(31) E. W. Gill, G. Jones, and D. K. Lawrence, *Biochem. Pharmacol.*, **22**, 175 (1973).
(32) R. Karler and W. Cely, *Fed. Proc.*, **33**, 540 (1974).
(33) E. W. Gill and D. K. Lawrence, *J. Pharm. Pharmacol.*, **25**, 988 (1973).
(34) B. T. Ho, V. S. Estevez, and L. F. Englert, *ibid.*, **25**, 488 (1973).
(35) F. H. Dost, "Grundlagen der Pharmakokinetik," Geog. Thieme Verlag, Stuttgart, Germany, 1968, pp. 41–43.

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Determination of Porosity and Pore-Size Distribution of Aspirin Tablets Relevant to Drug Stability

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Abstract □ Total porosity and pore-size distribution of aspirin tablets prepared from aspirin, starch USP, and precipitated colloidal silicon dioxide were determined using mercury porosimetry. The model represented a hydrolyzable drug substance in combination with simple excipients. The role of starch and silicon dioxide on the microstructure of the tablets was investigated, as was the chemical stability of various systems. In general, the porosity of tablets containing a constant quantity of starch increased linearly with silicon dioxide concentration. Examination of the pore-size distribution, however, revealed that at low concentrations silicon dioxide functioned primarily to reduce the size and volume of coarse pores representing the spaces between the agglomerates of starch and aspirin particles. This effect was optimum at 3%. A further increase in silicon dioxide concentration produced tablets with relatively larger pore sizes. Studies of changes in the porosity characteristics of tablets as influenced by water vapor over time showed distinct differences in this complex parameter. A unique trend in the change of the pore-size distribution was noted with tablets containing 3% silicon dioxide. These observations are discussed relative to the stability of aspirin tablets in which this concentration of silicon dioxide produced a maximum stabilizing effect.

Keyphrases □ Aspirin—tablets, stability, porosity and pore-size distribution determined, effect of colloidal silicon dioxide □ Porosity and pore-size distribution—aspirin tablets, effect of colloidal silicon dioxide □ Stability—aspirin tablets, porosity and pore-size distribution determined, effect of colloidal silicon dioxide □ Silicon dioxide, colloidal—effect on stability of aspirin tablets, porosity and pore-size distribution determined □ Dosage forms—aspirin tablets, stability, porosity and pore-size distribution determined, effect of colloidal silicon dioxide

In any systematic and fundamental approach to the design of solid dosage forms, a comprehensive elucidation of the physical characteristics of the solid components is necessary and basic. Knowledge of the physical nature of solid pharmaceutical systems is often confined to determinations of such properties as particle size, particle-size distribution, and true and bulk density. To relate the disintegration, dissolution, and stability characteristics to meaningful physicochemical parameters, a direct examination of the porosity characteristics also may be essential.

The pharmaceutical and chemical literature clearly documents that the pore-size distribution of solids is frequently among the controlling variables in processes involving disintegration, dissolution, adsorption, and diffusion. In tablet disintegration and dissolution, the initial stage is probably typically the penetration of the solvent medium into the tablet through the tablet pore system. The solvent penetration rate, in turn, was shown to be intimately related to the volume and size of tablet pores (1–6). From the standpoint of tablet stability, especially when dealing with hydrolyzable drugs, a physical description of the porous state of tablets may be important, since porosity and pore-size distribution affect the diffusion of water vapor and the accessibility of condensed vapor to the labile drug.

Studies on the porous nature of tablets have been conducted with increasing frequency recently (7). Porosity characteristics of tablets are primarily influenced by compression as a result of fragmentation, deformation, and consolidation of particles. Additional factors include particle size, particle shape, and the compressibility of particles (5, 6, 8).

As evident from review articles, the stability of aspirin in the solid state was the subject of numerous studies (9, 10). Many adjuvants have been proposed to enhance aspirin stability. Studies in these laboratories showed that inclusion of precipitated colloidal silicon dioxide into tablets prepared from aspirin and starch significantly improved aspirin stability. The maximum stabilizing effect of silicon dioxide occurred at 3%. Above this level, a continuous decrease in the stability of aspirin was observed. Although the major mechanism of this effect may be the large adsorptive capacity of silicon dioxide which functions as an internal tablet desiccant, an evaluation of tablet porosity characteristics may add significant dimensions in the interpretation of the physicochemical phenomena involved in aspirin decomposition.

The present study was undertaken to determine the influence of colloidal silicon dioxide on the porosity and pore-size distribution of aspirin tablets using mercury

Table I—Porosity and Related Characteristics of Aspirin Tablets

Silicon Dioxide Concentration, %	Apparent Volume, ml	True Volume, ml	Displacement Volume[a], ml	Total Porosity, %	Porosity in Pore Radius Range, %	
					$r > 0.006$ μm	$r < 0.006$ μm
Control	0.3508	0.3281	0.3504	6.46	5.54	0.92
0	0.3669	0.3426	0.3663	6.62	6.44	0.18
1	0.3684	0.3418	0.3682	7.21	6.85	0.36
3	0.3700	0.3392	0.3698	8.33	7.44	0.89
5	0.3732	0.3366	0.3730	9.81	9.14	0.67
10	0.3765	0.3302	0.3763	12.29	11.44	0.85
15	0.3830	0.3239	0.3825	15.43	15.24	0.19

[a] Determined by displacement of mercury at 1.6 psia.

porosimetry. Of major interest were the structural changes that occur in tablets under the influence of water vapor. On the basis of these observations, the role played by silicon dioxide was assessed and, inferentially, extended to the general utility and significance of pore-size distribution measurements in compressed pharmaceutical systems.

