral solvent molecule, the strong anion–solvent interactions due to hydrogen bonding lower the energy of anion relative to the electrically neutral solvent molecule. As a result, the equilibrium in water is shifted further to the right as compared to a solvent incapable of forming hydrogen bonding with the anions. Upon the addition of alkoxide ion as titrant during the titration, the solvated proton reacts quantitatively with alkoxide ion as shown in Scheme II(*a*). Consequently, salicylic acid and acetylsalicylic acid can be titrated individually in water as shown in Fig. 1 (curves 1 and 2). Parker (15) and Buckingham (16) have shown that anions are solvated to a greater extent



Solvolysis (Reaction with Solvent)
Scheme I



Titration of the Solvated Proton (Reaction with the Titrant)
Scheme II



Net Reaction
Scheme III

than cations in water and small anions are much more solvated than large anions. However, in water, the difference in energy between anions of salicylic acid and acetylsalicylic acid might not be great enough to permit the successful differentiation of a salicylic acid–acetylsalicylic acid mixture as evidenced by Fig. 1, curve 3.

When dimethylformamide replaced water as the solvent, the situation was changed. This is due to the fact that dimethylformamide and water possess different solvent characteristics. (*a*) Water is amphiprotic in nature, while dimethylformamide donates electrons but does not accept them. (*b*) The strongest base that can exist in water is the solvated hydroxide ion, while on the other hand, a much stronger base system is possible in dimethylformamide. (*c*) The dielectric constants of water and dimethylformamide are 80 and 36, respectively, and the higher the dielectric constant of a solvent, the lower the electrostatic interaction and less will be the chance for the dissolved species bearing an opposite charge to interact. (*d*) The positive charge on the small hydrogen atoms of water can come much closer to an anion than can a positive charge on an atom other than hydrogen. Hence, the possible interaction in dimethylformamide is of the weaker ion–dipole interaction, neither hydrogen bonding nor ion-pairing being operative. Consequently, the anions in dimethylformamide are much less solvated and more reactive than those in water. In other words, solute molecules dissolved in dimethylformamide yield anions which have much greater free energies than the anions produced from solutes dissolved in water. As illustrated in Scheme I (*c* and *d*), the forward reaction becomes less favorable relative to the backward

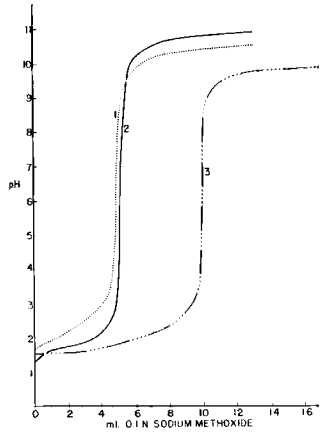


Fig. 1—Typical curves for the titration of salicylic acid and acetylsalicylic acid dissolved in water, individually and in combination, with 0.1 N sodium methoxide in benzene–methanol (10:1), using glass-modified calomel electrode system. Key: 1, acetylsalicylic acid; 2, salicylic acid; 3, salicylic acid and acetylsalicylic acid.

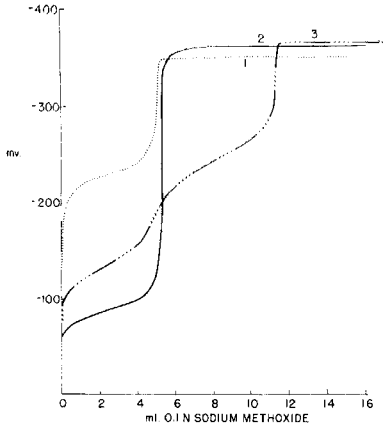


Fig. 2—Typical curves for the titration of salicylic acid and acetylsalicylic acid dissolved in dimethylformamide, individually and in combination, with 0.1 N sodium methoxide in benzene–methanol (10:1), using glass-modified calomel electrode system. Key: 1, acetylsalicylic acid; 2, salicylic acid; 3, salicylic acid and acetylsalicylic acid.

