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REVIEW ARTICLE

Determination of the Decomposition of Aspirin

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Keyphrases ☐ Aspirin decomposition—determination ☐ Decomposition products—aspirin ☐ Hydrolysis, aspirin—mechanism, kinetics, pH effect ☐ Analytical methods—salicylic acid in aspirin and aspirin products ☐ Stability—aspirin and dosage forms

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February 27, 1917, the day U. S. Patent No. 644,077 expired, a whole new vista opened to the drug industry. What was, and is, the best seller of all times could easily be classed as the wonder drug of all ages. From the latest available figures of the U. S. Tariff Commission,[1] there would be over 200 tablets available for every man, woman, and child in the United States if all the U. S. yearly production was made into tablets containing 325 mg. (5 gr.) of acetylsalicylic acid, more popularly known as aspirin.

Aspirin has the unique standing in the medical world of still being the most widely used drug, even with the advent of modern, highly potent therapeutic agents. Aspirin has superior qualities as an antipyretic and as a general analgesic, but more specifically in the relief of headaches, muscular pain, postoperative and traumatic pain, postpartum pain, dysmenorrhea, malignancy, colds and respiratory diseases, rheumatoid arthritis, acute rheumatic fever, and in the field of dental analgesia.

With such a versatile drug, it was obvious that aspirin would be combined with other drugs with the result that a more potent and effective preparation would result. It is the purpose of this review to emphasize the plight that continually plagues the analytical chemist in his constant search for a truly reliable method of measuring the decomposition of aspirin in the presence of other drugs or compounds. What makes this problem even more acute is that aspirin is highly selective of the type of compounds it intimately associates with. In fact, if aspirin acquires even a trace of moisture, it begins to fall to pieces. It thus becomes a problem of product development to finalize a stable formulation that will withstand a "normal" shelflife under all types of adverse conditions such as humidity, temperature, and interreactions with other components, even in the solid state.

DETERMINATION OF DECOMPOSITION OF ASPIRIN

When aspirin was first introduced as a drug, controversies ensued almost instantaneously as to how one could characterize truly good aspirin. Some became experts on detecting trace amounts of acetic acid and so classed the elegance of the aspirin accordingly. Even in those early days, the advertising agencies made the most

[1] From 1968 preliminary report: 31,248,000 pounds of aspirin by U. S. production.

of this purely subjective classification. Very few papers have been presented on the quantitative determination of acetic acid as a decomposition product of aspirin, chiefly because of the known volatility of acetic acid. Unless the original container was completely airtight, one would be measuring only the residual acetic acid, which would not be representative of the total acetic acid formed by the decomposition of the aspirin. General methods evolved in which dry air was passed over and through a thin layer of the finely powdered sample. The acetic acid vapor was trapped in the water and then titrated with very dilute sodium hydroxide. A simpler approach utilized a Conway micro diffusion cell. The more refined approach involved GLC.

It is interesting to note that visually the presence of any whiskers (very thin elongated crystals of salicylic acid) observed on the surface of a solid product containing aspirin is definitely an indication that some of the aspirin has decomposed and that the resulting salicylic acid has sublimed through the solid material. Here, again, if the container is not airtight, the possibility exists whereby the released salicylic acid, through sublimation, would leave the sample area and so not be measured. This crucial point, on the sublimation of salicylic acid, will be discussed more thoroughly in this review. As with most subjective tests, the evaluation of solid aspirin products by the appearance of salicylic acid whiskers is limited. Time is required for this sublimation to take place, so one would not normally apply it to fresh products. Therefore, one could actually have a poorly made aspirin tablet which, on the surface, showed no whiskers but internally had a high content of salicylic acid.

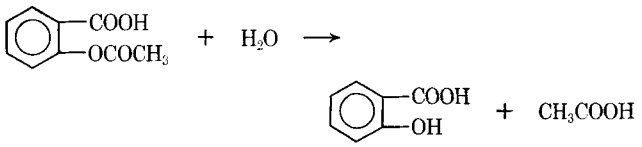
It is the intent of this review, therefore, to pursue a quantitative approach in the determination of the amount of decomposition of aspirin through the presence of salicylic acid rather than acetic acid.

No biological samples, such as blood, containing aspirin will be discussed in this review. Nor will this review include salts of aspirin, such as aluminum aspirin, solutions, or aspirin suspensions. The stability of aspirin in all these cases is definitely limited. The presence of any moisture (with the salts of aspirin, the water of hydration) results in the hydrolysis of aspirin at such a rate that the given formulation does not have a practical or useful shelflife. There is, however, a definite need in the field of pediatrics and geriatrics for a stable liquid formulation of aspirin, because there is no easier or simplier way to give a medicinal than by mouth to infants or to the feeble.

Even limiting this review just to solid preparations leaves a great deal confronting the analyst. A step-by-step evaluation of the approaches published in the scientific literature will be presented. One will readily see the tremendous need for a simple, reliable, universal test for the decomposition of aspirin which can be applied easily and rapidly to a completely unknown preparation containing aspirin.

HYDROLYSIS STUDIES

Decomposition of aspirin results from hydrolysis of the ester group, with the end products being acetic acid and salicylic acid. The oversimplified reaction for the hydrolysis of aspirin is presented only at this time, so one may visualize the overall picture of the decomposition of aspirin (Scheme I).



Scheme I

Those who have made a thorough study of the hydrolysis of aspirin under various well-controlled and stated conditions report that the reaction is very complex. Judged by the number of papers on this subject alone, the reaction is also highly controversial.

The first publication on the hydrolysis of aspirin in water was reported by Rath (1) who conducted his work at an extremely high temperature (100°). As was later found, the hydrolysis of aspirin is very sensitive to temperature changes, even near room temperature. The rate of hydrolysis was determined by titrating the total acidity at stated time intervals. The calculated values indicated a monomolecular reaction. Tsakalotos and Horsch (2, 3) also followed the hydrolysis of aspirin but at more reasonable temperatures (20, 50, and 60°). It took about 100 days to effect complete hydrolysis of aspirin in water at room temperature. Hydrogen ion was found to accelerate the hydrolysis rate, hydrochloric acid being more effective than sulfuric acid. Acetic acid and citric acid caused an initial increase in the rate of hydrolysis; but as the days passed, a decrease in the rate of hydrolysis was noted. The unfounded explanation given by these authors for this slowdown was that the salicylic acid produced was being acylated.

Wolf (4) substantiated the hydrogen-ion effect on the hydrolysis of aspirin by showing that the velocity constant in an acid medium (diluted hydrochloric acid) doubled over that of just water

Aspirin was solubilized in water at room temperature by Morton (5) with the aid of potassium and sodium citrates and acetates. The degree of hydrolysis was followed by titrating the samples with standard alkali at stated time intervals. The rate of hydrolysis was reported to be independent of not only the concentration of the aspirin but also of the solubilizing salt concentration.

Saponification (alkaline hydrolysis) of aspirin was reported by La Mer and Greenspan (6) at 25.000 ± 0.005°. The reaction was stopped by making the given sample (not an aliquot of the bulk solution as is the usual approach in hydrolysis studies) acidic with standard sulfuric acid. The excess acid was then titrated with 0.02 *M* sodium hydroxide. It is assumed that this back titration was conducted immediately; otherwise, hydrolysis of aspirin in the strongly acidic medium would become an unwanted factor in the calculation of the saponification rate. This study showed that aspirin underwent a simple ionic bimolecular reaction with sodium hydroxide in aqueous solution. Thus, a second-order rate constant was calculated.

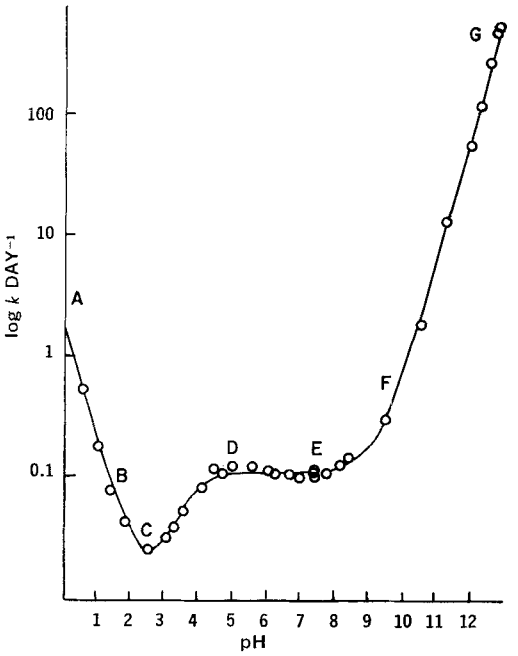


Figure 1—*pH–rate profile for hydrolysis of aspirin.* [*Reprinted, with permission, from L. J. Edwards*, Trans. Faraday Soc., **46**, *723(1950)*.]

Continuing this same approach, Sturtevant (7) analyzed the rate of saponification of aspirin at 35° calorimetrically with the aid of a thermocouple. His conclusion is not often seen in print other than by authors commenting about the previous contributions in relationship to their own work: "The results of these experiments have shown, however, that it would be very difficult to get accurate heat data on these reactions, and it has, therefore, not been considered worthwhile to carry the measurements any further."

A complete and thorough kinetic study of the factors involved in the hydrolysis of aspirin in dilute solution (3×10^{-3} *M*) was conducted by Edwards (8, 9). Using a UV spectrophotometric method for simultaneous determination of aspirin and salicylic acid, he observed the rate of decomposition to be first order at a fixed pH value (between pH 0.53 and 12.77) and constant ionic strength at 17°. Figure 1 depicts the relationship between velocity (rate) constant and pH. This curve was subject to only slight alteration with change in ionic strength. Temperature dependence of this aspirin reaction was studied between 10 and 50°. The pH–rate profile was of the same shape for every temperature, with displacement upward with increasing temperature. This plot of log *k* against pH helps to show visually that the hydrolysis was catalyzed appreciably by hydrogen ion (section AB of figure) and very strongly by hydroxyl ion (section FG of figure). Over the pH range 5–8 (section DE of figure), the rate was constant; in the pH range 2–3 (section C of figure), there was a pronounced minimum rate where the reaction velocity dropped to less than a quarter of the stationary value (DE) which is usually taken to represent the "spontaneous reaction." Edwards explained the relationship between the rate constant and pH on the assumption that the hydrolysis of aspirin may take place *via* the six simultaneous reactions shown in Scheme II.

Table I—Comparison of Hydrolysis Rates of Aspirin in Various Media

Investigator	*k*, Day^{-1}	Medium
Rath (1)	4.35×10^{-2}	Water
Edwards (8)	4.1×10^{-2}	Water
La Mer and Greenspan (6)	7.05×10^{3}	Sodium hydroxide
Sturtevant (7)	7.2×10^{3}	Sodium hydroxide
Edwards (8)	7.50×10^{3}	Sodium hydroxide
Morton (5)	0.103	Potassium citrate buffer about pH 7
Edwards (8)	0.117	pH 7

Through many relationships involving these six equations, the observed unimolecular (first-order) velocity constant could be expressed as a function of the six second-order constants (Eq. 1):

$$k = \frac{k_1 C_H + k_2 C_{H_2O} + k_3 C_{OH}}{1 + K/C_H} + \frac{k_4 C_H + k_5 C_{H_2O} + k_6 C_{OH}}{1 + C_H/K} \qquad \text{(Eq. 1)}$$

When each of the six components of *k* was plotted on a pH–log *k* diagram, four types of curves were obtained. The combination of these four individual curves into one overall curve resulted in a single final curve similar to that depicted in Fig. 1, including the previously inexplicable minimum (C in figure), which Edwards calculated as being at pH 2.44. (The observed minimum was at pH 2.5.)

A comparison of the results of this work (Table I) with those obtained by previous investigators showed good agreement in the rate constant when expressed in the same terminology and conditions.

The hydrolysis of aspirin was accounted for over the whole pH range by considering all the possible bimolecular reactions between the five species present in the equilibrium as: $2H_2O \rightleftarrows H_3O^+ + OH^-$ on the one hand and the equilibrium: $CH_3COOC_6H_4COOH \rightleftarrows CH_3COOC_6H_4COO^- + H^+$ on the other hand.

The mechanism of intramolecular catalysis of the hy-

	k, Day^{-1}
$CH_3COOC_6H_4COOH + H_3O^+ \xrightarrow{k_1} HOC_6H_4COOH + CH_3COOH + H^+$	1.9
$CH_3COOC_6H_4COOH + H_2O \xrightarrow{k_2} HOC_6H_4COOH + CH_3COOH$	$\gg 10^{-6}$
$CH_3COOC_6H_4COOH + OH^- \xrightarrow{k_3} HOC_6H_4COOH + CH_3COO^-$ *or* $HOC_6H_4COO^- + CH_3COOH$	$\ll 10^{6}$ and probably $< 10^{3}$
$CH_3COOC_6H_4COO^- + H_3O^+ \xrightarrow{k_4} HOC_6H_4COOH + CH_3COOH$	46.7
$CH_3COOC_6H_4COO^- + H_2O \xrightarrow{k_5} HOC_6H_4COOH + CH_3COO^-$ *or* $HOC_6H_4COO^- + CH_3COOH$	2.11×10^{-3}
$CH_3COOC_6H_4COO^- + OH^- \xrightarrow{k_6} HOC_6H_4COO^- + CH_3COO^-$	7.5×10^{3}

Scheme II

drolysis of aspirin in the pH region of 5–8 led Davidson and Auerbach (10) to investigate the behavior of aspirin in nonaqueous media. In the presence of dissolved base, aspirin possessed acid anhydride properties and functioned as an effective acetylating agent. They postulated the existence of a cyclic intermediate which results from the intramolecular nucleophilic attack by the ionized carboxyl group on the ester carbonyl. They also postulated that this base-catalyzed isomerization was the rate-controlling step for the hydrolysis of aspirin in the pH 5–8 range. This work was done with organic solvents, and there was no evidence for reactions of this type in aqueous solution.

Ferroni and Baistrocchi (11) measured the rate of hydrolysis of aspirin by determining the liberated salicylic acid fluorometrically. They reported that the reaction followed first-order kinetics and the rate constant was evaluated as 0.0122 $hr.^{-1}$ at 18.5° and pH 9.42.

Hydrolysis rate constants for aspirin, reported by Miyamoto *et al.* (12), agreed with the k_5 rate constant of Edwards (8): 2.625×10^{-3} at 24° and 6.909×10^{-3} at 37°. A general comment was made that aspirin hydrolyzed more quickly in simulated intestinal fluid than in simulated gastric fluid.

Garrett (13), extending the work of Edwards (8) to include a number of acyl salicylates in a very complete investigation, looked more thoroughly into the pH–rate profile of aspirin hydrolysis, particularly in the pH 4–8 range. Edwards' own demonstration that the hydrolysis was not catalyzed by acetate ion (varied from 0.005 to 0.3 *M*) was not consistent with the mechanism involving an attack by a water molecule on the aspirin anion, because the acetate ion is a considerably more powerful nucleophile than water. Garrett's work pointed rather to intramolecular nucleophilic catalysis by the ionized carboxyl group. Even though the carboxylate ion is an unfavorable case from the point of view of nucleophilicity, it apparently participates catalytically in a number of intramolecular catalyses of esters. The hydrolysis of aspirin may be regarded as a classical example. On increasing the alcohol concentration greatly in the pH-independent region of the pH–rate profile, a very unexpected increase was found.

In this light, alcohol would have to be considered a more active nucleophile than water. To clarify this anomalous enhancement of "spontaneous" hydrolysis with increasing alcohol content (0–60%), Garrett made a thorough study. The addition of alcohol to the solvent increased the rate of solvolysis; ethyl acetate was a resulting product. He ruled out the possibility that the rate increase was a generalized solvent effect by showing that the addition of dioxane had very little effect on the rate of hydrolysis of aspirin. He tried to explain his results by proposing a mechanism involving nucleophilic attack by alcohol on the tetrahedral carbon atom of an intermediate compound. This explanation has not been generally accepted. Nevertheless, the demonstration that the addition of alcohol increased the rate of solvolysis did suggest strongly that the question of the involvement of a molecule of solvent in the transition stage ought to be studied.

Using the same four acyl esters of salicylic acid as in the mentioned studies, Garrett (14) studied the stability of their saturated solutions. Prediction of stability in such solutions was made from separate studies of solubility rates and homogeneous rates on dilute solutions of these esters, since solvolytic degradation was a function of these two rates. This study did show that aspirin was the least stable of the esters studied.