EXPERIMENTAL

Materials—Aspirin USP (200–250-mesh powder), starch USP (80 mesh), and an experimental precipitated colloidal silicon dioxide meeting NF specifications were used in the preparation of aspirin tablets. Before use, starch and silicon dioxide were pretreated for about 12 hr by evacuation in a vacuum oven at 60 and 150°, respectively. The nitrogen surface areas of these substances were determined by the Brunauer, Emmett, and Teller (BET) method (11) using a commercially available instrument[1]. The corresponding values were 0.6 m^2/g for starch and 556 m^2/g for silicon dioxide. The true density measurements were performed by a helium-displacement technique utilizing the instrument[1] employed in the BET surface area determinations. The following values were obtained: aspirin, 1.40 g/ml; starch, 1.59 g/ml; and silicon dioxide, 2.29 g/ml.

Tablet Preparation—Several tablet formulations with various silicon dioxide concentrations (0, 1, 3, 5, 10, and 15%) were prepared. Each tablet contained 23 mg of starch in addition to the total aspirin–silicon dioxide content of 460 mg. Silicon dioxide concentrations were expressed on the basis of a 460-mg tablet weight, excluding the weight of starch. Tablets also were made from aspirin powder alone to serve as control samples. The components for 10 tablets of each formulation were weighed and mixed for 15 min in a miniblender of approximately 20-g capacity. The miniblender consisted of a V-shaped shell fabricated from clear plastic prescription bottles and was operated at 14 rpm by a motor.

An accurately weighed quantity of the powder mixture was then placed in a 12.7-mm flat-faced punch and die set and compressed at a calculated punch face pressure of 15.3×10^3 psi between the plates of a hydraulic press[2]. The weight of the compressed sample was again determined, and the tablet dimensions were measured with a micrometer. These physical measurements assured compliance of each tablet to identical compression conditions for consecutive samples and allowed the calculation of the apparent volume of the tablet.

To evaluate the chemical stability of tablets and the changes in their porosity characteristics with time, a relative water vapor pressure of 0.82 and a temperature of 40° were selected as the storage conditions. Constant relative pressure was maintained in a desiccator by a saturated potassium chloride solution (12). Samples were placed in the desiccator and stored in an oven until testing.

Evaluation of Porosity and Pore-Size Distribution—The total porosity of a tablet was determined from its apparent volume, its weight, and the true density of the individual tablet components. Pore-size distribution determinations were performed by intrusion of mercury using a commercial porosimeter[3] with a pressuring capacity of 15,000 psi. Approximately 20 increments of pressure were recorded in a typical determination, allowing sufficient time at each pressure for mercury to reach equilibrium in the sample. Equilibrium was assumed to have been reached when the liquid ceased penetration into the sample or penetrated at a rate of not more than 0.0001 ml/min.

In the derivation of pore-size distribution from basic intrusion data, the equation derived by Washburn (13) was used. The surface tension of mercury was taken to be 484 dynes/cm at 24°, the temperature of the measurements, as the best available value (14, 15). In the absence of specific data for the contact angle between mercury and the samples studied, this value was assumed to be 130°, a value commonly used for most solids (16, 17). Appropriate corrections were applied for the compressibility of mercury and of the residual air entrapped in the penetrometer in the initial evacuation step. Any volume change due to the compressibility of the sample itself was considered negligible. A correction factor for effective compressibility of mercury was determined by measuring the effect of applied pressure on the penetrometer (sample cell) filled entirely with mercury, *i.e.*, without any sample being present. Under the experimental conditions, the required corrections did not exceed 0.0075 ml. A description of the pore-size distribution calculations is presented elsewhere (18).

Evaluation of Aspirin Stability—The residual aspirin content of the tablets was determined by a spectrophotometric method (19). Basically, this procedure was based on a simultaneous determination of aspirin with salicylic acid in a pH 7.0 buffer solution at 296.5 and 262 nm.

RESULTS AND DISCUSSION

Effect of Silicon Dioxide on Porosity Characteristics—The results of pore volume and porosity determinations of aspirin tablets prepared with varying concentrations of silicon dioxide are outlined in Table I. The data are based on calculations from tablet dimensions and on results of mercury intrusion studies. The pore volume determined by intrusion of mercury represents the volume of pores in the pore radius range of about 60–0.006 μm. These limits of measurable pore radius are imposed by the pressure used in the initial introduction of mercury to the sample and by the maximum pressuring capacity of the instrument.

The close agreement seen in Table I between the measurements of apparent and displacement volumes of tablets shows that pores with radii larger than 60 μm were absent. The pore-size range determined by mercury intrusion represents virtually all of the pore volume present. The pore volume calculated to be present but remaining unintruded by mercury, in fact, constitutes an overestimate of the volume of pores smaller than 0.006 μm in radii. This unintruded volume also includes:

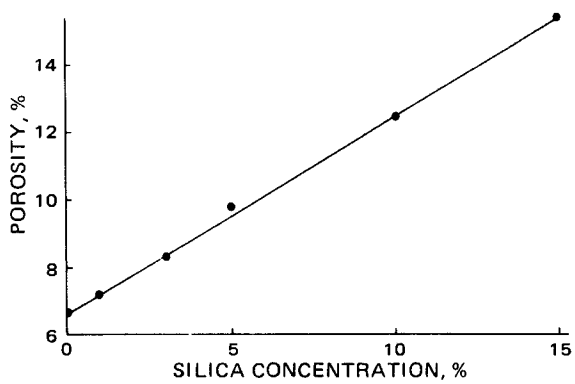

Figure 1—*Effect of colloidal silicon dioxide on total porosity of aspirin tablets.*

[1] Orr surface-area pore-volume analyzer, model 2100, Micromeritics Instrument Corp., Norcross, Ga.
[2] Carver laboratory press, model C, Fred S. Carver Inc., Summit, N.J.
[3] Aminco, Catalog No. 5-7121, American Instrument Co., Silver Spring, Md.