reaction when water is replaced with dimethylformamide. The equilibrium in dimethylformamide, therefore, does not lie as far to the right as it does in water. As the result, a greater sensitivity in end point detection was noted for the individual components with water (curves 1 and 2, Fig. 1) than with dimethylformamide (curves 1 and 2, Fig. 2) as titration solvent. Zaugg (17), Prue and Sherrington (18), and Parker (15) have pointed out that in dimethylformamide anions are much less solvated than cations, and large polarizable anions may be slightly more solvated than small anions. Even more important is the fact that the difference in energy between anions of different sizes and charge densities is much greater in dimethylformamide than in water. Consequently, differentiating titration of a salicylic acid–acetylsalicylic acid mixture can be readily achieved in dimethylformamide (curve 3, Fig. 2), but not in water (curve 3, Fig. 1). When the combination of drugs was titrated in dimethylformamide, two distinct inflections were obtained, the first corresponding to the salicylic acid content and the second representing the acetylsalicylic acid end point. In this connection, Kolthoff *et al.* (19) have emphasized that, in the interpretation of difference in acid strength in different solvents, one must consider the degree of anion solvation and the formation and dissociation of ion pairs in addition to the basic characteristics and dielectric constant of the solvent. Additional studies are presently underway concerning the basic behavior of salicylic acid and acetylsalicylic acid in water and dimethylformamide; the results will be presented in a future publication (20).

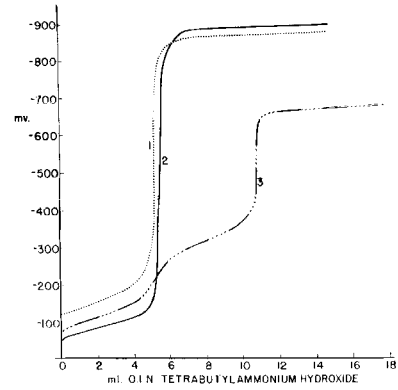


Fig. 3—Typical curves for the titration of salicylic acid and acetylsalicylic acid dissolved in dimethylformamide, individually and in combination, with 0.1 N tetrabutylammonium hydroxide in benzene–methanol (10:1), using glass-modified calomel electrode system. Key: 1, acetylsalicylic acid; 2, salicylic acid; 3, salicylic acid and acetylsalicylic acid.

With dimethylformamide as the titration solvent, the titration curves in Fig. 2 were obtained with sodium methoxide, whereas those in Fig. 3 were obtained with tetrabutylammonium hydroxide as the titrant. The sharp breaks in the titration curves (curves 1 and 2, Figs. 2 and 3) indicated excellent quantitative recoveries of acetylsalicylic acid and salicylic acid when titrated individually. As would be expected, a greater sensitivity in end point detection was realized for the individual components with the more basic titrant, tetrabutylammonium hydroxide, than with sodium methoxide titrant. A comparison of the sharpness of the inflection, as shown in Table I by the magnitude of $(\Delta E/\Delta \bar{V})_{max.}$, expressed in mv./ml., indicates that for acetylsalicylic acid the sharpness of the inflection was doubled when the quaternary ammonium hydroxide titrant replaced the alkoxide titrant. The steeper inflection given by salicylic acid over acetylsalicylic acid in dimethylformamide is predictable from their respective dissociation constants in water. When the combination of drugs was titrated (curve 3, Figs. 2 and 3), satisfactory differentiation was obtained with either titrant. When sodium methoxide was substituted by tetrabutylammonium hydroxide as titrant, as indicated in

TABLE I—DETERMINATION OF SALICYLIC ACID AND ACETYLSALICYLIC ACID, INDIVIDUALLY AND IN COMBINATION, IN DIMETHYLFORMAMIDE USING GLASS–CALOMEL ELECTRODE SYSTEM

Curve Ref.[a]	Compd.	Sodium Methoxide		Tetrabutylammonium Hydroxide	
		$(\Delta E/\Delta \bar{V})_{max.}$	Recovery, %	$(\Delta E/\Delta \bar{V})_{max.}$	Recovery, %
1	Acetylsalicylic acid	1000[b]	100.04 ± 0.32[c]	2500	99.50 ± 0.49
2	Salicylic acid	1450	99.94 ± 0.56	2800	99.98 ± 0.40
3	Salicylic acid	70	99.14 ± 0.36	80	100.54 ± 0.67
	Acetylsalicylic acid	550	99.87 ± 0.81	1850	98.98 ± 0.71

[a] Numbers correspond to curves in Figs. 2 and 3. [b] Expressed in mv./ml. of titrant added. [c] Standard deviation is based on at least 5 determinations.

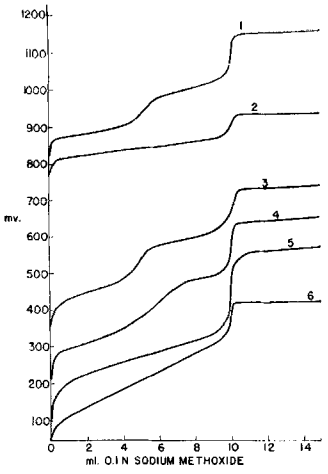


Fig. 4—Typical curves for differentiating titration of salicylic acid and acetylsalicylic acid in various solvents. Key: 1, dimethylformamide; 2, pyridine; 3, nitromethane; 4, nitrobenzene; 5, alcoholic solvent; 6, ketonic solvent. The titrant was 0.1 N sodium methoxide in benzene–methanol (10:1).

