icillin G (Na) in methanolic HCl. In this case, in addition to the penicillin G zone, two other spots were detected. One of these ($R_f$ 1.12 in System *A*) corresponded to the methyl α-D-penicilloate spot obtained with solutions in absolute methanol, while the substance giving the other unidentified spot ($R_f$'s: *A*, 0.49; *B*, 0.28) is presumably benzylpenillic acid (II) which is the normal rearrangement product of benzylpenicillin in acid solution. Spots corresponding in $R_f$ values to II have been detected in deteriorated procaine penicillin G solutions in water.

The TLC procedures described should be applicable to the detection of procaine and its degradation products in the presence of other drugs which are formulated with procaine, *e.g.*, epinephrine, ephedrine, morphine, scopolamine, paromomycin, dihydrostreptomycin. Since these latter compounds and penicillin G do not give color reactions with the Bratton-Marshall reagent (12), it should be possible to use this reaction as the basis for the assay of procaine in the presence of these compounds. Although the Bratton-Marshall reagent has been used for the assay of procaine penicillin G (13), the method appears to have been largely neglected by other workers. The usefulness of this reagent for the assay of procaine is presently being assessed in these laboratories.

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# Pharmaceutical Heterogeneous Systems IV: A Kinetic Approach to the Stability Screening of Solid Dosage Forms Containing Aspirin


**H. V. MAULDING, M. A. ZOGLIO, F. E. PIGOIS, and M. WAGNER**



**Abstract** □ The effect of common tablet excipients and physiologically active materials on the hydrolysis of aspirin in aqueous suspension has been studied. The relationship of hydrolytic rate to solvent concentration in these systems has been determined. The results obtained have been utilized in the stability ranking for various tablet mixes. The kinetic ranking is compared to the relative stability of tablets and powders. An attempt is made to correlate extrapolated kinetic data with stability results for the tablets.



**Keyphrases** □ Heterogeneous systems—pharmaceutical □ Aspirin dosage forms—stability screening □ Stability, aspirin dosage forms—kinetic ranking □ UV spectrophotometry—analysis □ Colorimetric analysis—spectrophotometer


Prior investigations have shown an inverse relationship between aspirin stability and the presence of moisture at ambient or somewhat higher temperatures although secondary phenomena as the pH of the water may alter the rate of degradation (1–6). The deleterious action of fatty acid lubricants on acetylsalicylic acid has been related to hydrolysis (4). It has been previously mentioned that one may compare an aspirin tablet to an aqueous suspension—approaching but not attaining zero water concentration—in order to facilitate explanation of this solvolytic degradation (3). However, the complexities rendered by the multiplicity of variables in heterogeneous systems of this nature make interpretation rather more difficult than in, for example, solution or homogeneous kinetics where the components and species can be accounted for in totality in many cases.

Increased rates of aspirin decomposition in the presence of several excipients and antacids have been reported several times in the literature (1, 3, 4, 7, 8). These agents reported were found to markedly accelerate salicylic acid formation.

More recently Guttman (9) has observed considerable degradation of buffered aspirin tablets in chloroformic solutions and this breakdown is apparently not proportional to water content of the chloroform.

Garrett suggested the possibility that normally first-order decomposition processes may become zero-order in saturated solutions (6) and this has been corroborated by others (3).

The purpose of this study was the evaluation of the effect of some commonly used tablet additives regarding their influence on the rate of generation of salicylic acid from aspirin.

**Table I**—Relative Stability Data for Acetylsalicylic Acid in Tablets and Powders[a,b]

| Mix No. | Additive | Mg. Salicylic Acid[c] Formed/200 mg. Aspirin in Sample | Relative[d] Ranking |
|---|---|---|---|
| | **Tablets** | | |
| C | 10% Hexamic acid | 0.38 | 1 |
| F | None | 0.79 | 2 |
| A | 10% Aluminum hydroxide | 2.03 | 5 |
| B | 5% Calcium stearate | 4.73 | 12 |
| E | 5% Magnesium stearate | 5.93 | 15 |
| D | 10% Magnesium trisilicate | 38.43 | 100 |
| | **Powders** | | |
| C | 10% Hexamic acid | 0.44 | 1 |
| F | None | 0.84 | 2 |
| A | 10% Aluminum hydroxide | 2.17 | 6 |
| B | 5% Calcium stearate | 4.65 | 13 |
| E | 5% Magnesium stearate | 5.98 | 16 |
| D | 10% Magnesium trisilicate | 36.60 | 100 |

[a] Time, 45 days, temperature, 40° (±0.25°). [b] Stored in 30-ml. vials sealed with rubber closures and aluminum crimp tops. [c] Salicylic acid formation corrected to 1% moisture for all mixes. [d] Ranking based on 100 for *D* with regard to salicylic acid formation and is only relative.