Okano and Kojima (15) investigated the effect of salicylic acid upon the rate of aspirin decomposition in solution. They observed deceleration of the aspirin hydrolysis between pH 2.2 and 7 with increasing amounts of salicylic acid being added to the medium. Below pH 2.2 the opposite effect was noted. These studies were conducted for 10 days at 35°, or 8 hr. at 50°, and the rate constants were calculated. The reversal effect of the salicylic acid on the hydrolysis of the aspirin is in the vicinity of the minimum shown by Edwards' (8) pH–rate profile of aspirin hydrolysis.

The hydrolysis of aspirin at pH 6 in water containing 4.3 atom % of ^{18}O produced, after 22 hr. refluxing, salicylic acid containing 6% of the excess ^{18}O in the water. This result was in agreement with the theoretical prediction made by Bender *et al.* (16) and gave backing to the hydrolysis mechanism of aspirin postulated by Garrett (14) and others. This involved an intramolecular attack of the carboxylate ion on the carbonyl carbon atom of the ester to produce acetylsalicyl anhydride, which subsequently hydrolyzes rapidly to produce acetate and salicylate ion, or alternatively that the addition of the carboxylate ion to the carbonyl group of the ester is followed by some reaction with water leading to the same products. It can be calculated from the relative rates of hydrolysis of ethyl acetate and ethyl salicylate that the reaction producing salicylic acid-^{18}O should occur to the extent of 2.5%. The 6% observed was considered to be reasonable and consistent with the postulated reaction in which water was involved.

Using a BPC mixture of aspirin, James (17) investigated the kinetics of the hydrolysis of aspirin from aqueous suspension by comparative, rather than quantitative, means. As long as there was a good excess of aspirin suspension present at the different temperatures, the hydrolysis rate was zero order. Thus, the more concentrated the suspension, the more stable was the aspirin. After 62 days at room temperature, suspensions of 3.3, 6.5, and 13.0% aspirin showed the following percent of intact aspirin remaining: 90, 94, and 97%, respectively. This follows, as the hydrolysis rate depends on the amount of aspirin in solution. Hence, suspensions show a low degree of hydrolysis relative to the total amount of aspirin in suspension.

While James used a titration procedure to follow the rate of hydrolysis in his study, Blaug and Wesolowski (18) used a more refined UV procedure in a pH 3 buffer. This pH was selected because it is the pH of a saturated solution of aspirin (approximately 4 g./l.). The effect of the following additives (calcium gluconate, glycerin, *N*-methyl-2-pyrrolidone, polyethylene glycol 6000,[2] polyvinylpyrrolidone, salicylic acid, sorbitol, pH 3.0 buffer, and water) on the stability of aspirin suspensions (6.5% of 100-mesh and 13% of 60-mesh aspirin) was followed

[2] Carbowax 6000, Union Carbide Corp.

by measuring the salicylic acid content at 298 mμ. The thermodynamic values reported in the paper were calculated from the data obtained with these various suspensions. The suspensions (in duplicate) were stored in a 50 ± 0.5° mechanical shaker for 24 hr. Samples were removed from the suspensions at hourly intervals for assay. Calcium gluconate accelerated the hydrolysis by increasing the pH of the medium, while the *N*-methyl-2-pyrrolidone or glycerin enhanced the solubility of the aspirin, thus increasing the hydrolysis. The presence of saturated salicylic acid did not affect the hydrolysis rate. The most promising additives were polyethylene glycol 6000 and polyvinylpyrrolidone, but physically they were unsatisfactory at this temperature as they formed gummy, insoluble masses of the suspension. Only sorbitol showed any potential stabilizing effect on the aspirin suspension.

In continuing his studies on the effect of alcohol on the hydrolysis rate of aspirin, Garrett (19) synthesized the mixed anhydride of aspirin and acetic acid to help establish his mechanism for the hydrolysis reaction. This compound did not form ethyl acetate as expected in the alcohol medium, so a logical explanation regarding the experimental evidence was still lacking.

Further studies with deuterium oxide solvent isotope effects in the nucleophilic reactions of phenylesters were reported by Bender *et al.* (20). One of the continuing problems associated with the hydrolytic reactions of carboxylic acid derivatives is to distinguish between nucleophilic and general basic catalysis of hydrolysis. The former involves the attack of a nucleophile upon a substrate, leading to the formation of an unstable intermediate which spontaneously breaks down to give the product and regenerates the catalytic entity. The latter catalysis involves the attack of a general base on the substrate removing a proton in a rate-determining stage. Either of these two processes may be carried out by a given substance which, by definition, is at one and the same time both a nucleophile and a general base. The deuterium oxide solvent isotope effect has been used to distinguish between these two possibilities.

The aspirin hydrolysis has been shown, on the basis of kinetic and isotopic experiments, to involve an intramolecular nucleophilic-catalyzed hydrolysis involving an anhydride intermediate. For the purpose of calculation, it has been assumed that the transition state of the reaction was one in which the carboxylate ion has been added to the carbonyl group of the ester, forming a tetrahedral addition intermediate. The formation of this intermediate is, in general, the slow step in the nucleophilic reactions of carboxylic acid derivatives. The authors concluded that the use of deuterium oxide solvent isotope effects as a criterion to distinguish between general base- and nucleophilic-catalyzed reactions was ambiguous; but when applied in a restricted sense, it may be empirically rewarding.

Nogami *et al.* (21) examined the effect of cationic (cetylethyldimethyl ammonium bromide and benzalkonium chloride), anionic (sodium lauryl sulfate), and nonionic (polyoxyethylene lauryl ether) surfactants on the suppression of the hydrolysis of aspirin which exists in anionic and undissociated forms in aqueous solution. The decomposition-rate constants in the buffer solutions (pH 1–7.5) were obtained, with or without the surfactant, and compared. Samples were kept at 37 ± 0.1°, with aliquots being removed at given intervals and assayed for salicylic acid with a ferric nitrate reagent. The color was determined spectrophotometrically at 530 mμ.

The hydrolysis of aspirin was found to follow a pseudo-first-order reaction in the media studied. In the pH 5–7.5 range, aspirin was chiefly in the anionic form. Due to electrostatic attraction, it formed a complex with the cationic surfactant which moved into micelles composed of excess surfactant. Thus, the hydrolysis of aspirin in this pH region was suppressed only by a cationic surfactant. In the pH 1–5 range, all the surfactants suppressed the hydrolysis of aspirin. Because the undissociated aspirin existed in this region, it moved into micelles and was less hydrolyzable. Near pH 1, only the anionic surfactant lost its effect on suppressing the hydrolysis of aspirin. This was explained by the promoting effect of sodium lauryl sulfate on the hydrolysis of aspirin, due to the attracted hydrogen ion on the micelle environment competing with the suppressing effect of the solubilization. Even though this report shows suppression of the hydrolysis of unionized aspirin by all the surfactants and the suppression of the anionic form by cationic surfactants, it by no means implies that these solutions could be used as a stable pharmaceutical formulation.

Nelander (22) reported the heat of hydrolysis of aspirin at 25° by a calorimetric procedure. The $-\Delta H$ (kcal./mole) for aspirin was 25.39 ± 0.03 in 0.8 *N* sodium hydroxide in water–alcohol, 2:3. The heat of solution in aqueous tromethamine solution, ionic strength 0.1, initial pH 8.05, for aspirin was 5.72 ± 0.08 kcal./mole.

The first application of ultrasonic energy in accelerated drug stability studies was published by Mario and Gerraughty (23). Duplicate samples of aspirin in the given buffer (pH 2.00, 4.00, or 5.95) were put in two constant-temperature baths at 21, 25, 35, and 45°, one with ultrasonic energy and the other (control) without. Aliquots from both baths were taken at stated time intervals and assayed for salicylic acid at 302 mμ; the content was calculated from standard curves of salicylic acid in the same buffer. Aspirin runs were done at two different concentration levels. The agreement of duplicate runs was good and indicated that the experimental technique was reproducible.

Pseudo-first-order rates were found at all pH values and with varying temperatures, both with and without ultrasonic energy. The hydrolysis rate constant, k, was calculated. The Arrhenius relationship was followed in all cases, and the heat of activation of the hydrolytic degradation of aspirin was not changed by the introduction of the ultrasonic energy. The increase in the rate found with the ultrasonic samples was equivalent to increasing the reaction mixture temperature within the range of 1.8–2.9°. This range was consistent, regardless of the pH or temperature used. Although the effects of ultrasonic energy were not startling on increasing hydrolysis, these studies did show the potential of this new technique, particularly with heat labile ingredients.

Table II—Apparent Zero-Order Rate Constants of Salicylic Acid at Constant pH

Aspirin plus Lubricant	*k*, mg. FSA/hr.	pH
None	0.123	2.60
Stearic acid	0.133	2.62
Hydrogenated vegetable oil	0.123	2.68
Talc	0.133	2.71
Aluminum stearate	0.281	3.16
Calcium stearate	0.986	3.75
Magnesium stearate	1.314	4.14

Needham and Gerraughty (24) pursued further the hydrolysis of aspirin in mixed solvent systems by ultrasonic energy. The solvent systems were: alcohol–water, 10, 30, 50, and 70%; ether–water, 3 and 5%; and ethylene glycol–water, 5, 10, 30, and 50%. The pH for all of the media was kept at 3.67. Since the thermal energy (use of dual constant-temperature baths) was kept constant for both ultrasonified and control systems, it was apparent that the ultrasonic energy was responsible for the increase in the kinetic rates. With the ethylene glycol–water system, as the concentration ratio was increased, the subsequent increase in viscosity apparently reduced the movement of molecules caused by the ultrasonic vibration, as shown by the smaller rate constants for the hydrolysis of aspirin.

In studying the interaction of aspirin with urea, Santopadre and Bolton (25) shook saturated solutions of aspirin in water at 30° with known varying amounts of urea (0 through 10 *M*) for 5 hr. Kinetic studies were made at pH 2.0, 2.5, 2.75, 3.0, and 3.5 at 30 ± 0.2°. First-order rate constants were calculated. Urea increased the rate of hydrolysis below pH 2.75 and decreased the rate of hydrolysis at pH values greater than 2.75. It is interesting to note that this "crossover" occurs at a pH corresponding to the pH of maximum stability, as reported by Edwards (8). This pH may thus represent a point where the hydrolysis mechanism changes, and this could provide an explanation for the change in the effect of urea.

Murthy and Rippie (26) studied the hydrolysis of aspirin in the presence of polysorbate 80. Saturated solutions of aspirin at 30 ± 0.1° were prepared in 0, 1, 2.5, and 4% solutions of polysorbate 80 at the following pH's: 2.63, 3.63, 4.10, 4.21, and 4.43. Kinetic studies were carried out on suspensions, saturated solutions, and half-saturated solutions for 48 hr. at 30 ± 0.1°. Samples were removed at stated times and assayed by the UV method described by Edwards (8).

With the suspensions, the observed increase in degradation-rate constants (pseudo-zero-order) with added polysorbate 80 was due to the instability of undissociated aspirin in the micellar phase. With the homogeneous solutions, the rate of hydrolysis of aspirin in the polysorbate micelles, while lower than in the aqueous phase, was not negligible. The solubility determinations in the various media showed the absence of dissociated aspirin in the micellar pseudophase of the polysorbate 80 solutions.

Hydrolysis of solubilized aspirin in the presence of the nonionic surfactant, cetomacrogol, was studied by Mitchell and Broadhead (27). All the studies were conducted at 37 ± 0.1° in the pH range of 1–7 on solutions of aspirin with cetomacrogol concentrations of 0.1 through 0.07 *M*.

The hydrolysis of aspirin proceeds as a first-order reaction, both in aqueous buffer and in buffered cetomacrogol solutions. Reaction rate constants were determined. At the pH of maximum stability, pH 2.27, where aspirin exists largely in the unionized form, the half-life increased with cetomacrogol concentration. In 0.07 *M* cetomacrogol, the half-life was approximately twice that in the control buffer. In the plateau region where aspirin is largely ionized, the rate of hydrolysis was independent of cetomacrogol concentration.

Kornblum and Zoglio (28) evaluated the commonly used tablet lubricants as to their effect on the stability of aspirin. Suspensions of aspirin with the various lubricants (talc, hydrogenated vegetable oil,[3] stearic acid, aluminum stearate, calcium stearate, and magnesium stearate) were prepared. The lubricants were also in excess to ensure saturation through the experiments.

The suspensions were maintained at 30°, with appropriate aliquots withdrawn at various time intervals for pH and salicylic acid determination by adding ferric chloride and reading at 540 mμ in a spectrophotometer. From the kinetic studies of these suspensions, the reaction rate appeared to be of zero order. The pH remained relatively constant through the 30-hr. study for the given suspension.

The results are summarized in Table II.

With both calcium and magnesium stearates, the rate of decomposition of aspirin was due to more than just the increase in pH. The authors showed that this increase was due to the high solubility of calcium and magnesium aspirin which were formed in these suspensions. The mechanism primarily involves a reaction of the alkali cation with aspirin in a solution to form a salt of aspirin which, in the presence of solvated aspirin, comprises a buffer system at a pH detrimental to the stability of aspirin.

Reduction of the water content in the aspirin–calcium stearate suspension was done to approach that found in a solid dosage form, the ultimate aim being the achievement of reproducible data which would permit subsequent extrapolation to the tablet or capsule dosage form. A major conclusion from this interesting study is that stearate salts should be avoided as tablet lubricants in preparing aspirin formulations.

In their study on salicylic acid sublimation, Gore *et al.* (29) determined the hydrolysis rates for aspirin at temperatures ranging from 17.2 to 30.2 ± 0.1° at pH 7.4 and reading the resulting salicylic acid at 296.5 mμ. Their data are summarized in Table III. These values are in close agreement with those reported by Morton (5) and Edwards (8).

It is only by pure coincidence, but certainly quite appropriate, that the last papers dealing with hydrolysis of aspirin in this review clarified the situation immensely. In an attempt to circumvent the problems raised by the kinetic equivalence of the several possible mechanisms, Fersht and Kirby (30, 31) looked first at

[3] Sterotex, Capitol City Products, Columbus, Ohio.

Table III—Rate Constants for the Hydrolysis of Aspirin in pH 7.40 Buffer Solution at Various Temperatures

Temperature	k, Day^{-1}
17.2	0.0937
21.3	0.1506
25.5	0.2067
30.2	0.3429

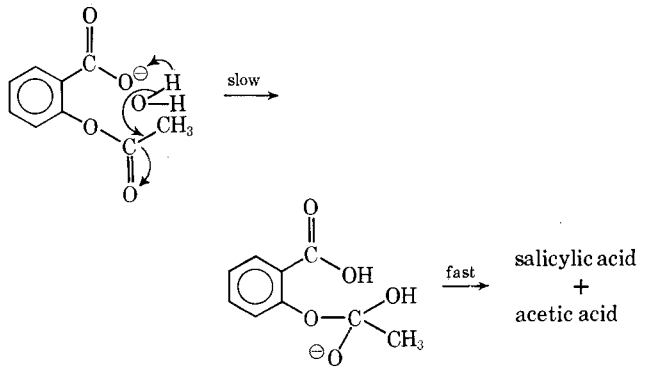
the reactivity toward hydrolysis of a series of substituted aspirins. The results suggested, unambiguously, that the most likely mechanism for the hydrolysis of aspirin was one in which the carboxylate group acts not as a nucleophile but as a general base.

The rate of hydrolysis of aspirin was measured in these studies by following the initial rate of release of salicylate at the isosbestic point, 298.5 mμ, and at 39.0 ± 0.03°. The ionic strength was maintained at 1.0 with added potassium chloride. It was this fact that showed why Edwards (8) failed to detect catalysis by acetate ion, because the ionic strength was not kept constant in his experiments. Fersht and Kirby (30, 31) found that the small acceleration due to the addition of a given concentration of acetate was almost exactly equal to the opposite effect of the increase in ionic strength. If Edwards had only known this fact, there no doubt would have been fewer controversial papers dealing with the mechanism of the hydrolysis of aspirin.

The pH–rate profile for aspirin hydrolysis, measured by Edwards (8), shows that the transition state for hydrolysis in the pH-independent region involves the aspirin anion, either alone in a unimolecular reaction or together with one or more molecules of solvent. Three mechanisms were consistent with this kinetic result for intramolecular catalysis of the hydrolysis of aspirin by the carboxyl group:

1. A unimolecular process in which the carboxylate group acts as a nucleophile. There was no longer any evidence that specifically supported the nucleophilic mechanism. It was not consistent with the effect of substituents on the reaction, and there were several indications that the rate-determining step was not a unimolecular process.