Table I, the sharpness of the inflection remained fairly unchanged for salicylic acid, while the sharpness of the inflection for acetylsalicylic acid was more than tripled. This may be explained, at least in part, by the fact that when tetrabutylammonium hydroxide is used as titrant, the potential span available is two to three times larger than when sodium methoxide is employed. The suppression of the sharpness of the inflections during differentiating titration of mixtures, as compared with the titration of individual components, is further shown from the data in Table I. With sodium methoxide as the titrant, the magnitude of $(\Delta E/\Delta \bar{V})_{max.}$ obtained during differentiation is suppressed about twentyfold for salicylic acid and approximately twofold for acetylsalicylic acid. When the mixture was titrated with tetrabutylammonium hydroxide, a more drastic suppression of the magnitude of $(\Delta E/\Delta \bar{V})_{max.}$ was observed with salicylic acid, giving a 35-fold reduction of the sharpness of potential break during differentiation as compared with the value obtained from the titration of salicylic acid alone. It is interesting to note that acetylsalicylic acid offers a sharper inflection than salicylic acid during the differentiating titration, in spite of the fact that salicylic acid demonstrates better inflection than its acetylated derivative when titrated individually.

Using a glass–methanol modified calomel electrode system, the effect of various solvents on the sensitivity of differentiation was investigated with either sodium methoxide (Fig. 4) or tetrabutylammonium hydroxide (Fig. 5) as the titrant. Basic solvents other than dimethylformamide, such as pyridine, while excellent for the individual components, do not permit differentiation in the case of acetylsalicylic acid–salicylic acid mixture. Figure 4, curve 2, indicates a single end point corresponding to the total acid present when titration is performed in pyridine. An explanation of this is that both acids react nearly completely with the pyridine which tends to level all acids so that their strengths become indistinguishable. This phenomenon has been termed the "leveling effect" (21). When sodium methoxide was used as the titrant, no resolution was observed in acetonitrile, the ketones, and alcohols. Using tetrabutylammonium hydroxide as titrant, similar results were obtained in these solvents with the exception of *tert*-butyl alcohol (curve 5, Fig. 5). When the titration is performed in nitromethane, differentiation is readily accomplished with the sodium methoxide titrant (curve 3, Fig. 4) but not with tetrabutylammonium hydroxide titrant (curve 4, Fig. 5). On the contrary, when the titration is carried out in *tert*-butyl alcohol, resolution is realized with tetrabutylammonium hydroxide titrant (curve

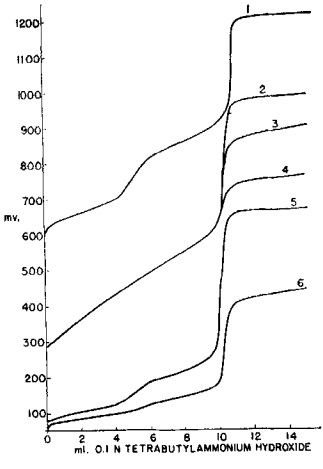


Fig. 5—Typical curves for differentiating titration of salicylic acid and acetylsalicylic acid in various solvents. Key: 1, dimethylformamide; 2, ketonic solvents; 3, acetonitrile; 4, nitromethane; 5, tert-butyl alcohol; 6, other alcoholic solvent. The titrant was 0.1 N tetrabutylammonium hydroxide in benzene–methanol (10:1).

TABLE II—DIFFERENTIATING TITRATION OF SALICYLIC ACID AND ACETYLSALICYLIC ACID IN VARIOUS SOLVENTS USING GLASS–CALOMEL ELECTRODE SYSTEM AND SODIUM METHOXIDE TITRANT

Solvent	No. of Determinations	Recovery, %: Salicylic Acid	Recovery, %: Acetylsalicylic Acid
Dimethylformamide	5	99.14 ± 0.36[a]	99.87 ± 0.81
Nitromethane	5	98.87 ± 0.49	100.06 ± 0.48
Nitrobenzene	5	100.81 ± 1.52	98.72 ± 0.98
Nitromethane–benzene (1:10)	3	99.12 ± 0.62	98.72 ± 0.65
Nitromethane–chloroform (1:10)	3	98.92 ± 0.75	100.81 ± 0.62
Nitromethane–dimethylformamide (1:10)	4	100.18 ± 0.40	99.42 ± 0.60
Dimethylformamide–nitrobenzene (1:10)	4	98.62 ± 0.64	100.70 ± 0.55
Dimethylformamide–benzene (1:8)	3	One end point[b]	
Dimethylformamide–chloroform (1:8)	3	One end point	

[a] Standard deviation is based on at least 4 determinations. [b] Corresponds to salicylic acid plus acetylsalicylic acid.