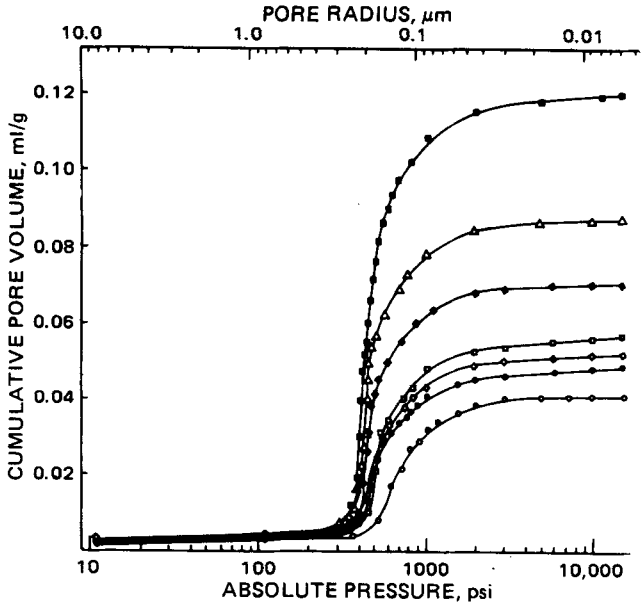

Figure 2—*Cumulative pore-size distribution of aspirin tablets containing various silicon dioxide concentrations. Key:* ○, *control;* ●, *0%;* ◇, *1%;* □, *3%;* ◆, *5%;* △, *10%; and* ■, *15%.*

(*a*) the volume of pores with radii larger than 0.006 μm but accessible to mercury only through smaller openings, and (*b*) the volume of pores that may be completely isolated from the pore network in the tablets. The total measured porosity (Table I) is in excellent agreement with the porosity calculated from the apparent volume and the true volume (Table I). However, the pore sizes determined by the mercury intrusion method represent the size of pore openings rather than the actual size of the pores.

A comparison of tablet porosity results given in Table I reveals that the total porosity of tablets prepared from aspirin only (control) was slightly lower than that prepared with 0% silicon dioxide. Since the latter formulation contained starch (5% of the total aspirin–silicon dioxide content), starch apparently did not alter significantly total tablet porosity under these compression conditions. On the other hand, the major effect of silicon dioxide on the porosity characteristics of aspirin tablets, containing a constant 5% level of starch, was immediately evident as a consistent increase in the total porosity with silicon dioxide concentration (Fig. 1). Despite its smaller specific volume, silicon dioxide contributed to the porosity of tablets to a greater extent than aspirin.

The manner by which silicon dioxide alters the porosity characteristics of aspirin tablets can best be inferred by examining the pore-size distribution of the samples shown in Fig. 2, where the cumulative pore volume intruded by mercury is plotted *versus* the pressure applied on the sample. The corresponding pore radius is also indicated on the upper abscissa. The data are presented in the form of a cumulative distribution function, which has the advantage that the fraction of pore volume between any arbitrary bounds of pore radii can be quickly assessed. The sigmoid shape of the cumulative distribution curves is characteristic of this determination. Although the pore-size measurements were originally made starting from a pore radius of about 60 μm, almost negligible intrusion was observed in the pore-size range of 60–10 μm. Therefore, these portions of the curves were omitted from the graph. The experimental points shown represent the average of at least two separate determinations. The reproducibility of the data was excellent.

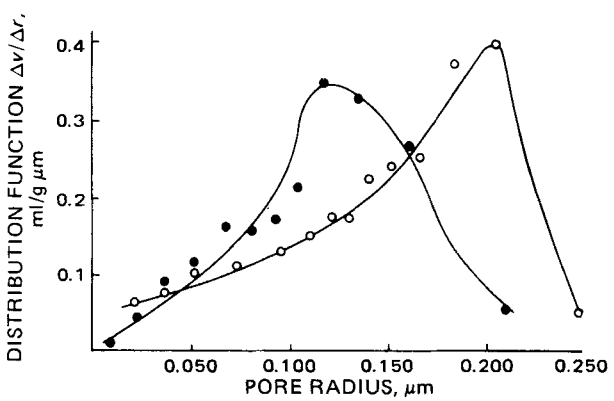

Figure 3—*Pore-size distribution of aspirin tablets. Key:* ●, *aspirin (control); and* ○, *aspirin with 5% starch (0% silicon dioxide).*

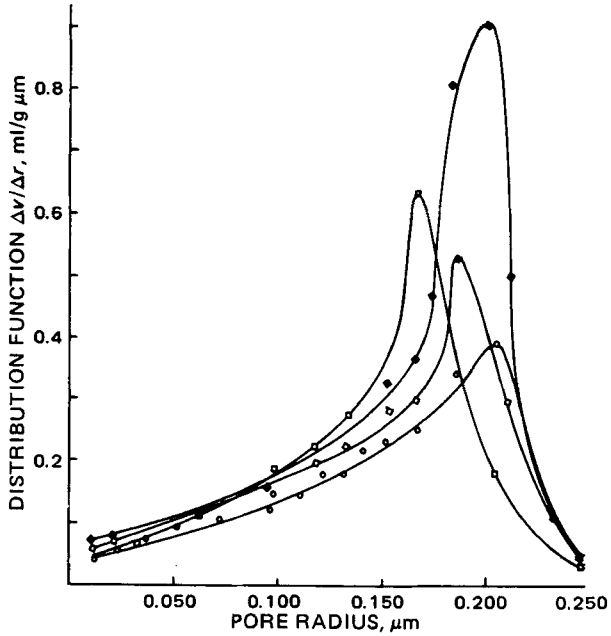

Figure 4—*Pore-size distribution of aspirin tablets containing various silicon dioxide concentrations. Key:* ○, *0%;* ◇, *1%;* □, *3%; and* ◆, *5%.*