2. A general acid catalysis of the attack of hydroxide ion by the undissociated carboxylic acid group. This mechanism was rejected because intermolecular general acid catalysis by the carboxy group of aspirin should be observed for attack by acetate as well as by the hydroxide ion.



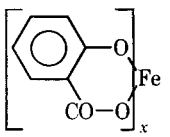
Scheme III—Mechanism of hydrolysis of aspirin as a classical general base catalysis

3. A general base catalysis of the attack of a water molecule by the carboxylate anion. There seems little doubt that the intermolecular reaction of acetate with the aspirin anion represents general base catalysis. There is even less doubt that intramolecular catalysis of hydrolysis by the carboxylate group of aspirin involves the same mechanism as the intermolecular reaction with acetate ion.

In actuality, it seems probable that the aspirin reaction lies close to the borderline between nucleophilic and general base catalysis (Scheme III).

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS WITH FERRIC IRON

Until 1965, the most prominent method of determining salicylic acid in aspirin, or products containing aspirin, was the reaction with ferric iron under a variety of conditions. The complex produced on mixing ferric ion and salicylic acid (a bidentate ligand) results in the formation of a series of intensely colored metal chelates having ligand–ferric-ion ratios of 1:1, 2:1, and 3:1. The formation of the cyclic chelate structure (I) involves the displacement of the weakly acidic, phenolic hydrogen by the metal, resulting in the formation of a six-membered ring by coordination of the metal through the phenolate and carboxylate groups of salicylic acid:



I

In the first of these publications, in 1911 (32), the sample containing aspirin was shaken with water or alcohol and filtered; then one drop of ferric chloride solution was added. On standing, the color changed from reddish to dark violet. This, in essence, was the beginning of the official compendia tests for salicylic acid in aspirin. It was noted very early by Melzer (33) that the presence of either sodium phosphate, tartaric acid, or borax masked the iron–salicylic acid color reaction. The useful suggestion was made that the tablets first be extracted with ether, since only the aspirin and salicylic acid would be extracted; thus a simple separation from the interfering compounds was easily accomplished. This immediately brings to mind that it would be easy to obtain false negative tests for salicylic acid if such a compound was incorporated in the aspirin tablet and no prior separation were made. Before the year was over, Linke (32) had refined the method for determining free salicylic acid in aspirin tablets by comparing the resulting iron–salicylate color to a series of salicylic acid standards. As little as 1 mcg. salicylic acid/ml. could be detected.

A limit test of 0.1% salicylic acid in aspirin was described by Leech (34), using a small volume of alcohol to dissolve the aspirin completely and then diluting with water before the addition of the ferric chloride reagent. This test became the basis for USP procedure when aspirin became official in 1926. It was emphasized that the standard salicylic acid tube should contain the same

amount of alcohol as the sample, because the final color was affected (decreased) by the presence of alcohol. Jones (35) stated that for tablets the limit test should be 0.15% salicylic acid and that there should be no turbidity in the final solutions.

Rather than compare the salicylic acid extract from aspirin, as described, Dahm (36) prepared a permanent color series containing various amounts of cobalt chloride dihydrate. After standardization with known quantities of salicylic acid, one could compare directly, under the stated conditions, aspirin extracts and "read" the percent free salicylic acid (FSA) directly. As these cobalt colors were of a permanent nature, it would save the analyst preparing a fresh salicylic acid reference standard.

Comments about the BP salicylic acid test made by Nutter-Smith (37, 38) brought forth that the official test was not effective below 0.04% salicylic acid, because the ferric chloride reagent was the limiting factor due to its own color. Using ferric ammonium sulfate corrected this situation. The presence of tartaric or citric acid in tablets (1%) has been shown to mask the presence of 0.2% salicylic acid. Thus, if one obtains a negative test for the FSA in unknown aspirin tablets, proper steps should be taken to separate the salicylic acid from the interfering material. Ruddiman (39, 40) added oxalic and tannic acids as masking agents of the iron–salicylate test and commented that sodium phosphate and borax did not interfere with the iron–salicylate test as had been believed. Incompatibility of aspirin with many drugs was proven by Snidow and Langenhan (41) using ferric alum reagent qualitatively.

Valentin and Lieber (42) showed that if ether was used to extract aspirin and salicylic acid from other materials, the evaporation step must be done with care, because too much heat results in high FSA values. They suggested chilling the ether and passing a stream of air over the solution to expedite the evaporation.

The use of a spectrophotometer in the determination of FSA was introduced by Hoffman (43) in 1929. Since the tablets being analyzed contained magnesium oxide, 4 *N* sulfuric acid was added during the grinding of the tablets. This was added to prevent hydrolysis (although not stated, it would also free any salicylic acid which might have been combined with magnesium ion) of the aspirin before the salicylic acid was extracted with a 1:1 mixture of ether and pentane. After evaporation of the clear extract, alcohol was added to dissolve the salicylic acid, and the iron reagent (ferric chloride) was added. This color was then compared with a standard series in the spectrophotometer.

Chloroform was introduced as a direct extractant of aspirin by Hitchens (44). This chloroform extract was shaken with 2% sodium bicarbonate aqueous solution to remove the aspirin (and salicylic acid). This, in turn, was made acidic with hydrochloric acid and extracted quantitatively with ethyl acetate. The ethyl acetate extract was evaporated under reduced pressure in a water bath maintained between 40–45°. The residue was dissolved in alcohol and diluted with water; ferric ammonium sulfate solution was then added. The resulting color was compared with standards of salicylic acid in the same medium. Because hydrolysis does take place in alkaline medium, Hitchens showed that, at 20° for 1 hr. in the sodium bicarbonate solution, about 0.25% of the aspirin was hydrolyzed. At 30°, about 0.35% of the aspirin was hydrolyzed. Since the procedure described took less than 20 min. in the alkaline medium, the error caused by this alkaline hydrolysis was called negligible. With standard runs of aspirin USP, the FSA content was less than 0.15% by this extraction procedure. With various mixes and commerical tablets, the FSA found was never over 0.2%. The purpose of this extraction procedure was to isolate quantitatively the aspirin from compounds such as acetphenetidin, caffeine, acetanilid, antipyrine, amidopyrine, and phenylsalicylate.

In 1937, Banchetti (45) made a critical evaluation of many of the pharmacopeias in regard to their FSA tests which used various ferric iron reagents after extraction of the aspirin and salicylic acid. It was pointed out that the tests should be done at the lowest practical temperature and as rapidly as possible to minimize hydrolysis of the aspirin while conducting the procedure. If evaporation of a solvent extract is required, it should be done with as little heat as possible to avoid excessive FSA values.

The first reported humidity- and temperature-controlled experiments with aspirin tablets in different packagings was conducted by Canback (46). Tablets were stored in wood boxes, tins, impregnated paper, and glass bottles at 20 ± 0.2° for 1 year at various humidity stations (0, 19, 44, 59, 75, and 100% relative humidity). Using 2.5 *M* acid to acidify the pulverized powder, the aspirin and salicylic acid were extracted with a 1:1 mixture of ether and petroleum ether. An aliquot was evaporated, and the salicylic content was determined by dissolving the residue with diluted alcohol. Ferric chloride solution was added, and the resulting color was read in a colorimeter after standing 15 min. After a year at the various humidity stations, the aspirin tablets stored in glass showed very little change in FSA content. The other packagings were greatly inferior with the wooden one being the poorest in regard to aspirin stability. The higher the humidity, the larger and quicker the FSA values increased (other than in the glass bottles where little change was found at any of the humidity stations).

Using a Duboscq colorimeter, Tsuzuki and Sawada (47) measured the amount of FSA produced after aspirin had been heated at 110 and 128°. The heated sample (after 5–35 min. at the stated temperature) was dissolved in methanol, the ferric chloride reagent was added, and the solution was compared to standards. The relationship of increased FSA with a corresponding lowering of the melting point of the aspirin was shown.

Pankratz and Bandelin (48) made a systematic and comprehensive study of the optimum conditions for the reaction of ferric iron and salicylic acid and its reproducibility. Maximum absorption of the ferric–salicylate complex in a nearly aqueous medium was at 525 mμ. This complex was very sensitive to pH changes. On studying pH effect at one pH unit increments from 1.0 through 9.0, the maximum color was found between 3.5 and 8.0. Above pH 6.5 the color faded rapidly, so the useful pH range was between pH 4.0 and 6.0. This emphasizes the point that unless the pH of the sample and of the standard series are close, the equivalent

Table IV—Percent Decomposition of Aspirin of Varying Particle Size after 6 Months[a] at 37°

Crystal Size, mesh	Relative Humidity, % 42	59	84
20–50	0.07	0.08	0.16
50–100	0.08	0.09	0.21
100–200	0.08	0.10	0.59

[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi, *Ann. Rep. Shionogi Research Lab.*, 3, 112(1953).

amount of color will not result. One variable, the alcohol concentration, was not controlled. But at the levels used, it apparently did not affect the linearity of the color to concentration. With such a study, one would have expected a comparison of the different ferric salts, particularly those which have been used in the past such as ferric chloride and ferric ammonium sulfate. Instead, this is the first paper in which ferric nitrate was used.

A study involving just pure aspirin, by Yamamoto and Takahashi (49), answered many questions which arise when one seriously wonders under what conditions aspirin is stable or the most stable. The effect of the particle size of the aspirin on its decomposition rate was studied at 37° over a storage period of 6 months at three controlled humidities. The data in Table IV indicate that the finer the crystals and the higher the relative humidity, the more aspirin hydrolyzed. One may also conclude that under 60% relative humidity at 37°, the percent decomposition of aspirin did not change with particle size. With today's use of micronized aspirin, this is a valuable fact.

In studies at 60° for a total of 25 hr. at 30, 60, 80, and 100% humidities, decomposition was linear with time, and the linearity seemed proportional to the vapor pressure because the slope of each line increased with an increase in vapor pressure. Although the FSA content never exceeded 0.04%, this study showed that an increase in humidity did result in an increase in the decomposition rate.

At 90° and at low humidity, the authors found that, after a total of 12 hr., aspirin decomposition was linear with time but had not surpassed 0.1%. At 120° (low humidity), over 15% of the aspirin was decomposed within 5 hr., and the rate of decomposition was no longer linear with time. This study emphasized the fact that temperature increases alone accelerated the decomposition of aspirin.

Grinding of aspirin for 15–120 sec. did not increase appreciably the percent decomposition. On repeated compression (three times) of aspirin, the percent decomposition was found not to have increased appreciably.

In another paper dealing with the stability of aspirin when mixed with other compounds, Yamamoto and Takahashi (50) found that such mixtures should be stored at the lowest practical humidity to prevent excessive decomposition of the aspirin (Table V).

Table V—Stability of Aspirin in Various Powder Mixtures at 37° for 15 Days[a]

Compound Mixed with Aspirin	Ratio of Aspirin to Compound	Percent Loss of Aspirin at 42% Relative Humidity	84% Relative Humidity
Antipyrine	10:3	1.2	21.1
Aminopyrine	10:3	8.2	33.2
Hexamine	10:3	56.4	83.4
Ethyl aminobenzoate	10:3	32.6	60.8
Caffeine	10:3	0.01	0.08
Zinc sulfate	10:3	0.02	0.03
Sodium benzoate	10:3	3.2	80.2
Sodium salicylate	100:5	—	26.3
Calcium glycerophosphate	10:3	0.4	4.2
Pheniramine maleate	100:3	—	6.0
Phenindamine tartrate	100:3	—	6.4

[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi *Ann. Rep. Shionogi Research Lab.*, 4, 79(1954).

Table VI—Effect of Ethylenediamine Salts on the Stability of Aspirin[a]

Salt of Ethylenediamine	k_1	Loss of Aspirin, %
Hydrochloride	—	0.6
Maleate	1.0×10^{-2}	3.1
Succinate	6.4×10^{-5}	10.5

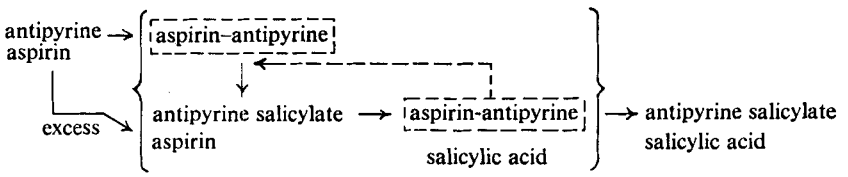
[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi, *Ann. Rep. Shionogi Research Lab.*, 4, 79(1954).

In a comparison of aspirin mixtures with various salts of ethylenediamine, the importance of acid strength on the decomposition of aspirin was shown (Table VI). This study was conducted at 37° and 84% relative humidity for 15 days with a 100:3 ratio of aspirin to ethylenediamine salt.

Further investigation of a 10:1 molar ratio of aspirin–antipyrine mixture stored at 37° and 84% relative humidity for 2 months produced salicylic acid and antipyrine salicylate. The mechanism proposed for this interaction is given in Scheme IV.

Scheme IV was explained in the following manner. First there was an acid–base reaction between aspirin and antipyrine; but because this salt was highly unstable, it decomposed rapidly to antipyrine salicylate. Since aspirin and salicylic acid have approximately the same acidity, the excess aspirin present was capable of reacting with the antipyrine salicylate, releasing salicylic acid and regenerating the unstable aspirin–antipyrine salt which, in turn, started the cycle again.

A similar reaction cycle was proposed for the decomposition of aspirin with pheniramine maleate (amine salt of a weak acid), while no reaction took place with pheniramine hydrochloride (amine salt of a strong acid)



Scheme IV

Table VII—Explanation of Symbols Used in Table VIII

Variable	High Level %	High Level Symbol in Table	Low Level %	Low Level Symbol in Table
Lubricants				
Glycerin monostearate	2	L	0.5	l
Magnesium stearate	2	L	0.5	l
Talc	4	L	1.0	l
Calcium stearate	2	L	0.5	l
Stearic acid	2	L	0.5	l
Mineral oil	4	L	1.0	l
Talc–mineral oil, 1:1	4	L	1.0	l
Pressure	Highest possible	P	Lowest possible	p
Moisture[a]	1.64	M	0.098	m
Aspirin	14-Mesh granules of 10% starch–aspirin granulation	A	40-Mesh crystals	a

[a] Refers to moisture content of the phenacetin–caffeine granulation only.

because aspirin was not capable of displacing the hydrochloric acid.

Without the aid of the computer age, Ribeiro *et al.* (51) undertook a massive, well-executed study of variables in the manufacturing of a stable APC tablet (aspirin, phenacetin, and caffeine). To study the probable causes of the decomposition of aspirin, a factorial experiment was set up testing all combinations of lubricants at two levels, pressure at two levels, moisture at two levels, and two types of aspirin; the entire series was repeated for each of the seven different lubricants (a $2^4 \times 7$ factorial experiment). These variables were selected because they seemed to be the most probable causes of aspirin breakdown in APC tablets. An Association of Official Agriculture Chemists (AOAC) (6th edition) procedure for salicylic acid was modified by using absolute alcohol to extract the salicylic acid from the pulverized tablets and to develop the final iron–salicylate color in a 35% alcohol medium. A spectrophotometer was used to read the resulting color at 537 mμ. Concentrations of salicylic acid were obtained from a standard curve of salicylic acid prepared exactly as the samples. These salicylic acid values were converted to aspirin values and reported in this paper as percent of aspirin content that had decomposed.

Before presenting the data in tabular form, a brief explanation of all the symbols used in the table is essential for proper interpretation of the percent decomposition of aspirin in the tablets after being stored in loosely capped bottles at 45° for 27 days. Tables VII and VIII summarize the results.

Interpretation of these results indicated that more stable combinations were possible if the lubricants used were talc, talc plus mineral oil, mineral oil, or glycerin monostearate rather than stearic acid, magnesium stearate, or calcium stearate. Compression pressure levels showed no effect. Moisture levels were not different enough to be consequential. The crystalline aspirin was superior to the starch–aspirin granulation.