TABLE III—EFFECT OF ELECTRODE COMBINATION ON DIFFERENTIATING TITRATION OF SALICYLIC ACID AND ACETYLSALICYLIC ACID

Curve Ref.[a]	Electrode Combination	End Points Observed,[b] No. Dimethylformamide: $NaOCH_3$	Dimethylformamide: $(Bu)_4NOH$	Nitromethane: $NaOCH_3$	Nitromethane: $(Bu)_4NOH$
1	Glass–calomel	2	2	2	1
2	Platinum–calomel	0	2	2	1
3	Antimony–calomel	1	2	2	1
4	Antimony–platinum	2	1[d]	1	1
5	Glass–antimony	2[c]	2	2	1
6	Glass–platinum	2[c]	2	2[c]	1[c]

[a] Numbers correspond to curves in Figs. 6 and 7. [b] Observation is based on at least 4 determinations. [c] Abnormal titration curve was observed. [d] One end point corresponding to salicylic acid plus acetylsalicylic acid.

5, Fig. 5) and not with the sodium methoxide titrant (curve 5, Fig. 4). Although it is possible to differentiate the acids in nitrobenzene (curve 4, Fig. 4), a smaller and less well-defined inflection is obtained for the stronger acid in the mixture. Consequently, the importance of the proper selection of the solvent–titrant combination for a successful differentiating titration is clearly exemplified by the plots in Figs. 4 and 5.

Pifer *et al.* (22) point out that the inclusion in the titration solvent system of an excess of a solvent having a low dielectric constant can increase markedly the sensitivity in the detection of the end point. This useful principle has been applied satisfactorily to the titration of acetophenetidin (23) and the differentiation of acetophenetidin–caffeine mixture (24). However, an attempt to sharpen the end points by the incorporation of an excess of benzene or chloroform in dimethylformamide, nitrobenzene, or nitromethane was not successful with sodium methoxide as the titrant. Although quantitative recoveries, as reported in Table II, were obtained for both components dissolved in nitromethane and mixed solvents containing a large excess of chloroform or benzene in nitromethane, the sharpness of the inflection remains fairly unaltered. The incorporation of an excess of a solvent having a low dielectric constant in dimethylformamide or nitrobenzene not only prevented the differentiation, but a single end point corresponding to the total acid present in the system was obtained.

The effect of six electrode combinations on the resolution of salicylic acid and acetylsalicylic acid was explored, using either sodium methoxide or tetrabutylammonium hydroxide as titrant. Dimethylformamide and nitromethane were employed as the titration solvents. The electrode systems and the number of end points subsequently observed for the differentiating titration are listed in Table III. It was observed that the feasibility of the differentiation and the characteristics of titration curves were markedly different from one titrant–solvent–electrode system to another. For example, with dimethylformamide as the titration solvent and sodium methoxide as the titrant, various degrees of differentiation were observed with six electrode couples investigated. As depicted in the family of curves on the left side of Fig. 6, two distinct inflections were obtained with the glass–calomel (curve 1) and the antimony–platinum (curve 4) electrode pairs, one potential break representing total acid was realized with the antimony–calomel (curve 3) electrode system, no discernible inflection was attainable with the platinum–calomel (curve 2) electrode couple, and abnormal titration curves near the second equivalent points were encountered with the antimony–glass (curve 5) and the platinum–glass (curve 6) electrode combinations. When tetrabutylammonium hydroxide replaced sodium methoxide as titrant, as shown in the family of curves on the right side of Fig. 6, all electrode systems, with the exception of the antimony–platinum (curve 4) electrode pair, gave satisfactory but different degrees of resolution for the salicylic acid–acetylsalicylic acid mixture dissolved in dimethylformamide. When titration was performed

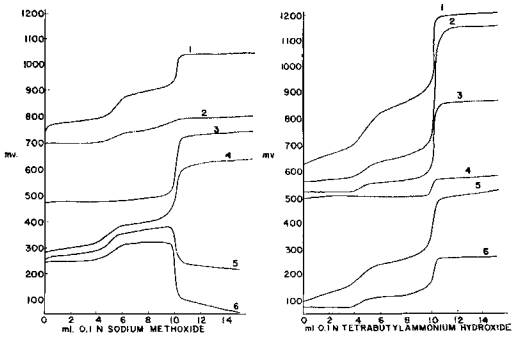


Fig. 6—Effect of electrode combination on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid dissolved in dimethylformamide. Right series of curves were obtained with sodium methoxide as titrant, whereas the left series of curves were obtained with tetrabutylammonium hydroxide. The numbers above the curves correspond to those in Table III.