Inspection of Fig. 2 shows the absence of significant pore volume for pores larger than about 0.2 μm followed by an abrupt upward trend, indicating that the distribution of pores was confined over a relatively narrow size range in all formulations. A greater portion of the pore volume was in the lower pore-size region as the silicon dioxide concentration increased. Judging from the slopes of the curves toward the lower pore-size limit of the instrumentation employed, essentially all of the pore volume present in tablets existed in accessible pores larger than 0.006 μm in radii.

The distribution curves display an apparent threshold radius characterized by a sudden, significant intrusion of mercury into the tablet pore system. The threshold radius may represent the minimum radius of pores that are essentially continuous throughout the tablet.

An alternative way of examining the results is to present the distribution curves as the derivative distribution function, *i.e.*, differential or derived pore-size distribution curves. Figures 3–5 represent typical distribution curves for the aspirin tablets investigated. The pore system developed in each formulation had a simple continuous distribution of pores. The pore-size distribution of tablets was drastically altered by the addition of 5% starch to the tablets, as judged from the shift in the maximum frequency toward a greater pore radius (Fig. 3). Apparently, starch promotes a tablet pore system characterized by a relatively larger size and volume of coarser pores. This observation seems to be rather significant in view of the fact that the influence of starch to total porosity is not quite so pronounced (Table I).

On the other hand, tablets containing 1% silicon dioxide in addition to starch showed a pore-size distribution with a maximum frequency at a smaller pore size (Fig. 4) than the tablets prepared from aspirin and starch only. This trend continued upon further addition of silicon dioxide, *i.e.*, up to 3%, but it was reversed at higher levels of silicon dioxide (Figs. 4 and 5). When the silicon dioxide concentration exceeded 3%, the general distribution moved consistently to relatively larger pore sizes with increasing silicon dioxide concentration. This concentration appeared to be rather characteristic, since the observed maximum frequency corresponded to the smallest pore size among tablets containing various silicon dioxide concentrations.

The major differential effect of incorporating silicon dioxide in the formulations was to decrease the size of coarser pores (Figs. 4 and 5). At a concentration level of 5%, the pore size corresponding to the maximum frequency of the distribution approached that of tablets containing no

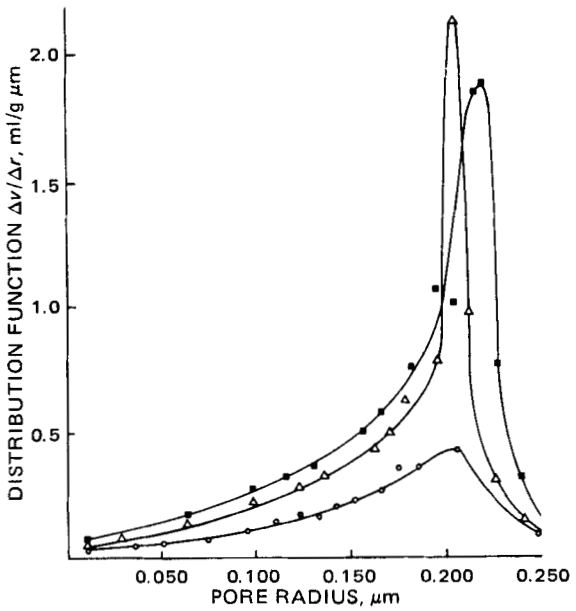

Figure 5—*Pore-size distribution of aspirin tablets containing various silicon dioxide concentrations. Key:* ○, *0%;* △, *10%; and* ■, *15%.*

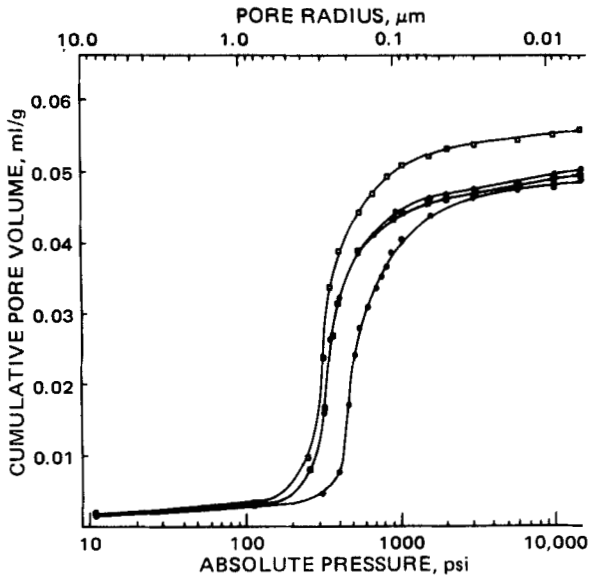

Figure 7—*Cumulative pore-size distribution of aspirin tablets containing 0% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key:* ○, *0 month;* □, *1 month;* ●, *3 months; and* ■, *5 months.*

silicon dioxide (Fig. 4). This finding suggests that the process of reduction in pore size by the addition of silicon dioxide was essentially completed at 3% silicon dioxide.