Since difficulties arose in applying the BP colorimetric test for the FSA, Edwards *et al.* (52) initiated an investigation of the kinetics of aspirin hydrolysis and of conditions affecting the formation and stability of the ferric–salicylate complex. Time, as already well known, was an important factor, because aspirin hydrolyzes continually once it is in a given solvent (here alcohol and later water). The need for fast filtration was obvious. Higher temperature expedited the hydrolysis of aspirin, so the lowest practical temperature should be used throughout the test. Constant pH for the test was a

Table VIII—Decomposition of Aspirin after 27 Days at 45° under the Stated Conditions Described in Table VII

Combination	% Aspirin Decomposed[a] Glycerin Monostearate	Magnesium Stearate	Talc	Calcium Stearate	Stearic Acid	Mineral Oil	Talc plus Mineral Oil
MPAL	1.17	8.67	1.10	20.95	8.03	4.11	2.97
MpAL	1.76	8.31	0.97	19.65	8.54	4.59	1.33
MPaL	0.67	7.06	0.55	21.21	0.98	0.99	0.30
Mpal	0.36	2.04	0.00	6.39	0.69	0.39	0.52
MPal	0.33	1.21	0.00	6.95	0.84	0.31	0.22
MpAl	1.63	3.20	2.76	6.20	3.48	4.70	0.87
MpaL	1.05	6.26	0.00	19.54	2.00	0.99	0.75
MPAl	0.99	2.28	2.21	3.84	1.27	2.40	1.16
mPAL	8.56	6.84	1.41	16.04	7.67	1.18	2.66
mpAL	8.59	7.71	2.65	18.35	12.51	2.03	0.58
mPaL	0.96	15.99	0.54	18.54	3.95	0.92	0.87
mpal	0.80	3.39	0.55	3.53	0.44	0.45	0.97
mPal	0.53	3.03	0.48	4.45	1.28	0.90	0.70
mpAl	0.82	2.45	0.49	8.09	2.09	0.68	0.63
mpaL	0.45	15.26	0.73	19.79	4.14	0.93	0.98
mPAl	1.00	3.22	0.73	6.31	3.84	1.06	0.16

[a] The freshly compressed tablets showed negligible decomposed aspirin.

must because the rates of hydrolysis of aspirin vary appreciably with pH. The intensity of the ferric–salicylate complex depends greatly on the pH of the medium. The maximum intensity was obtained between pH 2.5 and 3.5 and was best maintained using an acetic acid–ammonium monochloroacetate buffer. Below or above this pH range the intensity of the color decreased rapidly (intensity at pH 3 was over five times as strong as that at pH 1.5).

As the BP method for FSA is a limit test, a quantitative procedure was developed utilizing the maximum conditions described. If no known interfering materials were present, the aspirin and salicylic acid were dissolved in absolute ethanol diluted with water, all at 25°, and maintained by a thermostated water bath. The time (T_0) was noted when the buffer and ferric ammonium sulfate solution were added and mixed. The volume of the solution was made up to the mark with water, mixed, and kept at 25° for about 10 min. An aliquot was filtered, transferred into an absorption cell, and read at 530 mμ against a reagent blank; the time (T_1) was noted. At least three aliquots were withdrawn at intervals of not less than 10 min. apart. From these values (T_1, T_2, and T_3), a value for the extinction coefficient at zero time (T_0) was obtained by extrapolation. From a standard salicylic acid curve, the amount of salicylic acid at T_0 was thus obtained.

If interfering matter such as phosphate or citrate was present, the aspirin and salicylic acid were extracted from the powdered sample in a separator with benzene. The filtered and pooled benzene was then extracted with small volumes of a solution of the buffer and ferric ammonium sulfate reagent, until an aliquot showed no further coloration in the aqueous extract. The pooled aqueous solution was made to volume with the remaining buffered ferric ammonium sulfate solution and filtered, if necessary, before reading at 530 mμ. The FSA was calculated from a standard salicylic acid curve as before, but without having to calculate a T_0 value; the intact aspirin remains in the benzene layer, so no hydrolysis should be taking place in the aqueous layer.

It is of interest to note that the pH of the aqueous solution (2.95 ± 0.05) read at 530 mμ for both procedures was near the minimum of the hydrolysis rate of aspirin in regard to its pH–rate profile. On comparing the FSA values obtained by using these proposed procedures with those of the BP on several commercial products, the proposed procedure consistently found more FSA, which again raises doubt about the sensitivity of the official BP test for FSA.

Quite independently, Strode *et al.* (53) conducted a systematic study similar to Edwards, only this study involved modifying the USP XV free salicylic test from a limit test to a sensitive, reproducible procedure which was applicable in FSA testing not greater than 0.25%. Hydrolysis curves were constructed from transmittance measurements made at timed intervals on thermostated solutions of aspirin and ferric alum. Under the conditions of the spectrophotometric method, these curves indicated that salicylic acid increased at the rate of 0.0028%/min. at 20°, 0.0036%/min. at 25°, and 0.0054%/min. at 30°. This definitely emphasizes the need for reasonably close temperature control. To obtain reliable readings, 100-mm. cells instead of the usual 10-mm. cells were used. The ferric alum solution in 0.01 *N* hydrochloric acid was kept refrigerated and prepared fresh each week. The calibration standards were prepared so that they would be at the same pH and essentially of the same composition as the sample solutions being measured. This was accomplished by adding an aliquot of freshly prepared aspirin solution in alcohol (SD 30) and water at 25° to individual standard increments of salicylic acid, and noting the time between addition of the ferric alum solution and the reading of final solution (this should be within 5 min.). Through a simplified calculation, a correction for the hydrolysis of the aspirin during this short time interval may be applied. Thus, this is the first time where the salicylic standards contained essentially the same amount of aspirin as the samples being assayed. With colorless aspirin solutions or those from green-tinted formulations, the final iron–salicylate color was read at 515 mμ while the pink-tinted formulations were read at 575 mμ. Appropriate standard curves were run at these given wavelengths. Within the range of concentration measured, the precision and accuracy of this method were within 0.005% salicylic acid at the 95% confidence level.

They also developed a rapid visual method using matched Nessler tubes and a series of salicylic acid standards, which were prepared exactly as the sample in regard to pH, alcohol (SD 30) content, and ferric alum solution. These standard solutions were stable for 2 weeks. The aspirin sample (colorless for this test) was dissolved in alcohol (SD 30), the appropriate aliquot diluted with water cooled to 10°, then treated with the ferric alum solution, and compared within 30 sec. to the standard series of salicylic acid. The salicylic acid content was estimated visually to the nearest 10 mcg. of salicylic acid. By conducting this comparison test so rapidly and at 10°, the hydrolysis error appeared to be within experimental error.

In a thesis and later a publication, Leeson (54) and Leeson and Mattocks (55) made a very thorough study of the decomposition of aspirin in the solid state, utilizing a modification of the AOAC 6th edition procedure which improved the accuracy and sensitivity of the measurements. The aspirin and salicylic acid were dissolved in absolute alcohol. The final color of a given aliquot, which was developed in a 50% alcohol medium, was read in a spectrophotometer at 532 mμ (a slit width of 0.02 mm.) along with a series of salicylic standards treated exactly as the samples.

The step in which the sample or standard salicylic acid aliquot is diluted to exactly 50 ml. with absolute alcohol is an extremely important one. The original procedure consisted of adding the given aliquot to 50 ml. of absolute alcohol, but since the size of the aliquot varied, the concentration of alcohol in the final dilution was not constant. To determine the effect of alcohol concentration on the iron–salicylate color, three different concentrations of salicylic acid were made. They were read over a varying range of alcohol from 5 through 85% alcohol at increasing increments of 10% alcohol. It was readily concluded that the final concentration of alcohol had a significant effect on color intensity. With

Table IX—Stability of Various Aspirin–Antacid Mixtures (2:1)

Antacid	Over 1% FSA Stored for Stated Weeks at RT	Over 1% FSA Stored for Stated Weeks at 37.5°	% FSA after 1 Year at RT	% FSA after 1 Year at 37.5°
Dihydroxy aluminum aminoacetate	52+	52+	0.65	0.70
Calcium gluconate	52+	52+	0.80	0.78
Calcium carbonate	28	8	4.4	11.3
Aluminum hydroxide dried gel	12	4	3.9	6.9
Magnesium carbonate	12	2	11.0	42.9
Magnesium oxide	4	2	18.0	24.0
Magnesium hydroxide	2	2	19.5	38.6
Calcium lactate pentahydrate	36	6	71.0	100.0[a]
Magnesium trisilicate	4	2	100.0	100.0[b]
Dibasic sodium phosphate, anhydrous	16	2	100.0[b]	100.0[c]
Sodium bicarbonate	4	2	100.0[b]	100.0[d]

[a] Within 48 weeks. [b] Within 44 weeks. [c] Within 40 weeks. [d] Within 28 weeks.

the lowest salicylic acid concentration, alcohol content of 30 ml. instead of 25 ml. could introduce an error of 6.7% in FSA value. This error increases greatly with higher concentration of alcohol. For this reason, although a worker may select any alcohol volume desired, he must keep it constant throughout the study for both the samples and standards.

Under anhydrous conditions in sealed ampuls, aspirin (100–140-mesh) with and without calcium stearate (a lubricant in aspirin tablets which has been shown to expedite the decomposition of aspirin) were stored at 35, 45, 60, 80, 100, and 110°. Samples were removed at various time intervals over a period of 50 days and assayed for FSA content. Samples of aspirin alone showed little or no decomposition at 80° or below, while those with calcium stearate decomposed within 2 days to the extent of about 1% FSA and then remained near this level during the remainder of the study. At both 100 and 110°, with or without calcium stearate, the aspirin showed about 2% FSA in 5 days and then decreased gradually with time. As these samples both melted and changed color, it was not known whether the formation of a polymolecular salicylide accounted for the decrease in salicylic acid.

From these studies, it was believed that the small amount of decomposition found could have been caused by traces of moisture, which contaminated the dry aspirin during the filling and sealing of the ampuls. The amount of water necessary to account for the decomposition observed was approximately 10^{-5} moles. The conclusion was thus reached that below 80°, the decomposition of aspirin in the absence of moisture was of minor importance.

Consequently, the role of humidity, or more specifically vapor pressure, on the decomposition of aspirin was studied at various temperatures (50, 60, 70, and 80°) with vapor pressures varying from 46 through 232.5 mm. At various time intervals over a period of nearly 1 year, samples were taken from the given humidistats and assayed for FSA content. Decomposition was noted at all stations. The amount depended on the length of time in the given humidistat, temperature, and vapor pressure. The higher the temperature and vapor pressure, the more rapid was the decomposition.

Tablets containing aspirin, starch, and talc (washed and unwashed) were prepared and studied under similar conditions as the aspirin crystals. The effect of washed talc on the stability of the aspirin was not appreciably different from the talc. The complications arose at various humidistats in that the tablets would liquefy, particularly at the 80° stations (but not below 60°), once the salicylic acid content reached a critical level. Once liquefaction occurred, the salicylic acid content decreased sharply and the study with that humidistat was discontinued. Along with previous workers' conclusions, Leeson showed that the compression into tablets did not change the mechanism of decomposition.

The widespread use of aspirin in combination with various antacid compounds as buffering agents led Bandelin and Malesh (56) to study the stability of aspirin with 11 commonly used antacid compounds. Using a modification of the method of Pankratz and Bandelin (48), previously discussed, the FSA content of powder mixtures of two parts aspirin to one part antacid powder, after being stored at room temperature or at 37.5° for periods of time up to 1 year, were assayed at stated time intervals. The antacid powders were used directly from the commercial container so they were not pretreated or dried in any way before using. Table IX summarizes their results.

Both dihydroxy aluminum aminoacetate and calcium gluconate were definitely superior to the other antacids studied in regard to "available" FSA. The word available is used with the FSA reported in that the mixture assayed was extracted directly with acetone. It was not shown, or stated, if aluminum, calcium, or magnesium salicylate was formed during the decomposition of the aspirin, or if the acetone would dissolve these salts. Further, if they did dissolve, would the ferric iron replace the cation in the 50% acetone medium in which the iron–salicylate color was developed?

The unusual was done by Wirth (57) in that he followed the USP XV procedure for FSA *without* any modifications when assaying APC tablets. The FSA values reported were acceptable.

Using the procedure of Ribeiro *et al.* (51), Kral *et al.* (58) studied various mixtures of drugs commonly given with aspirin. Samples of the various mixes were kept at four different stations: room temperature, anhydrous state at room temperature, 97% relative humidity at room temperature, and 37° for periods up to 6 months. The individual mixtures of aspirin with phenacetin, caffeine, phenobarbital, dextrose, sucrose, or lactose were classed as being stable, while those with

Table X—Effect of Amphetamine Salts on the Stability of Aspirin

Amphetamine Salt	pK of Parent Acid	
Picrate	0.38	Increase in accelerating decomposition of aspirin
Acid oxalate	1.19	
Sulfate	1.92[a]	
Acid maleate	2.00	
Acid tartrate	3.02	

[a] Second dissociation constant.

antipyrine, amidopyrine, and quinine hydrochloride decomposed slightly. Mixtures of sodium bicarbonate, hexamethylenetetramine, and caffeine sodium benzoate decomposed rapidly.

In his thesis, Lippmann (59) used the colorimetric procedure developed by Leeson (54), but substituted 95% alcohol for absolute alcohol in his studies of aspirin decomposition with various amphetamine salts. Mixtures of 100 parts of aspirin (50–70-mesh) and 2.935 parts of amphetamine salt as base (60-mesh) were stored in humidity cabinets at 73° and 67% relative humidity. Amphetamine salts used were sulfate, diphenylacetate, *p*-aminobenzoate, 2-naphthoate, phthalate, and picrate. The phthalate salt increased the rate of decomposition of the aspirin the greatest; the order was as follows: phthalate > diphenylacetate = *p*-aminobenzoate = 2-naphthoate > picrate > sulfate.

A relationship between pK of the parent acid in the amphetamine salt and rate of decomposition of aspirin (Table X) correlated well with the acid strength discussed by Yamamoto and Takahashi (50).

Four different tablets of aspirin were manufactured by Nazareth and Huyck (60) to study the effect of calcium salts on the stability of aspirin. The tablets were stored at 9–12, 25–30, and 45° for a period of 8 weeks. Samples were removed weekly and assayed for salicylic acid by essentially the method of Pankratz and Bandelin (48), reading the final color in a 20% alcohol medium. Table XI summarizes their work.

It was noticed that when the percent FSA was over 6, needle-shaped crystals (whiskers) of salicylic acid appeared on the sides and neck of the container. This study showed that aspirin was unstable in the presence of either calcium carbonate or calcium succinate. This was in agreement with Yamamoto and Takahashi (50), who found that the presence of a salt of a weak acid accelerates the decomposition of aspirin.

Continuing the same type of study, Nazareth and Huyck (61) studied the stability of aspirin in four differently manufactured APC tablets. The tablets were stored for 5 weeks and samples removed weekly for the FSA assay.

Only Tablet A in Table XII showed a rapid decomposition of aspirin. It was the only tablet containing magnesium stearate as a lubricant.

As DeMarco and Marcus (62) did not require the sensitiveness described by Leeson (54), they modified the iron reagent to account for larger amounts of salicylic acid and still adhere to Beer's law (Table XIII).

Reagent No. 1 was used by Leeson and was shown to be very sensitive to the alcohol concentration. Reagent No. 4 was recommended, as it was not only insensitive

Table XI—Stability of Various Aspirin Tablets

Tablet	Weeks of Storage before 0.5% FSA Found at 9–12°	25–30°	45°	% FSA after 8 Weeks at 9–12°	25–30°	45°
I. Aspirin	8+	8+	7	0.3	0.4	0.5
II. Aspirin + calcium carbonate	7	2	1	0.5	1.2	14.0
III. Aspirin + calcium succinate	5	1	1	0.6	1.0	88.5
IV. Aspirin + calcium carbonate and succinate	4	1	1	0.6	1.2	96.7

to alcohol concentration changes, but the intensity of the iron–salicylate complex was greatly increased. Reagent No. 7 emphasized the need for acid in the color development medium.

Okano *et al.* (63) conducted a factorial experiment ($2^8 \times 4$) like Ribeiro *et al.* (51) and obtained essentially the same conclusions. Of the lubricants, talc, edible oil, and stearic acid were better than magnesium stearate or calcium stearate at 56°. Room relative humidity caused less decomposition than 84% relative humidity. Storage temperature at 45° caused more aspirin decomposition than room temperature (10–20°). Presence of lactose or diphenylpyraline hydrochloride resulted in an increase in aspirin decomposition. Little effect was noticed on the stability of aspirin, with or without starch, whether it was prism or needle form. The number of times the tablets were compressed at different pressures did not affect the aspirin.

In continuing their studies on the stability of aspirin with other drugs or compounds, Patel and Huyck (64) manufactured aspirin tablets with and without aluminum hydroxide dried gel USP. The tablets were stored for nine weeks and samples removed weekly for FSA assay as previously described.

The results in Table XIV are in agreement with the work reported by Bandelin and Malesh (56).

In two papers, Grabowska (65, 66) reported on the stability of aspirin with other drugs as powders or tablets after a year's storage. No decomposition of aspirin was reported when mixed with caffeine, quinine sulfate, codeine phosphate, phenobarbital, phenacetin, carbromal, urea, or *p*-aminobenzoic acid.