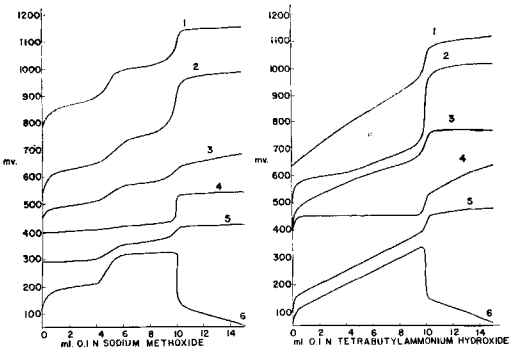


Fig. 7—Effect of electrode combination on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid dissolved in nitromethane. Left series of curves were obtained with sodium methoxide as titrant, and the right series of curves were obtained with tetrabutylammonium hydroxide. The numbers above the curves correspond to those in Table III.

in nitromethane, tetrabutylammonium hydroxide proved unsuitable as a differentiating titrant. As shown in the family of curves on the right side of Fig. 7, tetrabutylammonium hydroxide titrant did not permit the differentiation in any of six electrode combinations studied, whereas the sodium methoxide titrant allowed the differentiation to be accomplished in four out of the six electrode systems investigated, the exceptions being the antimony–platinum (one end point representing the total acid) and the glass–platinum (abnormal titration curve) electrode couples as demonstrated in the family of curves on the left side of Fig. 7. Lin and Blake (25, 26) reported the same phenomenon in the differentiating titration of acetylsalicylic acid and barbiturate combinations dissolved in methyl isobutyl ketone, using glass–calomel or antimony–calomel electrode pairs. They found that resolution was achieved with the sodium methoxide titrant but not with tetrabutylammonium hydroxide as the titrant. Further studies were performed using sodium methoxide as the titrant and acetonitrile as the titration solvent or using tetrabutylammonium hydroxide as the titrant and methyl isobutyl ketone as the titration solvent. However, no differentiation of salicylic acid–acetylsalicylic acid mixtures was observed with any of the six electrode systems investigated.

Quantitative recoveries, together with the corresponding maximum potential change per unit volume of titrant added, for both salicylic acid and acetylsalicylic acid obtained in various titrant–solvent–electrode combinations are tabulated in Table IV. Excellent results were obtained for all combinations. For each titrimetric condition, acetylsalicylic acid offers a sharper potential break than salicylic acid in the differentiating titration, although salicylic acid shows better inflection than acetylsalicylic acid when it is titrated separately. In general, the tetrabutylammonium hydroxide titrant gave sharper inflections than the sodium methoxide titrant for acetylsalicylic acid, the weaker acid presented in the acid mixture to be differentiated, while only slight differences in the sharpness of the inflections for salicylic acid was noted between the two titrants. Another example to illustrate the importance of the suitable solvent–titrant combination on the successfulness of the resolution can be easily deduced from Table III. With the antimony–calomel electrode system, for example, differentiation is readily achieved with a combination of dimethylformamide–tetrabutylammonium hydroxide or nitromethane–sodium methoxide but is unsuccessful with the dimethylformamide–sodium methoxide or nitromethane–tetrabutylammonium hydroxide system.

Since salicylic acid is one of the hydrolytic degradation products of acetylsalicylic acid, the effect of varying the ratio of concentrations of the components on the sensitivity of the differentiating titration was studied. The data for a series of titrations in which the milliequivalent ratio of salicylic acid to acetylsalicylic acid was varied from 1 to 1 to about 12 to 1 are reported in Table V. Typical titration curves obtained with the sodium methoxide titrant are shown in Fig. 8 and those obtained with tetrabutylammonium hydroxide titrant are depicted in Fig. 9. With sodium methoxide as the titrant, two discernible inflections in the titration curve were obtained when the milliequivalent ratio of salicylic acid to acetylsalicylic acid was not greater than about 5 to 1. When the ratio was greater

TABLE IV—DIFFERENTIATING TITRATION OF SALICYLIC ACID AND ACETYLSALICYLIC ACID IN VARIOUS TITRANT–SOLVENT–ELECTRODE COMBINATIONS

