REFERENCES

(1) Sadusk, J. F., Jr., "Changes and Trends in the Drug Regulatory Process," 4th annual Roland T. Lakey Honorary Lecture, Wayne State University College of Pharmacy, Detroit, Mich., Dec. 5, 1967.

(2) Glazko, A. J., Kinkel, A. W., Alegnani, W., and Holmes, E. L., *Clin. Pharmacol. Therap.*, **9**, 472(1968).

(3) Aguiar, A. J., Zelmer, J. E., and Kinkel, A. W., *J. Pharm. Sci.*, **56**, 1243(1967).

(4) Crane, R. K., and Wilson, T. H., *J. Appl. Physiol.*, **12**, 145(1958).

(5) Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques," 3rd ed., Burgess, Minneapolis, Minn., 1959, p. 149.

Keyphrases

Chloramphenicol capsules
Release, availability—chloramphenicol capsules
Deaggregation rates, capsules—turbidity measurements
Dissolution, capsules—*in vitro*
Intestinal sac, everted—drug permeation

Significance of Salicylic Acid Sublimation in Stability Testing of Aspirin-Containing Solids

By A. Y. GORE*, K. B. NAIK, D. O. KILDSIG, G. E. PECK, V. F. SMOLEN, and G. S. BANKER

The salicylic acid content, formed from the decomposition of aspirin, was found to be an unreliable basis for judging the stability of aspirin tablets. Under conditions of accelerated stability testing, the loss of salicylic acid from the system by sublimation can incur appreciable errors in the direction of overestimating aspirin stability. Since aspirin was not detected to sublime under these same conditions, its residual content is an improved indication of its stability. A method for its simultaneous determination with salicylic acid is presented.

SALICYLIC ACID was observed to be lost from aspirin tablets undergoing accelerated stability testing in this laboratory. This is illustrated in Fig. 1 where deposits, analytically identified as salicylic acid, are shown on the surface of aspirin tablets coated with a cellulosic film.

Considering that salicylic acid is a hydrolytic decomposition product of aspirin and sublimation is a commercial method for its purification (1), its volatilization under the elevated temperature and humidity conditions employed in accelerated stability testing could be anticipated. However, this phenomenon apparently has not been previously studied. An earlier investigation (2) either overlooked it or treated it as being unappreciable.

A more than negligible loss of the 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

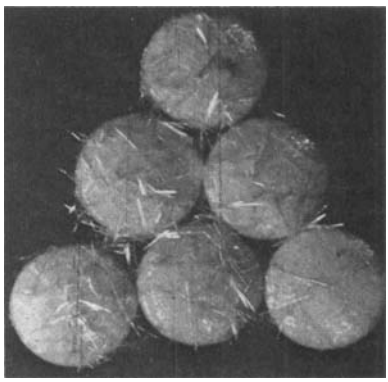

Fig. 1—Aspirin tablets coated with a cellulosic film and stored at 81.2% relative humidity and 50° for 98 days. The crystalline deposits on the surface were identified as salicylic acid formed from the decomposition of aspirin.

Received May 29, 1968, from the Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907

Accepted for publication July 18, 1968.

Presented to the Drug Standards, Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

This research was supported by a grant from the Robertshaw Chemical Co., Phoenix, Ariz.

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false confidence in the stability of the tested products.

The purpose of the present study was to determine whether the loss of salicylic acid by sublimation could introduce an appreciable error into stability tests based on its content within aspirin-containing solids. To achieve this objective it was first necessary to develop a method of gauging aspirin stability in solids which would be unaffected by any loss of salicylic acid.

MATERIALS AND METHODS

Reagents—All reagents were of analytical grade with the exception of aspirin USP. The aspirin used in the determination of Beer's law plots and spectral absorbance curves was purified by four successive recrystallizations. Each recrystallization involved dissolving 25 g. of aspirin in 25 ml. of anhydrous ether, filtration, addition of 20 ml. of petroleum ether, cooling to 0°, collection of crystals, washing with petroleum ether, and final drying in air. The purified crystals were stored in a vacuum desiccator. The recrystallized aspirin was found to be 100% pure as judged from the USP titration method for aspirin.