The aforementioned observations may be explained by considering that the small silicon dioxide particles were capable of filling the coarse interparticle voids developed by starch. Although the net effect of the presence of silicon dioxide in the formulations was to increase the total pore volume of tablets, in fact, silicon dioxide reduced the size and volume of coarser pores at low concentrations. This phenomenon is not at all in contrast with what is expected. From the value of the BET nitrogen surface area (556 m^2/g) and knowledge of the density of silicon dioxide (2.29 g/ml), it can be shown that the volume–surface diameter of spherical silicon dioxide particles corresponds to 4.7 nm. As compared to the size of aspirin and starch particles, which lie in the micrometer range, it does not appear unusual that silicon dioxide particles can easily undergo such a packing arrangement at low concentrations so as to limit the size of coarser pores.

In discussing the pore-size distribution of aspirin tablets, attention also must be focused on the compression characteristics of the tablet components. The absence of coarse pores in tablets prepared from aspirin powder alone can be attributed to relatively superior compressibility as well as deformability of aspirin particles. On the other hand, the development of coarser pores with formulations containing starch reflects the poor ability of this material to bond and compress. Similarly, the trend toward larger pore sizes with increasing silicon dioxide concentration presumably results from the resistance of silicon dioxide particles to consolidation during the compaction process.

Changes in Porosity Characteristics of Aspirin Tablets with Time—The susceptibility of the porosity characteristics of aspirin tablets to various changes with time is presented in Figs. 6–12 in the order of increasing silicon dioxide content. These figures depict the cumulative pore-size distribution of the samples exposed to a relative water vapor pressure of 0.82 at 40° for various times. The volume parameter is expressed in milliliters of pore space intruded by mercury per gram of sample previously outgassed in a vacuum oven at room temperature.

The mercury intruded pore volume of aspirin tablets (control) decreased consistently with increasing exposure to water vapor (Fig. 6). On the other hand, comparison of the distribution curves of the tablet for-

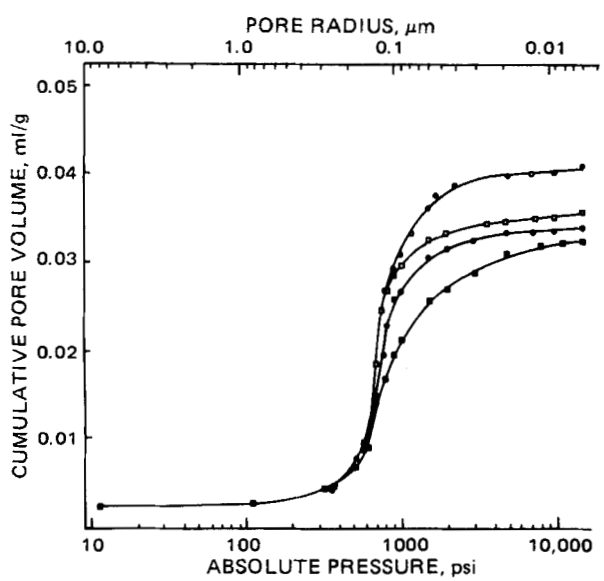

Figure 6—*Cumulative pore-size distribution of aspirin tablets (control) after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key:* ○, *0 month;* □, *1 month;* ●, *3 months; and* ■, *5 months.*

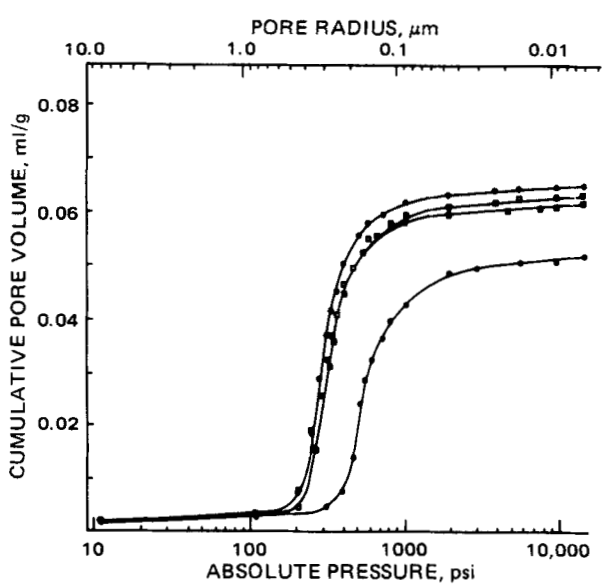

Figure 8—*Cumulative pore-size distribution of aspirin tablets containing 1% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key:* ○, *0 month;* □, *1 month;* ●, *3 months; and* ■, *5 months.*

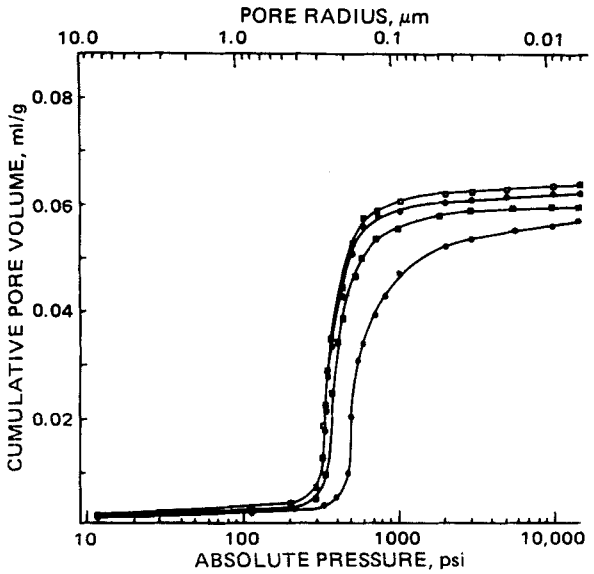

Figure 9—*Cumulative pore-size distribution of aspirin tablets containing 3% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key: ○, 0 month; □, 1 month; ●, 3 months; and ■, 5 months.*