Slight decomposition was reported with quinine hydrochloride or sodium benzoate. The presence of sodium phenobarbital, codeine base, or caffeine sodium benzoate greatly accelerated the decomposition of the aspirin. Four stabilizers, magnesium oxide, aluminum hydroxide, calcium carbonate, and calcium gluconate, were mixed individually with aspirin, sodium phenobarbital, and caffeine mix, and aspirin, caffeine, and so-

Table XII—Stability of Various APC Tablets

Tablet	% FSA after 5 Weeks at 9–12°	25–30°	45°
A	0.6	1.2	16.0
B	0.1	0.2	1.0
C	0.1	0.2	0.6
D	0.1	0.2	1.3

Table XIII—Effect of Iron Reagent and Percent Alcohol on the Iron–Salicylate Color

Iron Reagent	ml. Iron Reagent Added/100 ml. Solution	% Alcohol in Final Solution	Adherence to Beer's Law at 532 mμ; mcg. Salicylic Acid/ml.
1. 2% Ferric ammonium sulfate in 0.125 *N* hydrochloric acid	2	50	10–20
2. Same as No. 1	5	50	10–60
3. Same as No. 1	5	10	10–80
4. 1% Ferric chloride in 0.1 *N* hydrochloric acid	5	50	10–80
5. Same as No. 4	5	10	10–80
6. 0.5% Ferric chloride in 0.1 *N* hydrochloric acid	2	10	10–50
7. 0.5% Ferric chloride, no acid	2	10	None

dium benzoate mix. Magnesium oxide was best with the former mix, while aluminum hydroxide, calcium carbonate, or calcium gluconate was effective in stabilizing the aspirin in the latter formulation.

Control methods used in the Australian pharmaceutical industry for FSA in aspirin formulations were described by Green (67). The method of Strode *et al.* (53) was used in determining the FSA content in aspirin and aspirin–starch formulations. The results were in line with the BP FSA limits. If the formulation contained magnesium hydroxide, it was necessary to release the salicylic acid from its magnesium salt by first adding an aqueous acid and extracting the salicylic acid immediately with 1:1 pentane–ether solution. After evaporation of the mixed solvent the residue was dissolved in alcohol and assayed.

The effect of selected USP talcs on the stability of aspirin in tablets was reported by Gold and Campbell (68), utilizing a direct benzene extraction of the pulverized tablets. After vigorous shaking and centrifuging until clear, an aliquot was shaken with a ferric ammonium sulfate reagent. The clear aqueous layer, after centrifuging, was read in a colorimeter at 515 mμ. FSA content was calculated from a standard series of salicylic acid treated as the sample. Three series of tablets were prepared. The first used the talc, as is; the second used acid-washed talc. The third series used the best of the four talcs, Talc A, plus known amounts of various impurities (aluminum silicate, red iron oxide, calcium silicate, and calcium carbonate which were mixed individually with the talc to prepare the aspirin tablets).

The data indicated that the four USP talcs were different in regard to FSA formation after being stored at 40° and 90% relative humidity for 12 weeks (FSA varied from 0.8 to 25.8%). The decomposition, however, did not appear to be directly related to the pH of the talc. The acid washing of the talc before use did improve the aspirin stability greatly, particularly with Talc C. Of the added impurities to Talc A, the presence of aluminum silicate or red iron oxide did not significantly affect the stability of the aspirin, while both the calcium salts (carbonate being the worst offender) influenced appreciably the rate of decomposition of the aspirin.

Table XIV—Aspirin Dosage Form Stability

Tablet	% FSA after 9 Weeks at 10°	% FSA after 9 Weeks at RT	% FSA after 9 Weeks at 37.5°
Aspirin	0.13	0.13	0.16
Aspirin and aluminum hydroxide dried gel	1.16	2.04	3.30

Though the main interest of Troup and Mitchner (69) was on degradation of phenylephrine hydrochloride in tablet formulations containing aspirin, they did assay for FSA according to the Gold and Campbell (68) procedure. For the degradation of phenylephrine in aspirin-containing tablets to occur, the breakdown of the aspirin was prerequisite. This was accelerated greatly by the presence of magnesium stearate as a lubricant in the manufacturing of the tablets. For any one formulation held at elevated temperature (usually 70°), the increase in salicylic acid content plotted against the decrease in phenylephrine content gave a linear relationship.

The effect of four granulating solvents in the manufacturing of aspirin tablets was reported by Trose and Danz (70). Granulations prepared with 95 and 70% alcohol or spiritus gelatine did not increase the decomposition of the aspirin, while the use of 5% gelatin mucilage did. The FSA content was found by extracting the pulverized sample with alcohol, filtering, diluting with water, adding ferric chloride reagent, and reading the resulting color at 530 mμ in a colorimeter. If phosphates are present in the formulation, a preliminary extraction and evaporation must be made with 1:1 ether–petroleum ether mixture.

Jaminet and Evrard (71) evaluated the effectiveness of precirol (a glyceryl palmitostearate), a binder lubricant, in the manufacturing of aspirin tablets. After 3 months at 50° and 80% relative humidity, no FSA was reported from the tablets using this substance. The FSA was determined in the pulverized tablets by first extracting with 1:1 ether–petroleum ether solution, filtering, evaporating, dissolving the residue in alcohol, diluting with water, and adding a ferric ammonium sulfate reagent. The solutions were read at 520 mμ.

The effects of humidity on aspirin, on its mixtures and tablets, with five different fillers were reported by Wisniewski and Piasecka (72). The FSA was determined by dissolving the aspirin in alcohol, filtering if

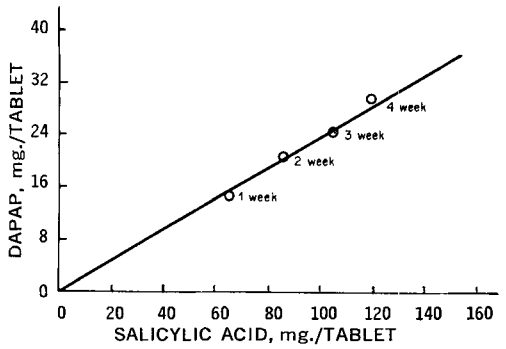


Figure 2—*Relationship between the rate of formation of salicylic acid and of DAPAP.*

required, and adding an aliquot to a Nessler cylinder containing water and the ferric ammonium sulfate reagent. These were compared with the prepared salicylic acid standard solutions. The samples were stored over a period of 4 months at 93, 58, and 20% relative humidity along with changing humidity conditions in the laboratory. The decomposition at 93% relative humidity took place in the following decreasing order of filler used: magnesium oxide > magnesium carbonate > calcium carbonate > magnesium stearate > no filler. This same decomposition order existed at 58 and 20% relative humidity, along with ambient conditions. It is one of the few times that magnesium stearate has not led the list in degree of decomposition of aspirin. During these studies, it was shown that alcohol concentration affected the final color, as did varying amounts of acetic acid. None of the fillers used altered the final color.

In a paper similar to that of degradation of phenylephrine (69), Koshy *et al.* (73) reported on the acetylation of acetaminophen (APAP) in tablet formulations containing aspirin. With tablets stored at 50° for a period of 6 weeks, the formation of diacetyl-*p*-aminophenol (DAPAP) indicated a linear relationship between the rate of formation of salicylic acid through a 4-week period (Fig. 2). The same trend existed in various samples stored at ambient conditions, although at lower salicylic acid and DAPAP levels. Commercial tablets of unknown age, purchased from retail outlets and containing both APAP and aspirin, showed this same relationship of aspirin decomposition and formation of DAPAP. As one of these lots showed high DAPAP, a study was conducted at 50° for 4 weeks on the effect of stearic acid in one and magnesium stearate in the other. The magnesium stearate mix produced nearly 1000 times as much DAPAP as the stearic acid or regular mix.

Continuing their study on the stability of aspirin, Jaminet and Louis (74) made tablets with various lubricants. On assaying these tablets after being stored 6 months at 50° and 80% relative humidity, the lubricants may be listed in order of increased amounts of salicylic acid being found. Magnesium stearate was the poorest lubricant in that nearly 90% of the aspirin was decomposed, the next being polyethylene glycol 6000 (about 15% being decomposed) > glycerol monostearate II > stearic acid > stearyl alcohol > glycerol monostearate I > precirol = geleol. The last two lubricants were the best in protecting the aspirin from decomposing under these conditions.

Table XV—Effect of Common Active and Inactive Aspirin Product Components on Color Development[a]

Component	Component–Salicylic Acid Ratio	Interference
Lactose	40:1	Nil
Meprobamate	100:1	Nil
Methylcellulose	40:1	Nil
Polyvinylpyrrolidone	20:1	Nil
Phenacetin	60:1	Nil
Calcium stearate	10:1	+20%
Magnesium stearate	10:1	+20%
Stearic acid	10:1	+30%
Alginic acid	10:1	Nil
Ion-exchange resin[b]	10:1	Nil
Caffeine	40:1	Nil
Ethoheptazine citrate	20:1	Nil
	100:1	−15%
Citric acid	15:1	−20%
Dihydrocodeine	20:1	Nil
Codeine phosphate	20:1	Nil
Phenergan HCl	3:1	+100%
Hydrogenated vegetable oil[c]	10:1	Nil
Talc	20:1	Nil
Meperidine HCl	40:1	Nil
Starch	70:1	Nil

[a] Reprinted, with permission, from L. F. Cullen *et al.*, *Ann. N.Y. Acad. Sci.*, **153**, Art. 2, Table 1(1968). [b] Amberlite, Rohm & Haas Co. [c] Sterotex.

It is befitting that the last paper using an iron reagent would be titled "An Automated Colorimetric Method for Determination of Free Salicylic Acid in Aspirin-Containing Products," by Cullen *et al.* (75). The number of salicylic acid determinations required in the development of a stable aspirin product, or in production quality control, can be overburdening. The need to automate the salicylic acid analysis was, and is, great, as the saving in analytical time, effort, manpower, and expense can be tremendous.

The method selected for automation was that of Pankratz and Bandelin (48), as they had reported on the optimum conditions needed in order to obtain the greatest accuracy and reproducibility in the quantitative determination of salicylic acid in pharmaceutical preparations. A flow diagram (Fig. 3) is presented at this time only to show the equipment arrangement utilizing a standard Technicon automated system which was programed at 15 samples/hr. Specificity studies are summarized in Table XV.

Turbidity accounted for the high values given by the presence of calcium and magnesium stearate and stearic acid. The quenching effect (negative interference) was brought about by citric acid which forms a nonionized salt with iron. Phenothiazine derivatives react directly with the ferric iron and so must be removed if present in the aspirin formulation. Linearity of the salicylic acid in the microaperture flow-through cells was excellent. The precision of standard salicylic acid showed a repeatability deviation of 1.5%, while replicate samples of a commercial tablet were within 2%. Good accuracy was demonstrated by recovery of known amounts of standard salicylic acid added to powdered commercial tablets and by comparing this to the manual procedure.

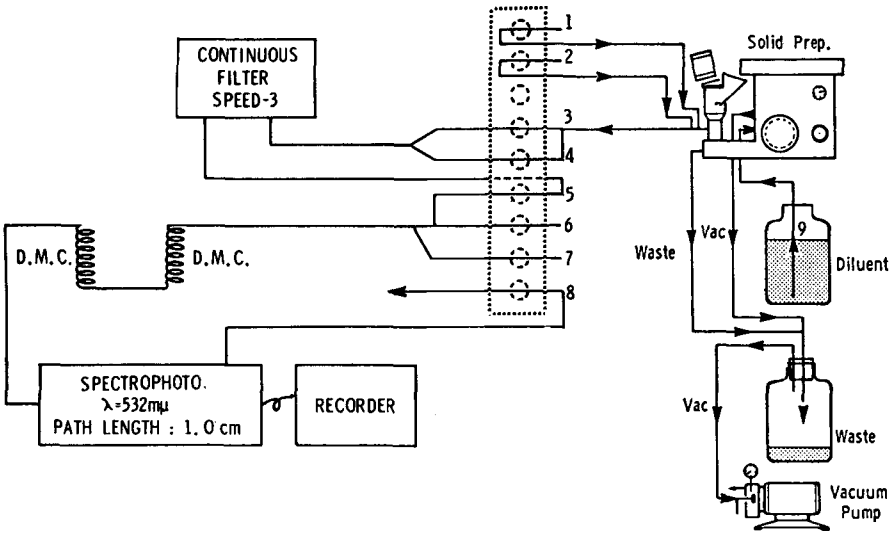


Figure 3—*Flow diagram for the determination of salicylic acid in aspirin-containing products. Key: 1, 0.8 ml./min. air; 2, 4.06 ml./min. SDA No. 30 alcohol; 3, 2.76 ml./min. sample; 4, 2.03 ml./min. sample; 5, 2.76 ml./min.; 6, 2.76 ml./min. $Fe(NO_3)_3$ reagent; 7, 2.00 ml./min. air; 8, 2.50-ml./min. flowcell; and 9, reservoir-SDA No. 30 alcohol. [Reprinted, with permission, from L. F. Cullen* et al., Ann. N. Y. Acad. Sci., *153, Art. 2, Fig. 1 (1968).]*

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS BY UV SPECTROPHOTOMETRY

Until Tinker and McBay (76) introduced their simple and rapid spectrophotometric method of analysis for aspirin and salicylic acid, two separate determinations had to be made for the intact aspirin and hydrolyzed aspirin. In selecting the solvent for the UV studies, they found that in using chloroform A.R. grade, both aspirin and salicylic acid had higher absorbances and stabilities than in either aqueous or alcohol solutions. Also, the greatest difference between maximum and minimum absorbance values for any given concentration was observed in chloroform. The maximum absorbance for aspirin and salicylic acid was found to be 278 and 308 mμ, respectively, as shown in Fig. 4. Beer's law was conformed to in the concentration ranges used. Equations were developed for this two-component mixture, and the application to aspirin, aspirin tablets, or capsules was valid and had an error of less than 0.2%. Samples of aspirin were dissolved in chloroform (filtered if necessary) and read at 308 mμ for salicylic acid content. A portion of the bulk solution was diluted 100 times and then read at 278 mμ for aspirin content. Both readings were against a chloroform blank.

Ebert (77) found that the existing methods for determining amphetamine sulfate, phenacetin, and aspirin in the corresponding tablets involved many laborious time-consuming extractions. After the separations were completed, lengthy titrations or a gravimetric procedure were required. With all this work, very little, if any, reliable information was available in regard to subtle changes such as the presence of salicylic acid which could be formed from the decomposition of the aspirin. Ebert, with meticulous care, designed a three-component spectrophotometric method for the simultaneous determination of aspirin, phenacetin, and salicylic acid. It was not only a more rapid method of analysis, but was found to give increased accuracy and precision. Alcohol was selected as the solvent of choice. In this medium, aspirin was found to have an absorption maximum at 226 mμ, salicylic acid at 235 mμ, and phenacetin at 250 mμ. As there was a great deal of overlapping of the three absorption curves, it was important to prove that the absorbance of this mixture, particularly where the aspirin showed 0, 50, and 100% hydrolysis, represented the sum of the absorbances of the individual compounds comprising the mixture at that wavelength. The validity of this was well proven by Ebert's work. From all these basic data, the appropriate validated equations were derived. The equations were then tried on tablets which had been extracted with ether. The three compounds in alcohol were then determined spectrophotometrically. The estimate of the accuracy and precision compared favorably with those methods used in the past. Though the major emphasis of this work was on the stability of sympathomimetic amine salts when combined with aspirin and phenacetin, interesting facts evolved about the stability of aspirin in these combinations. It might be added that in all the stability studies conducted here, phenacetin did not show any appreciable change.