For these comparisons aspirin powder mixes, tablets and suspensions were utilized in which a standard-type aspirin formulation was employed along with 5 and 10% of the various excipients and antacids examined.

## EXPERIMENTAL

**Powder Mix**—A basic powder mix containing the following relative amounts of ingredients was prepared: aspirin (40 mesh), 200 mg.; microcrystalline cellulose,[1] 25 mg.; corn starch, 24 mg.; stearic acid USP, 11 mg.; lactose (spray-dried), 10 mg.; total, 270 mg.

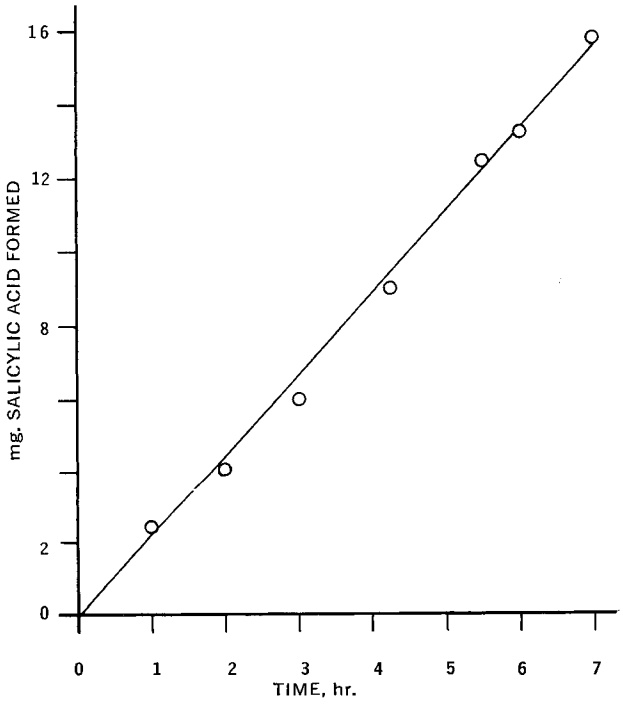


**Figure 1**—*Apparent zero-order plot for aspirin hydrolysis at 40° in aqueous suspension containing 6 ml. water and 594 mg. Mix* A *(samples equivalent to 400 mg. aspirin). 10% aluminum hydroxide is the additive.*

[1] Avicel, American Viscose.

**Table II**—Relative Rate Data for Salicylic Acid Formation from Powder Mixes in Suspension

| Mix No. | Additive | Mg. Salicylic Acid[a,b] Formed per Hour | Relative[c] Ranking |
|---|---|---|---|
| F | None | 0.054 | 1 |
| C | 10% Hexamic acid | 0.075 | 1+ |
| A | 10% Aluminum hydroxide | 0.329 | 6 |
| B | 5% Calcium stearate | 0.848 | 16 |
| E | 5% Magnesium stearate | 0.978 | 18 |
| D | 10% Magnesium trisilicate | 2.31 (5.70)[d] | 43 (105)[d] |

[a] The apparent zero-order rate constant for a one-ml. sample at 40° (±0.2°). [b] Sample used equivalent to 400 mg. aspirin. [c] Relative ranking based on *F* as 1. [d] The apparent zero-order rate constant was calculated for a 0.1-ml. sample and corrected to 1 ml. assuming saturation did not occur at higher volumes (0.5 ml.; Fig. 4).

The basic mix (Mix *F*, Table I) was stirred for 20 min. on a mixer (Hobart) and 2% by weight of water added at a rate of 1 ml./min. until it was thoroughly mixed with the other constituents. To this powder was separately added 27 mg./270 mg. basic mix or 10% of each of three substances: (*a*) aluminum hydroxide; (*b*) magnesium trisilicate; and (*c*) hexamic acid and 13.5 mg./270 mg. basic mix of (*a*) calcium stearate and magnesium stearate. Weighed samples of these powders were sealed in 30-ml. multiple-dose vials with rubber closures and aluminum crimp tops. These powder samples were stored at 40° and samples periodically removed for assay of salicylic acid. Moisture content for mixes and tablets were determined by the Karl Fischer procedure.

**Tablet Preparation**—Portions of the above mixes were tableted on a Stokes E machine using a 10-mm. standard punch to give 300-mg. tablets. No attempts were made to maintain a constant hardness due to difficulties with flow of some mixes. The tablets were stored in 30-ml. vials under the same conditions as the powder mixes and sampled after 45 days for aspirin breakdown.