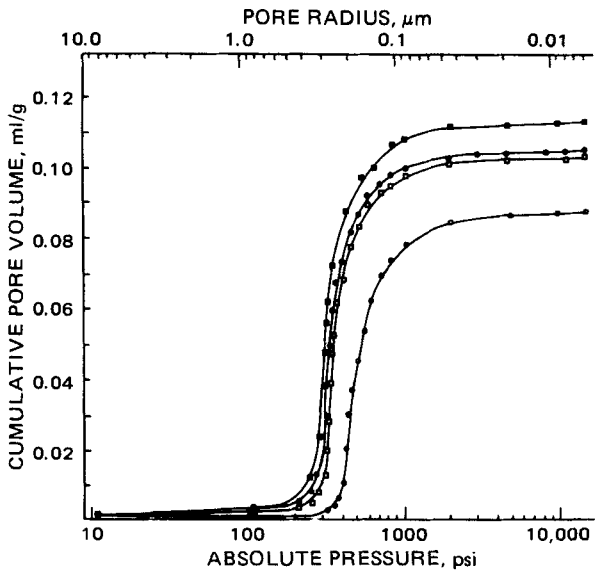

Figure 11—*Cumulative pore-size distribution of aspirin tablets containing 10% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key: ○, 0 month; □, 1 month; ●, 3 months; and ■, 5 months.*

mulation containing 0% silicon dioxide (Fig. 7) shows that the pore volume first increased on 1 month of exposure to water vapor and then decreased. This initial increase in the pore volume on exposure of the samples to water vapor for 1 month can also be seen by inspection of Figs. 8–12, which represent the cumulative pore-size distribution of aspirin tablets containing 1, 3, 5, 10, and 15% silicon dioxide. Further changes that occurred with time in the measured pore volume and the pore-size distribution differed depending on the silicon dioxide content of the samples.

At the end of 3 months, samples containing 1, 5, 10, and 15% silicon dioxide (Figs. 8 and 10–12) displayed additional increases in the pore volumes intruded by mercury, whereas the measured pore volume of the tablets having 3% silicon dioxide (Fig. 9) actually decreased with respect to the volume determined at the end of 1 month. Examination of the samples exposed to water vapor over 5 months revealed that the pore volumes of the tablets containing 5, 10, and 15% silicon dioxide continued to increase (Figs. 10–12) and those of the tablets having 3% silicon dioxide showed a further decrease (Fig. 9). Interestingly, as opposed to the previously observed trend that the mercury-intruded volume of tablets with 1% silicon dioxide increased with time, these samples appeared to have smaller pore volumes at the end of 5 months (Fig. 8).

Attention can be focused more sharply on these results with reference to Table II, where the values for the total pore volumes intruded by mercury are expressed as the percent of those obtained with corresponding samples not subjected to water vapor. The data may be explained as follows. When aspirin tablets are subjected to water vapor, water vapor is adsorbed on the surfaces of the tablet components. Since the tablet matrix is porous, and the relative pressure of water vapor is high enough for capillary condensation to occur, it is reasonable to expect that adsorption is accompanied by condensation of vapor to liquid in the small pores of the tablet. The quantity of water vapor adsorbed and subsequently condensed invariably depends on the adsorptive capacity of the particular formulation, which, as was experimentally confirmed, increases consistently with silicon dioxide concentration. Once the water vapor enters the tablet, decomposition of aspirin results, giving acetic acid and salicylic acid as the decomposition products. Acetic acid is highly volatile, but salicylic acid is partly sublimed through the tablet, as evidenced by the appearance of the crystals of this material on the sample

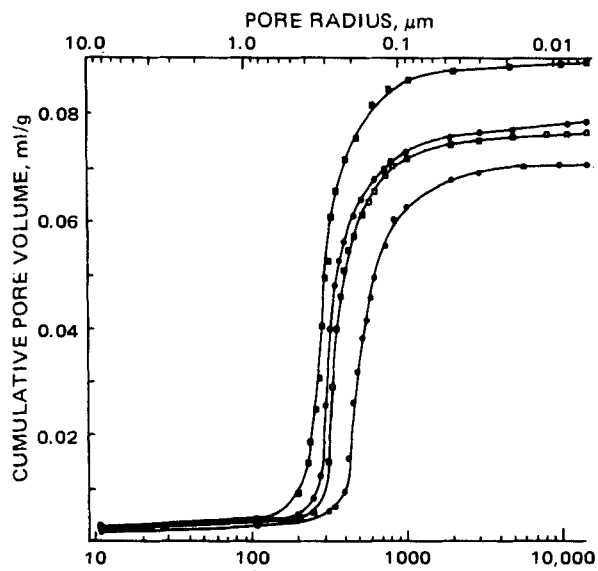

Figure 10—*Cumulative pore-size distribution of aspirin tablets containing 5% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key: ○, 0 month; □, 1 month; ●, 3 months; and ■, 5 months.*