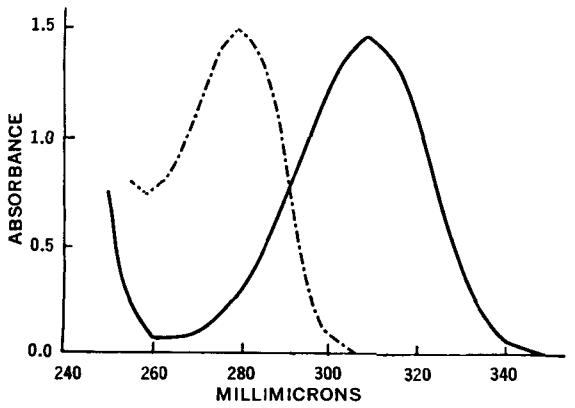


Figure 4—*UV spectrocurves of aspirin (– · –) and of salicylic acid (——) in chloroform.*

Stability studies on tablets containing amphetamine sulfate, aspirin, and phenacetin were conducted in regard to the effects of temperature, moisture, and lubricants on these compounds. All the tablets were packaged in loosely capped amber bottles. These bottles were stored under the following conditions: room temperature, 0% relative humidity; room temperature, 95% relative humidity; 43°, 0% relative humidity, and 43°, 95% relative humidity. After a year's storage and many assays, the important finding was that aspirin decom-

position appeared to be associated with the disappearance of the amphetamine sulfate. The aspirin decomposition appeared to be dependent upon the lubricant present in the following decreasing order: magnesium stearate > talc and magnesium stearate > stearic acid > talc. The increase of temperature accelerated the decomposition of aspirin, but the increase of humidity at the same temperature had a much greater effect. Methamphetamine hydrochloride was substituted for the amphetamine sulfate in a similar stability study, and the conclusions were the same as mentioned for amphetamine sulfate. It is of interest to note that no aspirin decomposition occurred in any of the formulas stored at room temperature, 0% relative humidity, regardless of the ingredients present. In another study, salicylic acid replaced aspirin in the tablet with the result that it had little or no effect on the amphetamine sulfate.

Ebert checked on the possibility that aspirin might be capable of acetylating amphetamine. Working with the pure compounds, he isolated acetylamphetamine which proved this belief. The addition of an equivalent amount of salicylic acid and acetic acid in place of the aspirin in that study did not result in the isolation of any acetylamphetamine. Isolation of any acetylamphetamine from the tablets studied was not successful. Today, with the aid of TLC and GLC, such a decomposition product could readily be detected quantitatively.

Though Leeson's work with aspirin (54) involved chiefly a colorimetric procedure, he used the UV procedure developed by Ebert. This was done to check that no loss of salicylic acid (through volatilization) from the vials on stability had occurred. As the tablets used here did not contain amphetamine sulfate or phenacetin, the interpretation required was simpler. Absolute alcohol was used in place of the 95% alcohol. This would assure better stability of the aspirin during the assay. Readings were taken at 226 mμ (slit width of 0.8 mm.) and at 235 mμ (slit width at 0.6 mm.) against absolute alcohol. On assaying many tablets randomly, the summation (in moles) of the aspirin and salicylic acid found accounted for all the aspirin originally employed. In short, no salicylic acid was being lost through volatilization under the storage conditions.

In the first of a series of many interesting and valuable publications dealing with the determination of the stability of aspirin in many products, Levine (78) introduced a rapid chromatographic assay for APC tablets which included the determination of salicylic acid. Previous papers using this column technique did not discuss the determination of salicylic acid.

The pulverized APC sample was extracted with chloroform containing a small amount of acetic acid (to convert any aspirin salt to the free acid and so be extracted by the chloroform). Without filtering off the insoluble excipient material, an aliquot was diluted with freshly water-washed ether and passed directly to the single duplex column which had just been washed using water-washed ether. The upper segment of the column contained 1 *N* sodium bicarbonate on diatomaceous earth[4] which acted as the supporting phase. The sodium bicarbonate trapped both the aspirin and salicylic acid. The lower segment of the column contained 4 *N* sulfuric acid on diatomaceous earth. The sulfuric acid retained the caffeine while the phenacetin passed through into an evaporating dish. Water-washed ether was passed through the column in small portions to elute quantitatively all the phenacetin. A volumetric flask was placed under the column and sufficient water-washed chloroform was passed through the column to elute quantitatively the caffeine. Immediately, the column was eluted with acetic acid in chloroform (previously water-washed before adding the acetic acid) into another volumetric flask. This last eluate was read immediately in a suitable spectrophotometer at 280 mμ for aspirin and 310 mμ for salicylic acid, as aspirin was not stable in this medium. The caffeine was read at 276 mμ while the phenacetin was evaporating. The residue in the evaporating dish was dissolved in a little chloroform and then diluted with isooctane in a volumetric flask and read at 285 mμ.

This entire procedure, from grinding of the tablets to the final UV readings, took less than 1 hr. Though it was not emphasized, the aspirin should not be left on the column any longer than necessary, as it has been found to hydrolyze readily. From experience it has been found that the phenacetin may be dissolved and diluted with chloroform; thus another solvent (isooctane) is unnecessary.

In calculating, standards of the three drugs and salicylic acid are read in the UV at the stated wavelength in the same medium as the sample. For practical purposes, it was assumed that aspirin's absorbance at 310 mμ was negligible (100 mcg. aspirin/ml. read 0.010). Thus, any reading at 310 mμ was calculated as salicylic acid. From this absorbance value at 310 mμ, the absorbance due to the salicylic acid at 280 mμ can be calculated from the values of the standard salicylic acid read at these two wavelengths and deducted from the total absorbance at 280 mμ; the remainder can be considered to be the absorbance of the intact aspirin.

This partition chromatographic procedure compared excellently with the old and very laborious NF X procedure. The fact of the matter is that Levine's work was so convincing (and deservedly so) that in the next revision of the NF (NF XI) his procedure replaced the old official method except for the FSA limit test. There were also other firsts in this paper; Levine broke tradition with conventional partition chromatography techniques by altering the nature of the immobile phase (neutralizing the sodium bicarbonate phase of the column with acetic acid) as a step in the process. In so doing, the versatility of partition chromatography was thus broadened to permit separations not previously possible. In earlier techniques, the constitution of the immobile phase remains unchanged.

In 1959 and 1960, Smith (79, 80) reported on an AOAC collaborative study of the Levine method for APC. As a result of these studies, the Levine method became official in the Tenth Edition of the Methods of Analysis of the AOAC. These collaborative tests did show a weakness in the procedure, in that the values reported for the salicylic acid content varied appreciably (from 0.15 to 3.17% as hydrolyzed aspirin). As stated before, with care the hydrolysis of the aspirin on the

[4] Celite 545, Johns-Manville Corp., New York, N. Y.

sodium bicarbonate column can be avoided, chiefly by eluting the column as quickly as possible. Smith suggested that the aspirin and salicylic acid content could be calculated by simultaneous equations rather than make the assumptions already discussed in the Levine paper.

Heuermann and Levine (81) and later Heuermann (82) expanded the usefulness of Levine's original work to the analysis of combinations of aspirin, phenacetin, and caffeine with other drugs. The other drugs were pyrilamine maleate, chlorprophenpyridamine maleate, phenindamine tartrate, methapyrilene hydrochloride, doxylamine succinate, thonzylamine hydrochloride, codeine sulfate or phosphate, phenobarbital, or cyclopentylallybarbituric acid. In neither paper were there any salicylic acid values reported, although discussion was made of its determination. An absorbance figure greater than 0.020 at 310 mμ indicated that partial hydrolysis of the aspirin had taken place and must be accounted for in the calculations of the aspirin content of the sample. Simultaneous equations were used in determining the intact aspirin and the FSA content of the sample.

In the application of his partition chromatographic procedure to just aspirin and aspirin tablets, Levine (83) required only the sodium bicarbonate segment of the column. With this paper, he clarified the salicylic acid status in that if 5% or more FSA was found, the aspirin and salicylic acid contents of the given sample were found simultaneously by the described UV procedure. If the FSA was under 5%, another technique was used. (This technique will be described later in this Review Article.)

With the UV procedure, it was noted earlier that aspirin was not stable in chloroform so a little acetic acid was added to acidify the medium. However, in the case of buffered tablets, a stronger acid was needed, not only to stabilize the aspirin but to protect it from the hydrolytic effect of the buffering agents present. This was accomplished by preparing a 0.24 *N* hydrochloric acid solution in methanol and adding a small amount of this solution to the original extraction medium of chloroform. The amount of acid present did not affect the efficiency of the chromatographic column, even if the tablets being assayed were not buffered. An aliquot of the aspirin solution was passed through the column with the aid of more chloroform (no ether was required as with APC tablets). The aspirin was eluted with acetic acid in chloroform and determined at 280 mμ, while the reading at 310 mμ showed the salicylic acid content. If the absorbance at 310 mμ was 0.075 or higher, it represented a concentration of 5% or more FSA. The aspirin reading at 280 mμ was corrected for this FSA absorbance so that the intact aspirin could be reported. If the FSA was below 5%, another method was used in reporting FSA since this spectrophotometric procedure resulted in too low an absorbance reading to be accurate. This procedure was successfully applied to regular aspirin tablets (white), pink aspirin tablets, orange-colored and flavored children's aspirin tablets, enteric-coated tablets, buffered aspirin tablets, and aluminum aspirin tablets.

The stability of aspirin compounded with 10 different kinds of antacids was reported by Kubo *et al.* (84). The samples were stored for a total of 90 days at: 5°, 52% relative humidity; 20°, 75% relative humidity; and 30°, 92% relative humidity. Samples were removed intermittently for assay by a UV spectrophotometric assay. The FSA content was calculated directly from the value at 308 mμ, while the intact aspirin value at 275 mμ had to be corrected for the salicylic acid absorbance value. The antacids studied were aluminum silicate, magnesium carbonate, magnesium trisilicate, calcium gluconate, calcium lactate, sodium phosphate, dried aluminum hydroxide gel, calcium carbonate, magnesium oxide, and sodium bicarbonate. Only the 5° station showed good stability with these antacids. At the 30° station, the last four mentioned antacids were completely incompatible with aspirin, as shown by the high FSA values.

Even though pyrilamine was known to interfere with the salicylic acid reading at 308 mμ, Siegel *et al.* (85) used the Tinker and McBay (76) UV procedure to expedite the analysis of tablets of pyrilamine resin adsorbate with aspirin and ascorbic acid. Tablets were stored at 60° for 1 week and for a total of 12 weeks at 45°. Samples removed at various time intervals showed an unexpected trend because the type of container closure and degree of fill were of prime importance for evaluating these products by accelerated temperature studies. Tablets stored in open bottles, or those with polyethylene snap caps, had greater stability than those with Bakelite screw caps. Filled containers appeared to have greater instability than partially filled ones; bottles that had been opened frequently, compared to those opened only once, appeared more stable. The following explanation was offered by the authors: the passage of air would remove moisture as well as gaseous acidic degradation products which promote instability in these tablets; thus, in tightly closed and well-filled bottles, this would not take place to as large a degree.

Chapman and Harrison (86) determined FSA in soluble aspirin tablets by dissolving the aspirin in glacial acetic acid, filtering, and reading the absorbance at 320 mμ against glacial acetic acid. The salicylic acid content was found from a standard calibration curve of salicylic acid run exactly as the sample. This solvent was selected because aspirin was not stable in chloroform for UV studies, and the results obtained were more reproducible than the BP procedure.

A more thorough study of the Tinker and McBay (76) procedure was reported by Ladomery (87). Chloroform was replaced with absolute spectral alcohol as the solvent for aspirin and salicylic acid. At the wavelength of maximum absorbance for salicylic acid, 300 mμ, Beer's law held through 80 mcg./ml. of absolute spectral alcohol. Similar equations were calculated, only using the absorption data acquired from the alcohol medium. Application of this method was acceptable and accurate.

For APC preparations which contain both barbituric acid derivatives and certain organic bases, it was the objective of Turi (88) to develop a single method rather than using two procedures as described by Heuermann and Levine (81). He was successful with capsules

or tablets containing aspirin, phenacetin, caffeine, itobarbital, and one of four phenothiazine derivatives (chlorpromazine hydrochloride, promethazine hydrochloride, thiethylperazine dimaleate, or thioridazine hydrochloride). No mention, however, was made of determining salicylic acid by this column procedure other than that a prolonged stay (on Column III) would result in a partial *in situ* degradation of the aspirin.

For the determination of aspirin, caffeine, and acetaminophen (APAP), Koshy (89) found that the reversal of the column arrangement described by Heuermann and Levine (81) was all that was required. The three active ingredients could be assayed beside the potential decomposition products, salicylic acid and *p*-aminophenol. After the two columns were prepared in the usual manner and placed in tandem, such that the top column contained the sulfuric acid as the immobile phase and the bottom column contained the sodium bicarbonate as the immobile phase, they were both washed with ether. The powdered sample was dissolved in ethyl acetate, an aliquot of which was then passed through the two columns and collected in a volumetric flask. The columns were further eluted with ether. This fraction contained intact APAP. The columns were then eluted with chloroform. This fraction contained the caffeine. The two columns were then separated, and the bottom column (sodium bicarbonate phase) eluted immediately with acetic acid in chloroform. This fraction contained the aspirin and salicylic acid and was assayed in the usual manner. The top column (sulfuric acid phase), containing *p*-aminophenol, was washed with ether to remove the chloroform. The diatomaceous earth support was then extruded from the column with air under pressure and collected in a beaker. The ether was evaporated from this material, and 0.1 *N* hydrochloric acid was added to dissolve the *p*-aminophenol. This extract was filtered and an aliquot was assayed colorimetrically using 1-naphthol as described by Greenberg and Lester (90).

The effect of water vapor pressure on moisture sorption and the stability of aspirin and ascorbic acid in tablet matrixes reported by Lee *et al.* (91) utilized the Tinker and McBay (76) procedure in evaluating the stability of the aspirin in these studies. Conclusions reached from these studies showed that the moisture adsorptive capacity of each compressed tablet formulation affected the stability of the two drugs to a great extent and were directly related to the moisture sorption and tablet hardness. (The harder the tablet, the less moisture it sorbed and the more stable the drug.) Of the six diluent systems studied, calcium sulfate and cellulose produced the most stable tablets of aspirin and ascorbic acid, while amylose produced the least stable. Under stress-storage conditions, screw-cap glass bottles proved to be a better moistureproof container than snap-top plastic vials. Cellophane and aluminum foil strip packaging materials were about equally effective. Both were more effective than the glass or plastic containers.

Reed and David (92) described a simple direct spectrophotometric determination of salicylic acid in either one complete capsule or one intact tablet of an aspirin containing medicinal, provided no interference by the other components with the salicylic acid absorption at 300 mμ was encountered. The entire dose unit is shaken with alcohol for 1 hr. along with a similar freshly prepared unit dose as a "blank" (as this was generally not available, an equivalent amount of fresh aspirin was weighed and used as the blank). This exposure to an alcoholic medium for 1 hr. could lead to generated hydrolysis in the case of aspirin tablets containing buffers as there is no mention of pH control here or in the aqueous dilution being read at 300 mμ. The sample was read against the blank so that the effect of the intact aspirin would be cancelled out, provided the difference between the two concentrations was not great.

Application of the Tinker and McBay (76) procedure was applied by Day *et al.* (93) in following the stability of two mixtures official in the BPC. They reported the accuracy of the method to be ±2.5%.

In a general paper on the use of UV for analysis of drugs in pharmaceuticals, Sattler (94) applied the method described by Ladomery (87) which was a modification of the Tinker and McBay procedure.

A direct spectrophotometric determination of five compounds, aspirin, salicylamide, caffeine, phenacetin, and salicylic acid, in tablets or powders without any preliminary separation was reported by Clayton and Thiers (95). The powdered sample was extracted with chloroform. From this extract, three aliquots (same volume size) were added to separate volumetric flasks, one to be an acidic medium, another to be a basic medium, and the third a hydrolyzed medium. A mixed solvent consisting of isopropanol, water, and a small amount of hydrochloric acid was added to each flask followed by a solution of 50% sodium hydroxide to the basic and hydrolyzed labeled flasks. After hydrolysis of the aspirin was complete at room temperature (about 15 min.), concentrated hydrochloric acid was added to the hydrolyzed labeled flask, rendering it acid again. On diluting all flasks to volume with the mixed solvent, the solutions were then read at the following wavelengths along with the appropriate reference blank solution. The acidic labeled solution was read at 250, 273, and 310 mμ, the basic labeled solution at 333 mμ, and the hydrolyzed labeled solution at 301 mμ. Using a variety of equations, the content of the individual components was calculated. The comment was made that if salicylic acid were absent, it was possible to omit one step, but the omission is not recommended, since this step provides a measure of any hydrolysis of aspirin which might have occurred during storage or manufacture of the product analyzed.