Determination of Salicylic Acid and Aspirin Sublimation—Samples of salicylic acid, aspirin, or mixtures of salicylic acid and aspirin were placed on the pan of a Cahn model RG electrobalance. The samples were maintained at constant temperatures of 40, 50, and 70 ± 1°, by placing the weighing chamber assembly within an oven. The initial sample weights were the same at each temperature studied. The powdered samples were spread evenly onto the balance pan so as to cover the entire pan thereby exposing a constant surface area. The loss of weight of the samples was continuously followed as a function of time with the aid of a Sargent SR recorder used in conjunction with the Cahn balance.

Development of a Simultaneous Assay Method for Aspirin and Salicylic Acid—A UV spectrophotometric method of analysis employing a pH 7.4 Clark and Lubs buffer as the solvent was developed. In comparison with the use of spectral grade chloroform as the solvent, this method provided improved precision. The method was adopted from Edward's study of aspirin hydrolysis (3). The pH of the buffer was chosen as 7.4. This value is sufficiently above the acidic pK's of salicylic acid and aspirin (3.49 and 2.97, respectively) that slight variations in the pH of the medium would not introduce an error into the determinations as a consequence of the differential absorption of ionized and unionized species (3). Furthermore, the slightly alkaline medium affords relatively rapid solution of solid aspirin and salicylic acid samples. The dissolution must be rapid in order to minimize aspirin hydrolysis during sample preparation and analysis. Edwards (3) has reported the apparent first-order hydrolysis of aspirin at 17° to be independent of pH in the range of 4–8.

In order to estimate the magnitude of the error resulting from aspirin hydrolysis in the pH 7.4 buffer prior to spectrophotometrically reading the samples, the hydrolysis rates were determined at temperatures ranging from 17.2 to 30.2 ± 0.1°. The aspirin hydrolysis was continuously followed with time using a Beckman DB spectrophotometer in conjunction with a Sargent SR recorder. The temperature of the reaction mixture was controlled by the use of a jacketed sample compartment through which water of the desired temperature was circulated. The reactions were followed for 1-hr. periods by recording the change in absorbance at 296.5 mμ (3). The results are summarized in Table I and by the Arrhenius plot of the apparent first-order rate constants in Fig. 2. The energy of activation was found to be 17.060 kcal./mole. This value is in fair agreement with the value of 17.40 kcal./mole reported by Garrett (4) for aspirin hydrolysis at pH 5.05 in 0.5% ethanol solution.

Table I—Rate Constants for the Hydrolysis of Aspirin in pH 7.40 Buffer Solution

Temp., °C.	k (Rate Constant), Day^{-1}	$\bar{k}$ (Average Rate Constant)
17.2	i, 0.08958 ii, 0.09786	0.09372
21.3	i, 0.1418 ii, 0.1595	0.1506
25.5	i, 0.1914 ii, 0.2221	0.2067
30.2	i, 0.3434 ii, 0.3425	0.3429

In accordance with the hydrolysis results, a delay of 13 min., at 25.5°, between the time of 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.

The selection of wavelengths for the spectrophotometric determination of aspirin and salicylic acid was made on the basis of the spectral absorption curves shown in Fig. 3. These curves were obtained using a Bausch and Lomb Spectronic 505 spectrophotometer and the pH 7.4 buffer as the solvent. Based on these curves in Fig. 3, wave-

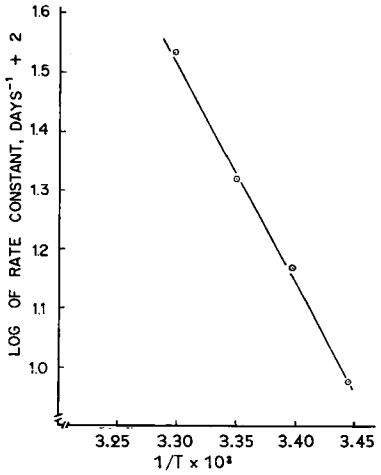

Fig. 2—Arrhenius plot for aspirin hydrolysis in pH 7.40 buffer solutions.