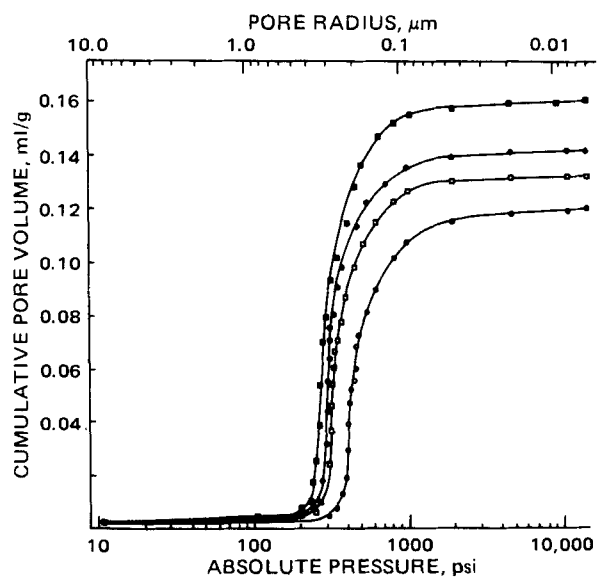

Figure 12—*Cumulative pore-size distribution of aspirin tablets containing 15% silicon dioxide after exposure to a relative water vapor pressure of 0.82 at 40° for various periods. Key: ○, 0 month; □, 1 month; ●, 3 months; and ■, 5 months.*

Table II—Percent Pore Volume Ratios of Aspirin Tablets Exposed to a Relative Water Vapor Pressure of 0.82 at 40° for Various Periods

Silicon Dioxide Concentration, %	Pore Volume Ratio after Indicated Period of Exposure, %		
	1 Month	3 Months	5 Months
Control	86.2	82.5	79.3
0	116.8	104.2	100.7
1	121.9	124.7	117.9
3	112.8	109.5	105.5
5	110.7	111.3	126.4
10	111.6	117.9	124.5
15	110.0	117.1	129.1

surface. A substantial portion of salicylic acid, however, is undoubtedly deposited in the pore system of the tablets. This result is evident from Table II, which shows a continual reduction with time in the pore volume ratios of control samples prepared from aspirin only.

Aside from influencing the porosity and pore-size distribution of the samples by causing chemical changes, water vapor also affects these parameters by physically altering the arrangement of pore spaces between the particles of the tablet components. The presence of starch in the formulations significantly increased their porosity, as reflected clearly by the increase in the pore volume ratios of the samples exposed to water vapor for 1 month (Table II). This result is naturally due to the swelling of starch grains upon penetration of water vapor into the tablet, which, in turn, distorts the arrangement and size distribution of pores. After exposure to water vapor for 1 month, tablets containing the lowest silicon dioxide concentration (1%) exhibited the maximum increase in pore volume. Further addition of silicon dioxide to the tablet formulations resulted in the reduction of the pore volume ratios with respect to this value. However, when the silicon dioxide concentration exceeded 3%, the pore volume ratios were essentially unaffected by silicon dioxide concentration. This trend presumably reflects the fact that the presence of silicon dioxide in tablets at concentration levels of 3% or above restricts the access of water vapor to starch in the tablet so that the swelling of this material is impeded.

Examination of the pore volume results obtained at the end of 3 months (Table II) shows that the samples containing starch but no silicon dioxide had a smaller pore volume ratio than that of corresponding samples analyzed after 1 month of exposure to water vapor. The deposition of salicylic acid in the tablet pores becomes the important factor in affecting the volume as well as the distribution of pores. Further reduction in the pore volume occurred when identical samples were exposed to water vapor for 5 months.

The situation, however, is quite different for tablets containing silicon dioxide, with the notable exception of tablets with 3% silicon dioxide. The data obtained at the end of 3 months show that the pore volume ratios of the samples with silicon dioxide were higher than their respective values determined after 1 month of exposure to water vapor. The divergence observed with samples containing 3% silicon dioxide indicates that in this particular formulation of the two opposing factors influencing porosity, *i.e.*, swelling of starch grains and deposition of salicylic acid in the pores, the latter predominates. Inspection of data resulting from the analysis of tablets at the end of 5 months showed that at the 3% silicon dioxide level the pore volume ratios continued to decrease. At this stage, a similar pattern is also evident with tablets containing 1% silicon dioxide.

Table IV—Stability of Aspirin Tablets under a Relative Vapor Pressure of 0.82 at 40°

Silicon Dioxide Concentration, %	Undegraded Aspirin after Indicated Period, %			
	1 Month	2 Months	3 Months	4 Months
Control	95.0	94.4	93.6	93.3
0	95.1	94.5	93.9	93.6
1	98.9	98.6	—	98.1
3	99.1	99.4	—	99.0
5	97.6	97.0	97.0	96.7
10	97.8	96.4	—	—
15	96.7	95.5	94.3	93.6

However, the increase in the pore volume ratios at higher silicon dioxide concentrations indicates that the effect of swelling of starch grains is more pronounced in these formulations. This suggests that the presence of a higher silicon dioxide content not only favors the adsorption of greater quantities of water vapor, but also as a result of this, the water vapor recedes from the silicon dioxide surface as it approaches the liquid state and becomes available for hydration of starch.

In discussing the changes in the pore characteristics of aspirin tablets with time, changes taking place in the pore sizes of the various formulations should also be considered. Therefore, the values of pore radii that may be used to characterize the pore-size distribution of aspirin tablets investigated are presented in Table III. With the exception of the control tablets, there was a general tendency for the pore sizes to attain greater values with increasing exposure of samples to water vapor. This observation is in accord with ordinary expectations based on the pore volume changes of the tablets. For a given formulation, the numerical change was the greatest between the untreated samples and samples exposed to water vapor for 1 month and diminished at longer periods of exposure. The trends evident in Table III are consistent with those obtained while considering the changes in the pore volume (Table II).

Table III also shows that the agreement between respective values of r_{med}, median pore radius, and r_{mode}, radius corresponding to the maximum frequency of pores in the sample, was reasonably close and that the pattern of change in their values was somewhat similar. Furthermore, after any period of exposure to water vapor, except for control tablets, samples containing 3% silicon dioxide exhibited the smallest values for r_{med} and r_{mode}. This finding reflects the apparent ability of this particular formulation to retain its unique feature irrespective of relative changes in the size and volumes of pores.