**Free Salicylic Acid Determination**—Samples of powder mixes or tablets were carefully and completely removed from the 30-ml. multiple dose vials in which they were stored. These samples were ground thoroughly in a mortar and a quantity equivalent to 200 mg. aspirin, along with 100 mg. citric acid dissolved in 10 ml. water-saturated chloroform with agitation (1 min.). This solution was

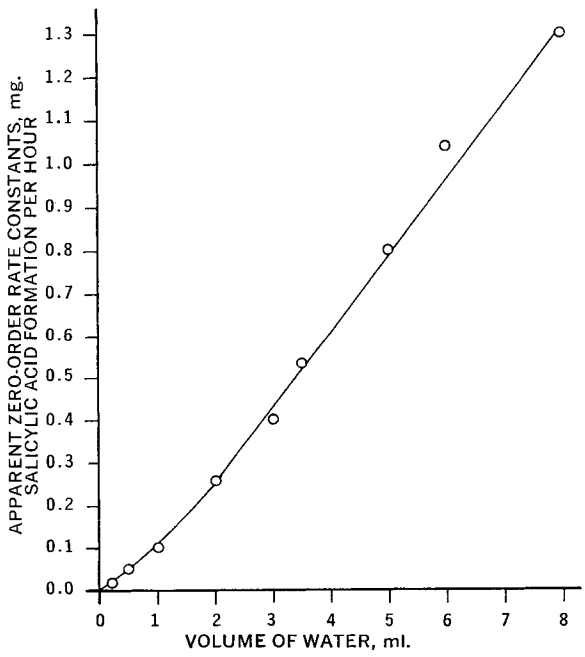


**Figure 2**—*Relationship of salicylic acid generation to the volume of water contained in Mix* A *suspensions. 10% aluminum hydroxide is the additive. Temperature, 40°.*

poured onto a column of 8 g. acid-washed diatomaceous earth[2, 3] previously mixed with 2% ferric chloride solution (8 ml.). The column was eluted with water-saturated chloroform (about 50 ml.) to remove aspirin and the purple complex eluted into a volumetric flask with 10% acetic acid in chloroform (10 ml.) followed by 1% acetic acid in water-saturated chloroform to remove the complex (10). The concentration of salicylic acid was determined by measuring the absorbance of the solution at 310 mμ.

**Suspension Studies**—Quantities of the powder mixes to which the 2% water had not been added were placed in 30-ml. vials. Varying amounts of water from 10 ml. down to 0.1 ml. were added exactly and a few glass beads introduced to promote mixing. The vials were sealed by rubber closures and aluminum crimp caps and placed on a rotating rack (6 r.p.m.) in a water bath at 40° (±0.2°). Samples for the free salicylic acid assays were taken in the following manner: water was added to the powder mix-water suspensions in the vials to give a total volume of water of 10 ml. The rubber closure was replaced and the vial vigorously shaken for 30 sec. to dissolve salicylic acid. A 3-ml. sample was removed through a pipet with a filter on its end (glass wool in rubber tubing) and this added to 3 ml. of 2% ferric chloride solution in a volumetric flask. The flask was filled with distilled water and the solution read against a blank of the same volume without the 3-ml. sample solution at 540 mμ (3).

## RESULTS AND DISCUSSION

The acceleration of aspirin hydrolysis by various common additives in both powders and tablets compressed from these powders was observed and the results are listed in Table I after allowing both powders and tablets to stand at 40° (±0.25°) for 45 days. The initial powder mix (Mix *F*) had 2% water added so aspirin degradation might proceed at a reasonably rapid and easily measurable rate. An attempt was made to use a standard type direct compression formulation although difficulties were encountered with flow of the two stearate salts and consequently their concentration was reduced to 5%. This had little bearing on the course of the study as goals were for relative ranking of various mixes rather than for any quantitative results at this time.

It is readily apparent from Table I that the tablets and powders fall into the same order regarding salicylic acid formation and thus the relative ranking numbers (between 1 and 100) show considerable similarity. The same behavior was demonstrated by samples stored at room temperature with the degree of degradation being less pronounced. This seems to indicate that the force of tablet compression has negligible effect on aspirin stability of the mixtures under scrutiny.

Table II gives the apparent zero-order rates with regard to salicylic acid formation in milligrams per hour for a saturated 1-ml. sample of the mixes. The relative ranking is seen to be the same as Table I.