Use of the isosbestic point as a base line in differential spectrophotometry was applied to aspirin and salicylic acid by Shane and Routh (96). When a series of concentrations of salicylic acid were used to prepare differential absorption spectra, monosodium salicylate (at pH 9) in the reference cell *versus* disodium salicylate (at pH 13.5) in the sample cell, two maxima at 246 and 319 mμ, two minima at 233 and 203 mμ, and two isosbestic points at 268 and 300 mμ were observed. If differential absorption spectra of aspirin solutions were prepared by the same procedure (monosodium acetylsalicylate in the reference cell *versus* disodium salicylate equivalent to the monosodium acetylsalicylate in the sample cell), a

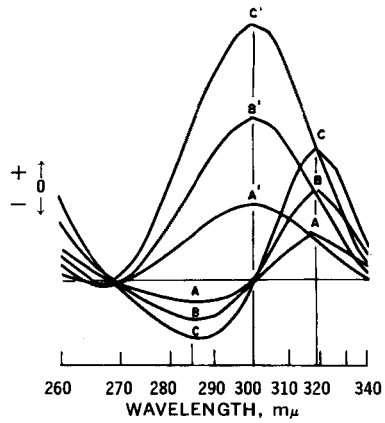


Figure 5—*Differential spectra of salicylic acid (A, B, C) and aspirin (A', B', C'). [Reprinted, with permission, from N. A. Shane and J. I. Routh,* Anal. Chem., **39**, *414, (1967).]*

maximum at 300 mμ, a minimum at 268 mμ, and an isosbestic point at 272 mμ were observed. Figure 5 illustrates the differential absorption spectra of three concentrations of salicylic acid (A, B, and C) compared to the spectra of three concentrations of aspirin (A', B', and C') in the region from 260 to 340 mμ. Each curve is constructed from the two spectra of the same concentration of either salicylic acid or aspirin obtained at pH 9 and 13.5 by subtracting one spectrum from the other spectrum, resulting in the differential absorption spectrum of the given compound. The isosbestic point in differential spectrophotometry would be the wavelength at which each difference curve crosses the zero line. The correlation of the isosbestic point at 300 mμ for salicylic acid, and the maximum at the same wavelength for aspirin, permits the use of the isosbestic point as the zero or base line for the quantitative determination of intact aspirin in the presence of salicylic acid. Thus, one would not need to do a FSA determination as the value reported for aspirin is for the intact aspirin.

A very much needed publication on the significance of salicylic acid sublimation in stability testing of aspirin-containing solids was presented by Gore *et al.* (29). A more than negligible loss of salicylic acid formed from the decomposition of aspirin would preclude the common practice of analytically determining changes in salicylic acid content in solid dosage forms of aspirin as a measure of degrading aspirin. The salicylic acid method could obviously underestimate the extent of decomposition of aspirin and, therefore, provide false confidence in the stability of the tested products. It thus became necessary to develop a method of gauging aspirin stability in solids which would be unaffected by any loss of salicylic acid.

A simultaneous spectrophotometric assay, based chiefly on the work of Edwards (8), was developed for aspirin and salicylic acid in a Clark and Lubs buffer, pH 7.4. It was found that this aqueous medium resulted in improved precision over the chloroform medium used by earlier workers. This slightly alkaline medium afforded a relatively rapid solution of solid aspirin and salicylic acid, and a medium in which slight variation in the pH would not introduce an error into the determination as a consequence of the differential absorption of ionized and unionized aspirin or salicylic acid. The rate of hydrolysis of aspirin has been reported (8) to be independent of pH in the range of 4 through 8. Hydrolysis rate constants were determined at pH 7.4 and 25.5°, and showed a delay of 13 min. between the sample preparation and reading on the spectrophotometer would cause an error of approximately 1%. The actual error was reduced to below 0.1% by maintaining the solutions below 15°, usually at 0°, and reading them within 5–10 min. of their preparation.

Table XVI—Constants for Aspirin and Salicylic Acid UV Assay

Compound	Concentration Range, mcg./ml. of pH 7.4 Buffer	Absorptivity Value at 262 mμ	Absorptivity Value at 296.5 mμ
Aspirin	0–160	3.2	0
Salicylic acid	0–10	3.3	—
Salicylic acid	0–30	—	26.0

Table XVI presents the pertinent data required in the construction of the calibration curves used here.

Before applying this UV procedure, several sublimation studies were conducted in which the need for such an assay was demonstrated quite convincingly. Using an electrobalance, the weights of salicylic acid were monitored continually at the stated temperatures for 12 hr. From this study the following rates of sublimation were calculated (Table XVII).

Table XVII—Sublimation Rates of Salicylic Acid at Various Temperatures

Temperature	Rate of Sublimation, mg./hr.
40 ± 0.05°	0.026
50 ± 0.20°	0.062
70 ± 0.20°	0.372

An Arrhenius-type plot of the apparent zero-order sublimation rates was shown. The slope of the curve was predominately determined by the enthalpy of sublimation of salicylic acid. The observed rates of sublimation may be expected to depend directly upon the area through which the mass transfer occurred. These results, therefore, were not intended to be quantitatively indicative of sublimation loss of salicylic acid during the stability testing, but merely substantiate that such a loss can occur even at moderately elevated temperatures.

A similar sublimation study of purified aspirin revealed no significant loss of weight up to 70°. It was concluded that aspirin does not appreciably sublime under the conditions of the experiment.

With a 9:1 mixture of aspirin–salicylic acid, the results from 12 hr. at 70° indicated that only salicylic acid was lost. This was verified by using the UV procedure discussed.

Aspirin tablets were stored at 50° and 81.2% relative humidity for a period of 98 days. At various time intervals tablets were removed and assayed by first grinding to a fine powder and dissolving in pH 7.4 buffer maintained at 0°. After filtration, an aliquot of the filtrate was further diluted with the cold pH 7.4 buffer and the absorbance measured at 262 and 296.5 mμ. In this application, an error of 0.003 absorbance unit at 262 mμ could contribute an error of approximately 1% in the

Table XVIII—Comparison of Aspirin Tablet Stability Testing Results at 50° and 81.2% Relative Humidity, Based on Determination of Aspirin and Salicylic Acid Content of the Tablets[a]

Time, Days	Aspirin Content Based on Analysis of Aspirin, %	Aspirin Content Based on Analysis of Salicylic Acid, %	Error Due to Sublimation of Salicylic Acid, %
0	100	100	0
15	99.1 ± 0.038	99.1 ± 0.028	0
30	98.4 ± 0.042	98.8 ± 0.215	0.4
45	97.2 ± 0.123	98.7 ± 0.075	1.5
60	97.1 ± 0.178	98.6 ± 0.023	1.5
98	95.0 ± 0.288	97.9 ± 0.311	2.9

[a] Each value is the average of four determinations recorded with ±1 standard deviation.

determination of the aspirin content of a solid consisting of 80% aspirin using a 50-mg. aliquot for analysis. The results of this study are presented in Table XVIII.

The last column does show that salicylic acid is lost from these tablets by sublimation under the stated conditions of the experiment.

By employing the UV procedure presented in this publication, the determination of the residual aspirin, rather than the apparent salicylic acid, in a solid can be used as a valid means of gauging the stability of the formulation. This method of analysis showed an accuracy within at least 1.5%.

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS WITH THE AID OF A FERRIC-ION CHROMATOGRAPHIC COLUMN

Analytically, the need has always been great to separate and isolate the salicylic acid from aspirin as well as other components in the pharmaceutical preparation. The potential susceptibility of aspirin to hydrolysis is constantly prevalent, so the more rapidly the intact aspirin is removed from the medium, once a given procedure is begun, the truer the reported FSA values will be.

Again Levine (83) appeared to be first in breaking away from the traditional methods of determining salicylic acid in aspirin formulations. On using the chromatographic columns he had introduced in 1957 with a diatomaceous earth–2% ferric chloride mix, the passage of a chloroform solution of aspirin and salicylic acid resulted in the trapping of the salicylic acid (shown by a purple zone on the column). The aspirin passed through, and by using several washes with chloroform, the aspirin was completely removed from the column. The bound salicylic acid was then eluted quantitatively with chloroform containing acetic acid. This eluate was read at 310 mμ for FSA. For the procedure to be valid, the purple zone should not reach the bottom of the tube during the washing out of the aspirin. If it does, the procedure must be repeated with another prepared column. As the original procedure was written, this was a major downfall in using this technique, because too many times the purple zone moved partially off the column during the aspirin removal step.

This procedure was applied to pink aspirin tablets, children's flavored and colored aspirin tablets, buffered aspirin tablets, and aluminum aspirin tablets. With the colored tablets, the dyes remained at the top of the column throughout the entire assay. With the buffered tablets, the chloroform-insoluble salts of aspirin or salicylic acid must be transformed to soluble acids in order that the FSA could be eluted in the proper fraction. Acetic acid could not be used since it would dissociate the ferric ion–salicylic acid complex on the column. Boric acid, however, was used for this transformation without affecting the column performance. A solution of boric acid in methanol was added to the sample, followed by chloroform to dissolve the freed aspirin and salicylic acid.

For enteric-coated tablets it was found best to mount in tandem a column containing just diatomaceous earth above the regular ferric ion–diatomaceous earth column. The plain diatomaceous earth column removed the surface-active agents present in these tablets, so the aqueous phase would not be stripped off the regular ferric ion–diatomaceous earth column during both the prewashing step and elution of the salicylic acid. This double column setup could be used also where dyes are present in the original chloroform extract, as well as for large amounts of excipient material in the sample being added to the column.

As will be seen with later papers dealing with this novel approach of Levine's, a delicate balance was being maintained with the ferric chloride content on the diatomaceous earth. A sufficiently low concentration of ferric chloride must be maintained to trap the salicylic acid, but at the same time a sufficiently large quantity of ferric chloride must be present to provide an excess over the amount removed during the washing step. It is thus recommended that the modifications of this procedure, to be discussed in this review, be used rather than the method described in this publication. Just the introduction of this approach to the literature served a very worthwhile purpose as the official compendia now use a procedure based on this original study.

Green (67) applied this described procedure by Levine and as he did not present any comments in his paper, it could be assumed that he did not experience any major difficulty with the columns.

In 1961 Weber and Levine (97) made note that "several investigators have encountered difficulty with the published method" (83). During the elution of the aspirin, the salicylic acid migrated slowly down the column (as evidenced by the position of the purple complex) and spread out into a diffuse band, which sometimes becomes difficult to discern.

In this publication, this was rectified by modifying the ferric chloride reagent. This radical improve-

ment in the chromatographic separation was achieved by having a high concentration of urea in the ferric chloride solution. The resultant effects were tremendous: the band of the ferric–salicylate complex obtained with this new reagent was more deeply colored than that obtained with just the simple ferric chloride reagent. The dense, sharply delineated band migrated only slightly during the elution of the aspirin.

It, therefore, became feasible to use a shorter column which did not require extraordinary care in packing. This column even accommodated larger samples of aspirin.

Optimum conditions were obtained with an immobile phase containing 5% ferric chloride solution which was 10 *M* with respect to urea. The pH must be maintained between 3.1 and 3.3. At lower pH levels the salicylic acid band became diffuse and more loosely retained, while at higher pH levels, recovery of salicylic acid from the column may be incomplete using the specified volume of eluant.

For aspirin or aspirin tablets, the pulverized sample was dissolved with chloroform and passed through the column with the aid of several more portions of chloroform to wash the intact aspirin through the column. A volumetric flask containing some hydrochloric acid in methanol was placed under the column, and the column first eluted with acetic acid in ether followed by chloroform. Concomitantly the absorbance of this solution and of the standard salicylic acid in the same medium was determined at 306 mμ.

For APC tablets and flavored tablets, a column containing a small pad of cotton was placed in tandem over the regular ferric chloride–urea column. The sample in the chloroform was passed first through the cotton column to remove the dyes and any insoluble excipients. Another portion of chloroform was passed through the two columns, and then the top column was discarded. The regular column was then washed with chloroform and the salicylic acid eluted.

As moderate amount of urea was eluted together with the salicylic acid from the column, the hydrochloric acid in the receiving flask was added to maintain acidity of the eluate. The methanol was present to achieve miscibility of the acid with the eluting solvent.

With buffered aspirin tablets, the boric acid was replaced with oxalic acid in methanol, as the oxalic acid was more effective in releasing the bound salicylic acid from the antacid. The oxalic acid solution recovered quantitatively salicylic acid from its calcium salt. However, neither this nor any other reagent thus far tested quantitatively released salicylic acid from aluminum hydroxide gel without causing extensive hydrolysis of aspirin.

Excellent reproducibility on replicate analyses of a wide variety of commercial samples was shown.

Though Weber's publication (98) on the analysis of salicylic acid and benzoic acid does not deal with aspirin, *per se*, it had a valuable innovation regarding the ferric chloride–urea–diatomaceous earth column. When eluting salicylic acid of larger quantities than usual, the occasional leakage of ferric chloride was noted which invalidated the assay. This was seen visually by the presence of a yellow color rather than the usual colorless clear eluate. If any yellow is present in the solution read, the reading at 306 mμ would have to be voided. Weber found that a small layer of diatomaceous earth with 30% phosphoric acid as the immobile phase placed at the bottom of the column on top of the glass wool support, and then followed by the regular ferric chloride–urea–diatomaceous earth mix, resulted in no ferric chloride leakage. The phosphoric acid retained any ferric chloride which might be removed during the elution step of the salicylic acid.

In their study on the formation of acetylcodeine from aspirin and codeine, Jacobs *et al.* (99) used the original Levine procedure (83) in isolating the salicylic acid which resulted from the previously mentioned reaction.

On discussing pharmaceutical heterogeneous systems, Zoglio *et al.* (28) have presented four papers regarding the hydrolysis of aspirin. The salicylic acid resulting from the degradation of aspirin in the various formulations was determined by a modification of Levine's iron–diatomaceous earth procedure (83). The first paper [Kornblum and Zoglio (28)] is discussed in the *Hydrolysis Studies* portion of this review. They demonstrated that calcium or magnesium stearate accelerated the production of salicylic acid from aspirin through the solubilization of aspirin as a calcium or magnesium salt. More aspirin would thus be in solution which in turn would hydrolyze in the existing pH which was conducive to hydrolysis. This effect was not as pronounced when aluminum stearate was used as a lubricant, as the aluminum salt of aspirin has a low solubility in water.

On pursuing this alkali stearate effect, Zoglio *et al.* (28) prepared capsules containing aspirin (20 parts), magnesium stearate (1 part), and 0, 1, 2, 5, 10, and 20 parts of hexamic, maleic, malic, or tartaric acids, or maleic anhydride. These capsules were stored at 22, 40, and 50° for 30 days. A minimum of 20% by weight of hexamic, maleic, or malic acid was required to retard the hydrolysis of aspirin in these capsules. Tartaric acid or maleic anhydride was not effective at the higher temperatures. Besides the lower desired pH contributed by the added acid, the mechanism of inhibiting the degradation of aspirin involved the additive acid and aspirin competing for the magnesium ion.

Maulding *et al.* (28) showed that stearic acid USP, which is nearly a 1:1 mixture of stearic and palmitic acids, promoted the decomposition of aspirin more than either reagent grade stearic or palmitic acid. On preparing various synthetic mixtures of reagent stearic and palmitic acids, the one simulating the amounts of the two acids in stearic acid USP behaved similarly as the stearic acid USP in accelerating the decomposition of aspirin. This ratio of stearic and palmitic acids was also the melting point minimum of the various mixes of these two acids. The possibility exists, therefore, of a liquid or semiliquid being present in formulations containing stearic acid USP which might serve as the medium for aspirin hydrolysis.

The fourth paper dealt with the acceleration of aspirin hydrolysis by various common additives (hexamic acid, aluminum hydroxide calcium stearate, magnesium stearate, or magnesium trisilicate), at a 5 or 10% level of the powder mix or tablet. The formulations con-

taining magnesium trisilicate resulted in the largest amount of FSA (about 13%) after 45 days at 40°. Hexamic acid, under these storage conditions, retarded aspirin hydrolysis. The salicylic acid values resulting from these various formulations were in good agreement with values obtained from extrapolating apparent zero-order rates of aspirin suspensions of the same powder mix as used in the tablet. These studies show that stability prediction for solid dosage forms from apparent zero-order rates of suspensions is feasible and informative.

The determination of the FSA in buffered aspirin tablets by Levine and Weber (100) explored further the usefulness of their ferric chloride–urea–diatomaceous earth column. It was essential that the entire amount of aspirin and salicylic acid be dissolved in the mobile phase before the chromatographic treatment, and that the aspirin was not hydrolyzed during the preparation of that solution. These two requirements are not readily achieved in the case of buffered aspirin tablets. Chloroform solutions of aspirin, in the presence of basic materials such as those which comprise the buffering components of the tablets, undergo hydrolysis together with aspirin anhydride formation. Boric acid stabilizes the solution, at least with respect to the hydrolysis of aspirin, but does not achieve the necessary release of the aspirin and salicylic acid from the buffer components to permit their complete solution in chloroform.