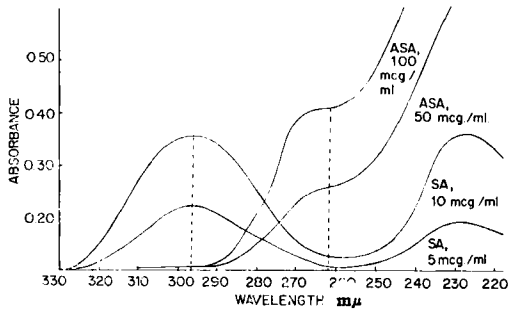

Fig. 3—Wavelength-absorbance spectra for aspirin and salicylic acid in pH 7.40 buffer solution.

lengths of 296.5 and 262 mμ were selected to be routinely employed for the assay. It may be noted that at 296.5 mμ aspirin absorbance is independent of concentration. The small absorbance noted in this region for aspirin may be merely due to the base line of the instrument. The absorbance of a mixture of aspirin and salicylic acid at 296.5 mμ is therefore attributable to that of salicylic acid plus a constant independent of the amount of aspirin present. The data pertinent to the construction of the required Beer's law calibration curves is presented in Table II. Variations from

TABLE II—CONSTANTS FOR ASPIRIN AND SALICYLIC ACID ULTRAVIOLET SPECTROPHOTOMETRIC ASSAY

Drug	Concentration Range, Mcg./ml.	Absorptivity —Abs./mg./ml.— 262 mμ	296.5 mμ
Aspirin	0–160	3.2	0
Salicylic acid	0–10	3.3	—
	0–30	—	26.0

the means of triplicate absorbance values were found to be within 0.002 absorbance units. A Beckman DU-2 spectrophotometer was employed. In order to correct for any variations in instrument response the apparently constant, concentration independent, absorbance of aspirin at 296.5 mμ was determined each experimental day. At 262 mμ the calibration curve for salicylic acid deviates from Beer's law below a concentration of 2 mcg./ml. Absorbance values of 0.006–0.007 were commonly observed below this concentration. When this condition arose, a known amount of salicylic acid was added to the sample solution to raise its concentration to approximately 6 mcg./ml. and therefore into a region where Beer's law is followed. Raising the concentration of salicylic acid in this manner allows the absorbance of aspirin at this wavelength to be more precisely determined.

Assay Procedure—A solid sample was comminuted and 10–50 mg. of the powder was dissolved in 25 ml. of pH 7.4 buffer maintained at 0°. Following filtration, 3.0 ml. of the solution was diluted to 50 ml. and the absorbance determined as described earlier. The entire procedure was completed within a period of 5–10 min. The absorbance measured at the 2 wavelengths and the constant absorbance for aspirin at 296.5 allowed the concentrations of aspirin and salicylic acid to be readily calculated (5).

Preparation and Stability Testing of Aspirin Tablets—Aspirin tablets containing 10% starch and 3% talc, having a total weight of 624 ± 5.71 mg. (*SD*) were prepared using a 1.11 cm. (7/16 in.) diameter punch. Tablets having a thickness of 0.088 cm. (0.2236 in.) ± 2.09 × 10^{-3} and a hardness of 32.95 ± 1.27 lb. were produced. Prior to stability testing the tablets were stored in a vacuum desiccator. For stability testing the tablets were placed on a fiberglass screen within a desiccator containing a saturated potassium chloride solution which maintained the humidity at 81.29% relative humidity (74.20 mm. Hg) at 50°. The desiccator was placed within an oven maintained at 50 ± 1°. At selected time intervals four tablets were removed for analysis. Under these test conditions, no moisture condensation was observed in the storage desiccators.

RESULTS

Verity and Error in the Method of Analysis—The developed assay procedure was applied to solutions of known salicylic acid and aspirin concentration. The agreement between known and determined concentrations of aspirin and salicylic acid was exact within the number of figures significant in the experiment. The data are presented in Table III.

The assay procedure was developed for the determination of aspirin in solids. In this application an error of 0.003 absorbance units at 262 mμ can contribute an error of approximately 1% in the determination of the aspirin content of a solid consisting of 80% aspirin using a 50-mg. aliquot for analysis. A single analysis of a 9:1 powdered mixture of aspirin and salicylic acid revealed an error of 1.09% and 1.52% in its aspirin and salicylic acid content, respectively. These results are shown in Table IV.