Titrant[a]	Solvent	Electrode Pair	Salicylic Acid $(\Delta E/\Delta V)_{max.}$	Salicylic Acid Recovery, %	Acetylsalicylic Acid $(\Delta E/\Delta V)_{max.}$	Acetylsalicylic Acid Recovery, %
Sodium methoxide	Dimethylformamide	Glass-calomel[b]	70[c]	99.14 ± 0.36[d]	550	99.87 ± 0.81
		Antimony–platinum	50	100.72 ± 0.69	800	100.02 ± 0.50
	Nitromethane	Glass–calomel	100	98.87 ± 0.49	300	100.06 ± 0.48
		Platinum–calomel	80	99.50 ± 0.81	400	99.62 ± 0.60
		Antimony–calomel	45	100.71 ± 0.45	60	100.40 ± 0.38
		Antimony–glass	55	98.62 ± 0.48	80	99.89 ± 0.72
Tetrabutylammonium hydroxide	Dimethylformamide	Glass–calomel	80	100.54 ± 0.67	1850	98.98 ± 0.71
		Platinum–calomel	60	101.17 ± 0.50	3150	100.06 ± 0.45
		Antimony–calomel	45	99.42 ± 0.48	2100	99.62 ± 0.82
		Antimony–glass	55	98.62 ± 0.46	900	100.80 ± 0.54
		Platinum–glass	50	97.86 ± 0.72	1000	100.42 ± 0.65
	tert-Butyl alcohol	Glass–calomel	50	100.32 ± 0.52	2500	98.71 ± 0.66

[a] Titrant was prepared in 10:1 benzene–methanol solvent mixture. [b] Electrolyte bridge in calomel electrode was saturated potassium chloride in methanol. [c] Expressed in mv./ml. of titrant added. [d] Standard deviation is based on at least 4 determinations.

TABLE V—EFFECT OF SALICYLIC ACID-TO-ACETYLSALICYLIC ACID RATIO ON SENSITIVITY OF DIFFERENTIATING TITRATION

Curve Ref.[a]	Amt. Weighed, meq. Salicylic Acid	Amt. Weighed, meq. Acetylsalicylic Acid	Recovery, %: Sodium Methoxide Titrant — Salicylic Acid	Recovery, %: Sodium Methoxide Titrant — Acetylsalicylic Acid	Recovery, %: Tetrabutylammonium Hydroxide Titrant — Salicylic Acid	Recovery, %: Tetrabutylammonium Hydroxide Titrant — Acetylsalicylic Acid
1	0.50	0.50	100.71 ± 0.54[b]	98.62 ± 0.68	101.04 ± 0.42	99.92 ± 0.60
2	0.50	0.40	99.46 ± 0.64	99.60 ± 0.50	100.75 ± 0.77	99.77 ± 0.54
3	0.50	0.33	100.40 ± 0.38	99.62 ± 0.71	100.80 ± 0.65	98.42 ± 0.68
4	0.50	0.20	98.79 ± 0.71	101.37 ± 0.82	99.98 ± 0.54	98.32 ± 0.57
5	0.50	0.17	99.98 ± 0.78	100.04 ± 0.62	98.78 ± 0.80	100.57 ± 0.60
6	0.50	0.13	100.84 ± 0.78	99.00 ± 0.92	99.37 ± 0.64	100.10 ± 0.71
7	0.50	0.10	100.32 ± 0.66	100.94 ± 0.90	100.60 ± 0.50	100.94 ± 0.65
8	0.50	0.07	One end point[c]		99.72 ± 0.84	98.70 ± 0.72
9	0.50	0.40	One end point		One end point	

[a] Numbers correspond to curves in Figs. 8 and 9. [b] Standard deviation is based on at least 4 determinations. [c] Corresponds to salicylic acid plus acetylsalicylic acid.

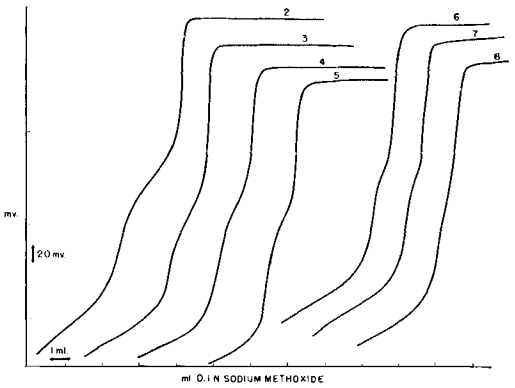


Fig. 8—Effect of salicylic acid-to-acetylsalicylic acid ratio on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid dissolved in dimethylformamide. The numbers above the curves correspond to those in Table V. The titrant was 0.1 N sodium methoxide in benzene–methanol (10:1).