In designing a valid assay procedure, the acid which is used must: (*a*) produce only minimal hydrolysis of aspirin under the conditions of the assay; (*b*) rapidly and completely release aspirin and salicylic acid from the buffer components of the tablet; (*e*) be soluble in chloroform; and (*d*) be readily removed from the chloroform, so that the ferric–salicylic acid complex will not be dissociated during the following step of the analysis.

These requirements were fulfilled by 98–100% formic acid. The distribution of formic acid between chloroform and inorganic acids was greatly in favor of the aqueous phase; therefore, the formic acid was removed from the chloroform solution by passage over dilute hydrochloric acid.

In the analysis of buffered aspirin tablets, two columns are prepared and placed in tandem. The top column consists of diatomaceous earth with 0.05 *N* hydrochloric acid as the immobile phase. It is here that the formic acid in the chloroform is removed. The packing of this column should be such that a flow rate of chloroform of 12 ml./min. is obtained.

The bottom column consists of a layer of diatomaceous earth with 30% phosphoric acid as the immobile phase. The phosphoric acid retains any ferric iron which may be eluted during the procedure. The upper stage of this column consists of the 5% ferric chloride and 10 *M* urea as the immobile phase on diatomaceous earth.

In the procedure, water-saturated solvents were used throughout. To a ground sample of tablets in a volumetric flask, the 98% formic acid was added with swirling to wet the sample completely (not more than 30–45 sec.) followed by chloroform. This mixture was shaken for 10 min. The extent of hydrolysis of aspirin during the period of contact of the sample with the formic acid before dilution lies in the range of 0.01–0.02%/min., so here the extent of hydrolysis will be in the order of 0.01%. After dilution with the chloroform, the hydrolysis of aspirin sharply decreased to an average of only 0.03%/hr. Thus a negligible amount of hydrolysis occurs during the 10-min. shaking period for dissolving the aspirin and salicylic acid. On diluting to volume with chloroform and mixing, the solution was filtered through a loose plug of glass wool. An aliquot of this filtrate was then passed through the double columns which were washed with more chloroform to remove the aspirin. The eluate and the top column were discarded. A receiver containing hydrochloric acid in methanol was placed under the remaining (bottom) column, and ether containing acetic acid was passed through the column followed by chloroform containing acetic acid. This eluate was read at 306 mμ along with a salicylic acid standard in the same medium.

By this method, great strides have been made in regard to freeing salicylic acid from various antacids. It was shown that the formic acid treatment readily recovers salicylic acid from its calcium or magnesium salts but that aluminum salicylate was quite refractory to this treatment. As dried aluminum hydroxide gel is present in several of the commercial buffered aspirin tablets, the need is still acute for a method which will recover salicylic acid from its aluminum salt.

On studying the USP XVII limit test for salicylic acid in aspirin tablets containing buffers, Guttman (101) obtained spurious and nonreproducible results. Low recoveries were explained by an adsorption phenomenon. Significant adsorption of salicylic acid occurred when solutions of chloroform were in contact with chloroform-insoluble agents which are commonly employed as buffers in aspirin tablets. The affinity of salicylic acid for magnesium carbonate and aluminum glycinate was high, but the capacity of these solids for the acid was rather low. These two compounds held tenaciously to the salicylic acid, as it was impossible to elute completely adsorbed material by repeated contacts with fresh solvent.

High recoveries of salicylic acid resulted from a surprisingly rapid transformation of aspirin to a product having the chromatographic characteristics of salicylic acid. This was observed only when solid basic material (here magnesium carbonate) was suspended in the chloroform solution of aspirin. This transformation of aspirin was thus surface catalyzed.

On repeating this experiment with different commercially available buffered aspirin products, the same phenomenon occurred except with one—and it contained citric acid monohydrate besides two antacid compounds. With this sample, the salicylic acid content did not increase appreciably with time of contact with the solid suspension. With all these suspension studies, it was of interest to note that, upon filtration, immediate cessation of salicylic acid production resulted. This was shown by the readings in the UV at 278 and 306 mμ on the chloroform filtrate or when the ferric chloride–urea–diatomaceous earth column procedure (USP XVII) was used.

Table XIX—Results Obtained when Various Methods Were Employed to Estimate Salicylic Acid (SA) Contents of a Number of Commercial Buffered Aspirin Products

Product	Buffers Present	% SA Found Using: USP Procedure	Citric Acid Procedure for FSA	Weber and Levine (97) Procedure	Citric Acid Procedure for Total Nonaspirin Salicylates
1	Aluminum hydroxide Magnesium hydroxide	2.19 2.80 3.03	4.02 4.02 3.89	6.59 6.53	6.64 6.58 6.62
2	Aluminum glycinate Magnesium carbonate	Not detectable	0.211 0.236 0.219	0.64 0.69 0.62 0.67	0.728 0.718 0.739
3	Aluminum hydroxide Glycine Magnesium carbonate	0.455	1.01	3.79 3.91 3.52	3.78 3.58 3.58
4	Calcium phosphate Sodium bicarbonate Citric acid	—	5.22	—	5.92
5	Aluminum hydroxide Glycine Magnesium carbonate	—	0.682	—	2.81
6	Aluminum hydroxide Magnesium hydroxide	—	1.82	—	3.39
7	Calcium carbonate Magnesium carbonate	—	1.96	—	3.37

On further studies with aspirin in chloroform and magnesium carbonate in suspension with and without an equal weight of citric acid monohydrate, it was found that no salicylic acid was produced in 200 min. at 25° with citric acid present. After 60 min. of magnesium carbonate being in contact with the aspirin solution, the addition of citric acid monohydrate resulted in no further salicylic acid production. It was visually observed that the addition of citric acid had a pronounced effect on the nature of the suspension. Marked flocculation of particles was apparent immediately after the addition of citric acid monohydrate. Citric acid which had been previously oven-dried showed a much less marked effect in inhibiting salicylic acid production. This showed that presence of water from the hydrate was essential in this reaction with suspended material. Applying this citric acid monohydrate to the previously studied commercial buffered tablets on an equal weight basis showed that in all systems studied here, the citric acid was effective in markedly reducing the catalytic ability of suspended solids in the production of salicylic acid from the aspirin in chloroform.

Repeating the magnesium carbonate–aspirin adsorption experiment discussed earlier, only this time with and without an equal amount of citric acid monohydrate, it was found that in the presence of citric acid there was no appreciable adsorption of the aspirin from the chloroform. It is also of interest that treatment of antacid compounds in suspension in chloroform with citric acid monohydrate significantly reduced their capacities for adsorbing salicylic acid.

Guttman's study demonstrated very convincingly that finely divided solids of antacids do catalyze a conversion of aspirin to a product which, by the analytical method employed, was determined as salicylic acid. The reaction occurred under essentially anhydrous conditions, and adsorption of the aspirin was apparently a prerequisite for the transformation. The presence of citric acid monohydrate was shown to be effective in inhibiting the formation and adsorption of salicylic acid in the systems reported here. A feasible explanation was given: citric acid monohydrate, which is essentially chloroform insoluble, inhibits the reaction by releasing water of hydration to the surface of the adsorbent. A neutralization reaction takes place in the hydrate layer which modifies the surface characteristics of the adsorbent so as to destroy the adsorption sites. Thus, the catalytic production of salicylic acid from aspirin is never initiated.

Results from studies presented in this paper showed that addition of citric acid monohydrate to the official chromatographic procedure in the original extraction of the sample resulted in almost quantitative recovery of salicylic acid. It was also noted that this citric acid treatment was not effective in displacing salicylic acid from metallic salts which are known to form in buffered aspirin products.

In applying the procedure suggested in the preceding paper, Guttman and Salomon (102) compared its usefulness (and superiority) over the existing USP XVII FSA test for buffered tablets of aspirin. The method consisted of treating, by trituration, a powdered sample with an equal weight of citric acid monohydrate, and dissolving the aspirin and the FSA from the powder mass with chloroform. The remaining residue was treated with an aqueous solution of a strong acid (hydrochloric acid), and this solution was extracted with chloroform. The two chloroform extracts (one containing the FSA and the other containing the nonaspirin salicylates) were combined, and the salicylic acid content determined by the chromatographic method of Weber and Levine (97). With the assumption that the citric acid treatment results only in desorption of salicylic acid and aspirin and does not cause conversion of salicylate salts to free acid, one would have a method available for the estimation of the FSA content

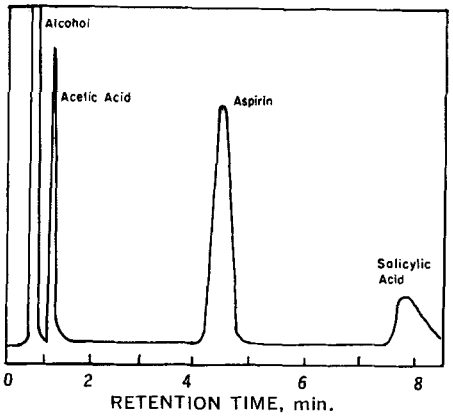


Figure 6—*Gas chromatogram of aspirin in alcohol. [Reprinted, with permission, from J. G. Nikelly,* Anal. Chem., **36**, *2248 (1964).]*

as well as total nonaspirin salicylate contents of buffered tablets of aspirin. Table XIX summarizes the results on applying four different procedures to commercially available buffered aspirin tablets: USP XVII (using boric acid in the initial extraction), Guttman's citric acid procedure, Guttman and Salomon's citric acid procedure for total nonaspirin salicylates, and the Levine and Weber procedure (100) which used formic acid in the initial extraction medium.

Inspection of this table makes obvious that the FSA values, as determined by the USP procedure, were consistently and significantly lower than those determined by the other methods. It is logical to assume that these low values resulted from adsorption of significant amounts of salicylic acid during sample extraction, and to the insensitivity of the procedure to salicylic acid which is present in the sample in the form of salts. The latter two methods appear to be equally precise. It is interesting to note the difference between the citric acid procedure for FSA and the citric acid procedure for total nonaspirin salicylates. The differences reflect the fact that significant amounts of salicylic acid can be present in buffered tablets as chloroform-insoluble salts. It is also apparent that no relationship exists between the FSA content and total nonaspirin salicylate content of these tablets.

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS BY MISCELLANEOUS METHODS

Schulek and Burger (103) stated that on using BrCl as a brominating agent, salicylic acid could be brominated quantitatively in 2 min., even in the presence of aspirin. With the usual amounts of FSA present in aspirin products, it is debatable whether such a titration would be sensitive enough to be of value.

The application of differential thermal analysis (DTA) and thermogravimetric analysis (TGA) to aspirin was published by Wendlandt and Hoiberg (104). No mention was made of the effects of the presence of salicylic acid, but application of this approach would definitely be of interest in evaluating the purity of aspirin.

Gas chromatography has a great potential in the analysis of aspirin products, but only two papers have mentioned the possibilities. Crippen and Freimuth (105) stated that their proposed procedure would differentiate aspirin from salicylic acid only if the temperature of 100–125° and a 1.23–1.83-m. (4–6-ft.) column were used. Nikelly (106) showed (Fig. 6) that under the stated conditions used in the determination of aspirin, the amount of acetic and salicylic acids could be estimated.

In applying an IR spectrophotometric assay of aspirin anhydride, Garrett and Johnson (107) concluded that the insignificance of salicylic acid in these lots of varied purity showed that little definition was lost on ignoring it. Though samples of aspirin were not used here, the usefulness of IR studies on the purity of aspirin in regard to salicylic acid would be of value.

The need for actual physical separation of salicylic acid from aspirin is great. The work by Vietti-Michelina (108) and Wagner (109) on paper chromatographic or paper electrophoretic separation of aspirin and salicylic acid might be applied successfully by using the latest techniques available in this field.

A too often neglected technique in drug studies in the United States is the application of polarography. In 1949 Korshunov *et al.* (110) reported on the reduction of weak acids at a dropping-mercury cathode. The half-wave potentials were given as 1.52 to 1.65 (for 1–7 mmoles/l.) for aspirin and 1.66 to 1.83 (for 1–9 mmoles/l.) for salicylic acid in 0.05 *N* tetramethylammonium iodide. With the more sensitive polarographs of today, such an approach in the analysis of aspirin products could prove to be useful.

Quantitative separation of aspirin and salicylic acid by sephadex G gel filtration was reported by Lee *et al.* (111). No detectable (fluorometrically) hydrolysis of aspirin on the column was noted.

TLC should be an invaluable tool in aspirin stability studies. By using a mixture of hexane, glacial acetic acid, and chloroform (20:5:5), Reimers (112) separated aspirin and salicylic acid. After resolving the mixtures of aspirin–salicylic acid on thin-layer plates [using ascending technique and a hexane, glacial acetic acid, and chloroform mixture (85:15:10), R_f values of 0.2 and 0.35 were obtained for aspirin and salicylic acid, respectively], the spots were removed from the dried plates, packed in an appropriate cell, and determined by UV reflectance spectroscopy. A linear relationship at 302 mμ between absorbance and the square root of the concentration was observed by Frodyma *et al.* (113) with spots containing up to 3.0 μmoles of either compound.

An exhaustive paper on differentiating nonaqueous titration of salicylic acid and aspirin by Lin (114) showed that such a procedure was feasible. Dimethylformamide was the solvent of choice along with the titrant of 0.1 *N* tetrabutylammonium hydroxide in benzene–methanol (10:1) using either a glass–calomel or platinum–calomel electrode pair.

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RESEARCH ARTICLES

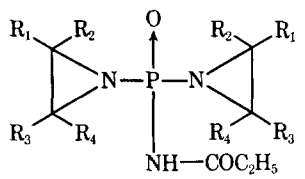
Mechanisms of Reactions of Ring-Substituted Bis(1-aziridinyl)phosphinyl Urethan Antineoplastic Agents

C. K. NAVADA, Z. F. CHMIELEWICZ, and T. J. BARDOS*

Abstract □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan (IV) was synthesized and compared with the corresponding *cis*-2,3-dimethyl derivative (III). The comparative alkylating activities and rates of hydrolysis of these two stereoisomeric aziridine derivatives, III and IV, were determined and compared with corresponding data for the monomethyl derivative (V) and two other clinically tested members of this series of antineoplastic agents (dual antagonists), AB-100 (I) and AB-132 (II). The structures of the final hydrolysis products of III, IV, and V were determined and confirmed by direct synthesis. The results indicate that the mechanisms of hydrolysis of III, IV, and V (as that of the unsubstituted aziridine derivative, AB-100) are essentially S_N2, in contrast to the much faster hydrolysis of the 2,2-dimethylaziridine analog, AB-132, which involves a carbonium-ion mechanism. These studies give further support to the hypothesis that the unique pharmacologic properties of AB-132, as compared to other members of this series, may be related to the unique chemical properties of the 2,2-dimethylaziridine moieties.

Keyphrases □ Antineoplastic agents—reaction mechanisms □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan—synthesis □ Alkylating activity—*cis*-, *trans*-2,3-dimethylaziridine analogs □ Hydrolysis mechanism—ring-substituted aziridine derivatives □ IR spectrophotometry—identity □ NMR spectroscopy—identity

The synthesis of a series of bis(1-aziridinyl)phosphinyl carbamates, termed "dual antagonists" (I and its analogs containing different carbamate moieties) (1, 2), and their antineoplastic activities in experimental animals (3, 4) and in man (5–9) were previously reported. In an effort to decrease the hematologic toxicity due to the "alkylating" aziridine groups, derivatives were synthesized in which the C-atoms of the aziridine rings were substituted with methyl or ethyl groups (10). One member of this new series, ethyl bis(2,2-dimethyl-1-aziridinyl)phosphinyl carbamate (AB-132, II), has been studied to a considerable extent experimentally (11) as well as clinically (12–17). Its interesting pharmacologic properties [*e.g.*, cholinesterase inhibition (18–20)] and its radiation potentiating effect (21–23) suggested that this compound may act by a different mechanism than the C-unsubstituted aziridine derivatives (24). This conclusion was supported by chemical studies of its hydrolytic and alkylation reactions (11, 25), which indicated that the unique properties of II may be related to the ability of the 2,2-dimethylaziridine group to participate in S_N1 reactions with its substituted carbon (by forming a tertiary carbonium ion) and, alternatively,



I. $R_1 = R_2 = R_3 = R_4 = H$ (AB-100)
II. $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$ (AB-132)
III. $R_1 = R_3 = CH_3$; $R_2 = R_4 = H$ (*cis*) (AB-144)
IV. $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$ (*trans*) (AB-145)
V. $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$ (AB-143)