TABLE III—VERIFICATION OF THE ASSAY OF ASPIRIN IN MIXTURES OF SALICYLIC ACID AND ASPIRIN

Buffered Solution, pH 7.40	Known Concentration, mcg./ml.	Absorbance, 262 mμ	296.5 mμ	Concentration Determined, mcg./ml.
Salicylic acid (SA)	10	0.034	0.260	10
Aspirin (ASA)	104	0.336	0.012	104
Mixture equal vol. SA and ASA	5(SA) 52(ASA)	0.184	0.138	5(SA) 52(ASA)

TABLE IV—MASS BALANCE AND RESULTS OF ASSAYING A KNOWN 9:1 MIXTURE OF ASPIRIN AND SALICYLIC ACID BEFORE AND AFTER STORAGE FOR 12 HR. AT 70°C.

	Zero Time	After Storage at 70°C. for 12 hr.
Actual weight of sample[a]	55.35	51.90[b]
Assayed aspirin content	50.42	50.60
Assayed salicylic acid content	4.69	1.33
Total sample weight on basis of assay	55.11	51.93
Aspirin, %	91.09	91.42[b]
Salicylic acid, %	8.48	2.40[b]

[a] Weights are in mg. determined with a Cahn model RG electrobalance. [b] The loss of sample weight was 3.35 mg. Based on the assay results 3.18 mg. is attributable to salicylic acid and a weight gain of 0.18 mg. occurring for aspirin.

TABLE V—SUBLIMATION RATES OF SALICYLIC ACID AT 40, 50, AND 70°C.

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

Sublimation of Salicylic Acid and Aspirin—Sublimation rates of salicylic acid were studied at 40, 50, and 70°. The rates, under conditions of constant exposed surface area, were independent of time for the 12-hr. period in which they were recorded. The values are presented in Table V. An Arrhenius type plot of the apparent zero-order sublimation rates is presented in Fig. 4. The slope of the curve in Fig. 4 is likely predominately determined by the enthalpy of sublimation of salicylic acid. The observed rates of sublimation may be expected to directly depend upon the area

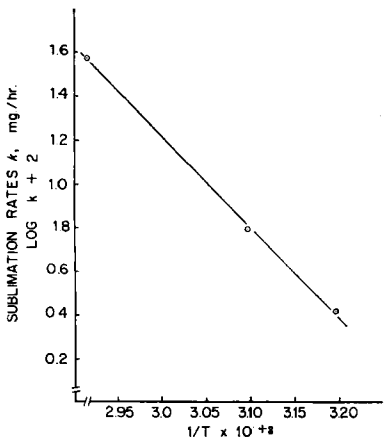

Fig. 4—Arrhenius type plot for sublimation of salicylic acid.

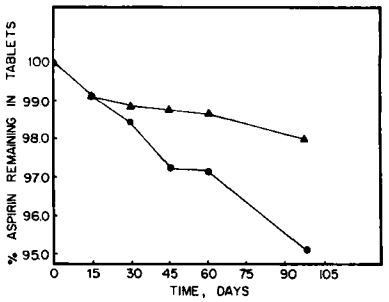

Fig. 5—Hydrolysis of aspirin in tablets at 50° and 81.20% relative humidity (74.20 mm. Hg water vapor pressure). Key: ▲, calculated on basis of salicylic acid content; ●, calculated on basis of aspirin content.

through which the mass transfer occurred. These results therefore are not intended to be quantitatively indicative of sublimative loss of salicylic acid during stability testing, but merely substantiate that such loss can occur even at moderately elevated temperatures.

A similar sublimation study of purified aspirin revealed no significant loss of weight up to 70°. The sensitivity of the Cahn electrobalance is 1×10^{-4} mg. It was therefore concluded that aspirin does not appreciably sublime under the conditions of the experiment.

The observed lack of sublimation of aspirin and appreciable loss of salicylic acid was further confirmed in mixtures. The loss of weight of a mixture of 9 parts aspirin to 1 part salicylic acid was followed at 70° for 12 hr. The mixture was assayed for aspirin and salicylic acid before and after storage. The results presented in Table IV indicate that only salicylic acid was lost from the sample.