than this, only one end point representing total acid was realized. The curves in Fig. 8 demonstrate clearly the effect of the ratio on the resolution of the two end points. With tetrabutylammonium hydroxide as the titrant, satisfactory resolution was realized when the salicylic acid-to-acetylsalicylic acid ratio was as high as 7 to 1. However, the salicylic acid inflection began to blend with the acetylsalicylic acid inflection at an 11 to 1 ratio. In Fig. 9, curve 8, two end points are clearly defined and recoveries of both components are quantitative. However, in curve 9, only one end point corresponding to the total acid is discernible. Conceivably these ratios could be extended in dimethylformamide by using either more diluted titrants or larger sample sizes.

Water is present as a common impurity in nearly all nonaqueous solvents. The presence of water in the titration solvent can interfere in the titration of acids in protophilic (basic) solvents, of bases in protogenic (acidic) solvents, and of acids and bases in inert solvents. This is due to the amphiprotic nature of water. However, the presence of water in the quaternary ammonium titrant has shown to exhibit a profound stabilizing action on the titrants at a sacrifice of its basic strength (27). The weak acid properties of water are evident in basic solvents, such as dimethylformamide (28, 29), pyridine (30), and ethylenediamine (28). In this investigation, the effect of adding varying quantities of water into the dimethylformamide titration solvent on the sensitivity of differentiation was examined. Equal milliequivalent concentrations of salicylic acid and acetylsalicylic acid were used for all titrations. This requirement was necessary in order to prevent any concentration effect (31). A suitable quantity of water or dimethylformamide was delivered from microburets into the beaker containing accurately weighed samples of the acids. Since a well-defined inflection will be realized for the potentiometric titration of the stronger acid if the solvent employed has good differentiating power, a new and simple method was employed to define the resolution power of the solvent system. This is depicted in Fig. 10 for sodium methoxide titrant and in Fig. 11 for tetrabutylammonium hydroxide titrant. Only a representative set of curves obtained are shown for clarity. The magnitude of the inflection for the stronger acid which is the determining factor for satisfactory differentiation was determined by extrapolating the two straight portions of the

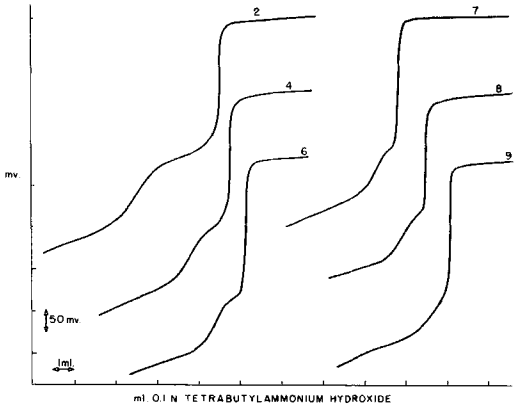


Fig. 9—Effect of salicylic acid-to-acetylsalicylic acid ratio on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid dissolved in dimethylformamide. The numbers above the curves correspond to those in Table V. The titrant was 0.1 N tetrabutylammonium hydroxide in benzene–methanol (10:1).

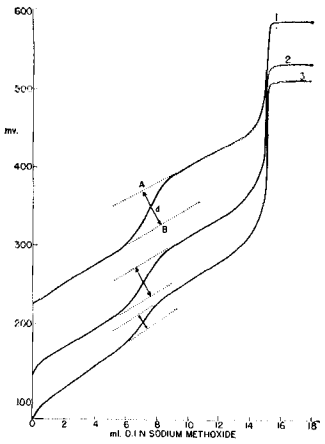


Fig. 10—Effect of water in water–dimethylformamide solvent mixture on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid. Key: 1, 0% v/v water; 2, 6% v/v water; 3, 15% v/v water.

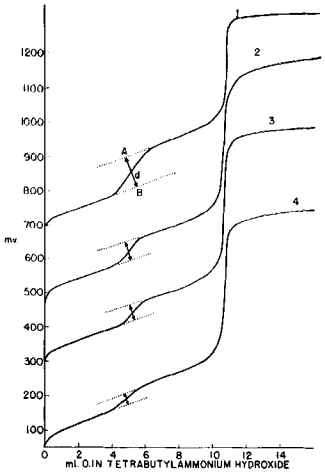


Fig. 11—Effect of water in water–dimethylformamide solvent mixture on sensitivity of differentiating titration of salicylic acid and acetylsalicylic acid. Key: 1, 0% v/v water; 2, 6% v/v water; 3, 10% v/v water; 4, 20% v/v water.