Figure 1 illustrates a typical plot of salicylic acid generated *versus* time for a suspension of Mix *A* containing 594 mg. powder in 6 ml. water. The apparent zero-order rate constant can be obtained as usual from the slope of the straight line. Experiments of this sort (Fig. 1) were performed where the amount of water present in suspension was reduced to approach that of the solid dosage forms. Composites of these rate constants *versus* volumes of water in milliliters are shown in Figs. 2 and 3 for Mixes *A* and *C*, respectively. The tendency toward the anticipated zero rate of reaction at zero water concentration is obvious. Figure 4 gives the plots of suspensions with low water contents (0.1, 0.2, and 0.5 ml.) of the fast-reacting magnesium trisilicate system (*D*) in the usual units of milligrams salicylic acid (amount) *versus* time. The slope ratios deviate from the 1:2:5 which is expected from a process following the zero-order rate law. The low value obtained from the apparent zero-order rate constant in Figure 4 for the higher amount of water (0.5 ml.) is probably a result of unsaturation of the system with regard to all components. If such a system were saturated a graph of rates *versus* water volume would be linear as is the case in Figs. 2 and 3.

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

[3] When the samples showed considerable degradation a proportionally greater amount of Celite and ferric chloride solution had to be used.

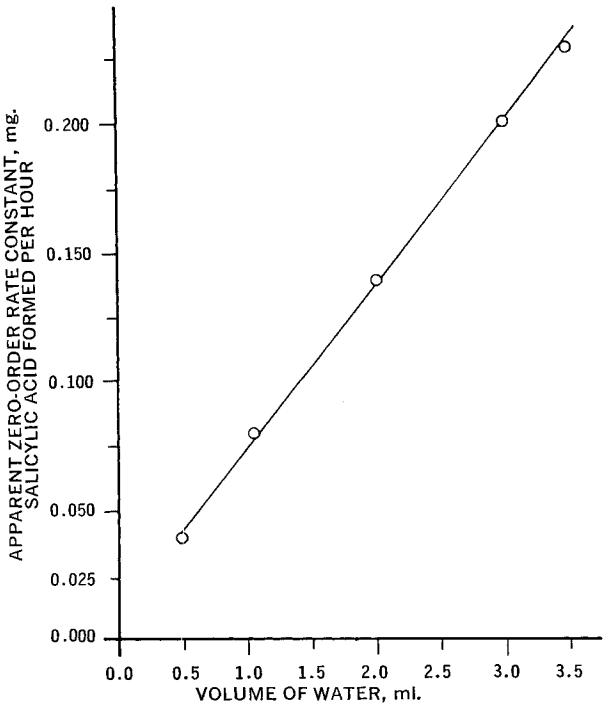


**Figure 3**—*Relationship of rate of salicylic acid generation to the volume of water contained in Mix* C *suspension. 10% hexamic acid is the additive. Temperature, 40°.*

The comparatively high reaction velocities encountered in systems as *D* as well as *B* and *E* (Tables I and II) may be partially accounted for by pH phenomena manifest in the suspension and in moisture present in solid dosage forms as previously mentioned by Zoglio and

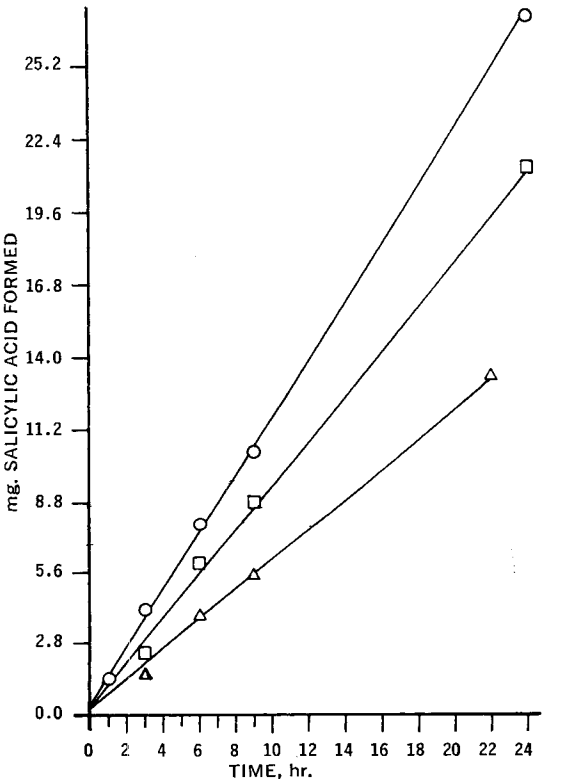


**Figure 4**—*Apparent zero-order plots for aspirin hydrolysis in aqueous suspensions containing 594 mg. Mix* D *(samples equivalent to 400 mg. aspirin). 10% magnesium trisilicate is the additive. Key:* ○, *slope 1.16, 0.5 ml.* $H_2O$; □, *slope 0.93, 0.2 ml.* $H_2O$; △, *slope 0.57, 0.1 ml.* $H_2O$.