If the 9:1 mixture of aspirin and salicylic acid had been a solid dosage form of aspirin, which had decomposed to the extent of 10%, a serious underestimation of its extent of decomposition would occur if it was based upon salicylic acid content rather than residual aspirin content. On the basis of residual aspirin content, following 12 hr. storage, the extent of decomposition would be recorded as 8.58%.[1] Under similar conditions the extent of decomposition based on salicylic acid content would be determined as only 3.12%.[1]

Sublimation of Salicylic Acid from Aspirin Tablets Undergoing Stability Testing—The average percentages of original aspirin content, present at zero time, remaining in tablets stored at 81.2% relative humidity and 50° are presented in Fig. 5. The upper plot is based on the salicylic acid content of the tablets. The lower curve is based upon determinations of the aspirin content of the tablets. The disparity between the two curves is noted to increase with time. The vertical difference between the two curves represents the percent error involved in assuming the salicylic acid content of the tablets is representative of the extent of aspirin decomposition. This same vertical distance between the curves also represents the salicylic acid lost by sublimation from the tablets. The upper curve in Fig. 5 appears to possess some tendency to approach constancy. This observation could

[1] These values are the result of a single determination.

TABLE VI—COMPARISON OF ASPIRIN TABLET STABILITY TESTING RESULTS AT 50°C. AND 81.2% RELATIVE HUMIDITY, BASED ON THE 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 4 determinations recorded with ±1 standard deviation.

be interpreted as a trend toward equilibrium within the tablet (2). Judging from the lower curve, no such trend toward equilibrium is apparent. The data pertaining to Fig. 5 is summarized in Table VI.

SUMMARY AND CONCLUSIONS

Previously reported studies of the stability of aspirin-containing solids have utilized the salicylic acid content of the solids as a measure of aspirin decomposition. The underestimation in the extent of decomposition of the aspirin which can result from such a practice is clearly demonstrated by the results of the present work. The error can be expected to become increasingly serious with storage time and the severity of the temperature and humidity conditions under which the solids are stored. This error can be circumvented by employing a method of determining decomposed aspirin which is independent of the loss of salicylic acid from the solid due to sublimation. Since aspirin was found not to sublime, the determination of its residual content in a solid is a valid means of gauging the stability of the formulation. A method of analysis for aspirin with an accuracy of at least 1.5% was developed for application to testing of aspirin stability in solid dosage forms.

REFERENCES

(1) Nord, M., *Chem. Eng.*, **58**, 157(1951).
(2) Leeson, L. J., and Mattocks, A. M., *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 329(1958).
(3) Edwards L. J., *Trans. Faraday Soc.*, **46**, 723(1950).
(4) Garrett, E. R., *J. Org. Chem.*, **26**, 3660(1961).
(5) Willard, H. H., Merritt, L. L., and Dean, J. P., "Instrumental Methods of Analysis," 4th. ed., D. Van Nostrand, Princeton, N. J., 1965, pp. 94–95.

Keyphrases

Aspirin stability—salicylic acid sublimation
Salicylic acid sublimation—aspirin tablets
Temperature effect—aspirin tablets stability
UV spectrophotometry—analysis

Stability of Cyanocobalamin in Film-Coated Multivitamin Tablets

By JAMES T. JACOB, ROBERT J. NESSEL, and JACK BLODINGER

Multivitamin tablets containing each of two commercially available protected forms of cyanocobalamin showed considerable loss of vitamin B_{12} after exposure to methanol vapor for 1 month at room temperature. Other solvents commonly used in film coating such as acetone, *n*-butanol, butyl acetate, isopropanol, and methylene chloride did not affect the stability of vitamin B_{12}. Tablets containing vitamins B_1, B_{12}, ascorbic acid, and niacinamide, alone and in combination with one another after exposure to methanol vapor at room temperature showed considerable loss of vitamin B_{12} only in presence of ascorbic acid and/or niacinamide.

THERE ARE SEVERAL reports in the literature on the stability of cyanocobalamin (vitamin B_{12}) in liquid multivitamin preparations. Decomposition products of other vitamins, pH, heat, and light can in most cases contribute to the degradation of vitamin B_{12}. Feller and Macek (1) reported that decomposition of vitamin B_{12} occurs at elevated temperatures in the presence

Received June 13, 1968, from the Product Development and Service Laboratories, Merck & Co., Inc., Rahway, NJ 07065
Accepted for publication July 25, 1968.
Presented to the Industrial Pharmaceutical Technology Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.