TABLE VI—EFFECT OF WATER CONCENTRATION IN TITRATION SOLVENT ON SENSITIVITY OF DIFFERENTIATING TITRATION

% Water in Water–Dimethylformamide Solvent Mixture	d, cm. Sodium Methoxide Titrant	d, cm. Tetrabutylammonium Hydroxide Titrant
0	2.55[a]	5.08
1	2.40	4.63
2	2.33	4.30
4	2.12	3.75
6	1.97	2.94
8	1.80	2.50
10	1.65	2.12
12	1.41	1.82
15	1.08	1.51
18	0.90	1.33
20	0.75	1.07
25	0.56	0.95

[a] Average of 3 determinations.

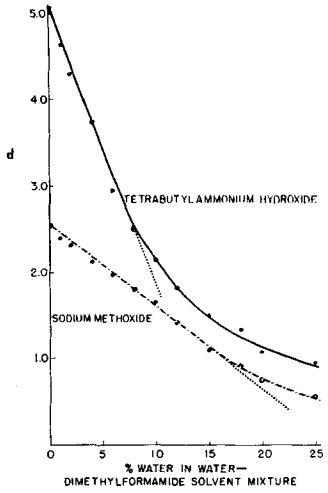


Fig. 12—Relationship between d and concentration of water in water–dimethylformamide solvent mixture during differentiating titration of salicylic acid and acetylsalicylic acid. Glass-modified calomel electrode system was employed.

titration curve and then drawing a straight line through the end point and verticle to the extrapolated line to obtain interception points A and B. The distance of the line AB = d is measured in cm. The magnitude of d obtained in various water–dimethylformamide solvent mixtures is reported in Table VI. The higher the concentration of water in the solvent mixture, the smaller the d value, and the weaker the differentiating ability of the solvent mixture. As expected, water has a detrimental effect on the differentiation. The presence of only a small percentage in the solvent is sufficient to reduce markedly the size and sharpness of the inflections obtained. The potential jump for salicylic acid was reduced gradually upon the progressive addition of water. This is well illustrated in Figs. 10 and 11. Although the recovery is quantitative for both components in the mixture, higher recoveries were obtained when the solvent mixture contained higher water concentrations. However, this is coincided with the increasing blank obtained as the water concentration is increased. When the magnitude of inflection of the stronger acid was plotted *versus* per cent v/v of water in the water–dimethylformamide mixed solvent, Fig. 12 was obtained. At the same concentration of water, larger d values were obtained with the tetrabutylammonium titrant than with the sodium methoxide titrant. This indicates that relatively larger inflections were obtained with the former titrant than with the latter titrant at any particular level of water concentration in the mixed solvent. A linear relationship exists up to 15% of water when the sodium methoxide titrant was employed, as compared to 8% of water when the quaternary ammonium hydroxide titrant was used. The slope of the linear portion of the curve for the quaternary

ammonium titrant is approximately three times steeper than that of the sodium methoxide titrant. This correlation demonstrates that the rate of water tolerance with the sodium methoxide titrant is threefold as resistant as with the quaternary ammonium hydroxide titrant. Since this investigation was designed to provide potentiometric differentiating data for analytical purposes rather than accurate fundamental data, the volume shrinkage during mixing of the solvents and the dielectric constants of such solvent mixtures were not precisely controlled. It is possible that the dielectric constants of such mixtures as measured in the ordinary manner do not represent the effective dielectric constant in the immediate surroundings of the ions. The greater polarity of the water as compared to the dimethylformamide may cause the titration in the immediate vicinity of the ions to be richer in water than the bulk of the solution (32–34). The qualitative pictures obtained in Table VI and Fig. 12 indicate that a progressive increase of dielectric constant with increasing water concentration has a harmful effect on the sensitivity of differentiation.

The proposed nonaqueous differentiating procedures make possible the simple and accurate determination of the combination of acetylsalicylic acid and salicylic acid. Preliminary treatment of the sample is unnecessary, and tedious separation and extraction techniques are obviated. The differentiating titration is achieved in various solvent–titrant–electrode combinations. The technique is applicable even when there is a disproportionate concentration of the components. This work suggests that, by a proper combination of solvent, titrant, and electrode system, it should be possible to titrate potentiometrically both components of acidic or basic mixture whose dissociation constants not only approach but are well below the theoretical limit of 16 proposed by Auerbach and Smolczyk (10).

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