Kornblum (3). The two mixes containing magnesium trisilicate and magnesium stearate result in generation of the freely soluble magnesium salt of aspirin producing a buffer with aspirin (3) of relatively high pH—3.5 and above—which is a poor environment for aspirin. When 1.188 g. Mix *D* was stirred in suspension with 20 ml. water the pH increased from 3.5 to 3.85 in 6 hr. while Mix *E* remained constant at 3.45 for the same time period. Over this interval of time the magnesium titer of *D* goes up to three times its initial value while *E* remains approximately at its original figure. Almost the same values hold for simple aspirin–magnesium stearate and aspirin–magnesium trisilicate suspensions. This helps explain the increased pH as it seems to be a partial result of a large amount of magnesium aspirin in solution. The pH profile of acetylsalicylic acid shows there is a considerable difference in the rate constants for degradation at these two values (3.85 for *D* and 3.45 for *E*) with the ratios being 2:1 at 25° (11). The high water content of magnesium trisilicate USP probably contributes to the degree and rate of Mix *D* relative to the others. It should be pointed out that in systems like these it is extremely unlikely that only one variable is operative but that several factors are working concomitantly to lead to the observed results. The 5% calcium stearate preparation (Mix *B*) exhibits somewhat less aspirin decomposition than 5% magnesium stearate which has previously been shown to be the case with simpler mixtures (3). The 10% aluminum hydroxide mixture (*A*) shows relatively little aspirin breakdown possibly due to the comparative insolubility of the aluminum salt of aspirin thus lowering the pH close to the saturation pH of aspirin (about 2.6) which is a fairly stable range for aspirin (11). Aluminum hydroxide is also well known as an adsorbent which may play some role in preventing aspirin decomposition.

The basic mix (*F*) and the 10% hexamic acid (*C*) show relatively little degradation as might be expected from pH effects (5).

In Table I the milligrams of salicylic acid formed in the tablets and powder mixes are expressed as the amount formed per 1% moisture. The adjustment was made when a variation of water content was found by Karl Fischer analysis (1.0 to 1.8%) rather than the expected 2%. Corrections were based on the assumption that for these systems apparent zero-order rates and therefore amounts of salicylic acid formed were directly proportional to moisture content. The adjustment to equal moisture levels in this manner provided a more successful degree of ranking.

Implicit in work of this nature is the ultimate aim of stability prediction for solid dosage forms from apparent zero-order rates obtained from suspension studies along with other pertinent information concerning the system under investigation. Long-term predictions were not attempted in this study but it is shown that relative results comparing powder mixes, tablets and suspensions exhibit very good correlation in the systems under observation as listed in Tables I and II.

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# Microculture Assay for the Rapid Determination of Antifungal Activity


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**Abstract** □ Determination of hyphal growth rates in microslide cultures has been utilized to determine concentrations of griseofulvin. At concentrations between 0.001 mcg. and 0.01 mcg./ml. griseofulvin in Sabouraud liquid medium, the curve showing growth rate as a function of log dose is linear. In this concentration range the rate of hyphal elongation is constant with respect to time, and curling or distortion of hyphae is not seen except at the upper limit of the range. Microculture assays are compared to simultaneous assays performed using the current USP agar cup procedure. The microslide technique requires a combined incubation and reading period of 24 hr. It can be used to determine accurately one thousandth the concentration of griseofulvin required for the official procedure.



**Keyphrases** □ Antifungal activity, determination—microculture slide technique □ Griseofulvin activity, assay—agar cup *versus* microculture technique □ Hyphal growth rates—griseofulvin concentration


Although chemical assays are available for most antifungal agents, microbiological assays are the accepted standards for potency determinations (1). Microbiological assays use minute quantities of drugs with solvent extraction being the only usual preparative step required for dosage forms or biological specimens. While compounds possessing analogous chemical structure frequently interfere with chemical assay methods, this is not a problem with microbiological methods, unless the analogs also possess biological activity. Microbiological assay techniques have been extended to the determination of compounds possessing antifungal activity. Turbidimetric and agar diffusion methods have been most widely accepted as antifungal assays (1). Although several methods have been developed to observe the growth of fungi microscopically, few have been developed sufficiently to be useful as assay procedures. The objective of this investigation is to develop the microculture slide technique of Elliott *et al.* (2) as a rapid, sensitive, and efficient assay procedure for antifungal agents. Because of its potency