Interpretation of Stabilizing Properties of Colloidal Silicon Dioxide in Hydrolytic Decomposition of Aspirin—The pore-size distribution results discussed previously clearly indicate that this parameter is of significant value in following the microstructural changes that occur in aspirin tablets as a result of exposure to water vapor. Furthermore, the examination of such changes sheds considerable light on the stabilizing properties of silicon dioxide in the hydrolytic decomposition of aspirin in tablets (Table IV).

The results obtained from a direct examination of the changes in porosity characteristics indicate that the desiccant effect of silicon dioxide apparently was not the only factor favoring aspirin stability in these formulations. The pore volume data (Table II) indicate that the presence of silicon dioxide in the tablets did not preclude the access of water vapor to starch. Water vapor continued to be available for aspirin hydrolysis. The availability of water vapor to starch and, presumably, to aspirin was

Table III—Characteristic Pore Radii (Micrometers) of Aspirin Tablets

Silicon Dioxide Concentration, %	Untreated Samples		Samples Exposed to Water Vapor[a]					
			1 Month		3 Months		5 Months	
	r_{med}[b]	r_{mode}[c]	r_{med}	r_{mode}	r_{med}	r_{mode}	r_{med}	r_{mode}
Control	0.130	0.118	0.140	0.136	0.135	0.118	0.115	0.136
0	0.175	0.206	0.275	0.315	0.254	0.268	0.254	0.268
1	0.168	0.185	0.290	0.328	0.299	0.328	0.293	0.300
3	0.164	0.167	0.234	0.240	0.241	0.257	0.241	0.257
5	0.177	0.200	0.250	0.277	0.268	0.277	0.304	0.321
10	0.184	0.205	0.244	0.283	0.259	0.283	0.276	0.305
15	0.194	0.220	0.248	0.283	0.262	0.292	0.280	0.336

[a] At a relative pressure of 0.82 at 40°. [b] Median pore radius, the radius corresponding to 50% of the mercury intruded pore volume. [c] The radius corresponding to the maximum frequency of pores in the sample.

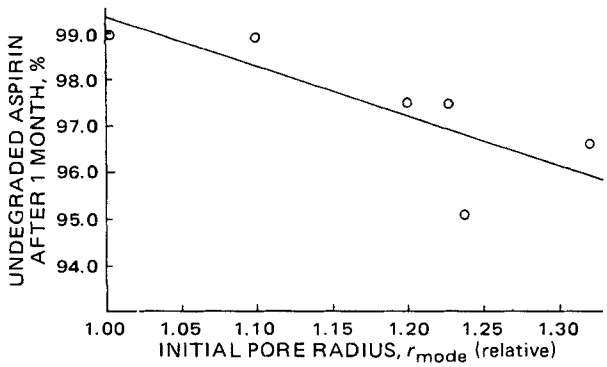

Figure 13—*Relationship between aspirin stability and pore size.*

not a simple function of silicon dioxide concentration in the tablets studied. Undoubtedly, the precise mechanism by which silicon dioxide affected the stabilization of aspirin did not involve the simple retention of water vapor by silicon dioxide to limit the availability of water vapor to aspirin *per se*. A complex combination of factors was possibly responsible for the optimum stability observed at the 3% silicon dioxide concentration.

Aspirin decomposition in tablets involves a heterogeneous reaction, the rate of which may be controlled both by diffusional processes and the hydrolytic reaction at the unreacted aspirin surface. The two general diffusional processes of importance include the diffusion of water vapor into the tablet and the transfer of salicylic acid in and out of the tablet pores. As the present experimental data verify, salicylic acid is deposited in the tablet pore system. In this process, salicylic acid may easily form a protective layer on the aspirin surface, which, in turn, results in slow diffusion of water vapor through this layer. It is also possible to conceive that the hydrolytic reaction may approach an equilibrium state. In either event, the aspirin degradation rate becomes diffusion controlled, and the complex internal pore structure and the nature of individual pores may become determining factors in the accessibility of water vapor to the aspirin surface. The characteristic pore-size distribution pattern observed with tablets containing 3% silicon dioxide may, therefore, be helpful in interpreting the relatively small extent of aspirin degradation in this formulation.

To determine whether a simple correlation existed between aspirin stability and the pore size attained in the formulations, the stability results shown in Table IV were plotted against the values of r_{mode}, the pore radius corresponding to the maximum frequency of pores in the tablet. The trend representing aspirin stability at the end of 1 month as a function of initial pore size is illustrated in Fig. 13, where r_{mode} is expressed in normalized form relative to that of the tablets having the smallest pore size (Table III). Figure 13 shows that aspirin stability is apparently related to the initial pore size.

An extension of the relationship seen in Fig. 13 to stability data over longer periods did not appear feasible, because of the complexity of the interactions of various variables involved in aspirin stability. When a similar plot was prepared for the 3-month data, for example, the trend remained the same, but the correlation was relatively poor. The deviation occurred primarily due to the formulation containing 1% silicon dioxide, which had relatively greater stability despite the larger pore size attained in this formulation with time (Table III).

This apparent lack of direct correlation between aspirin stability and pore size suggests that the water vapor accessible to starch is not equally available to the aspirin surface. Presumably, at low silicon dioxide concentration, starch itself retains the water vapor, resulting in its greater hydration and an increased pore volume and pore size in the tablets. With increased silicon dioxide concentration, however, starch grains are isolated from each other; silicon dioxide adsorbs the water vapor to a greater extent, since it has a much larger surface area and greater adsorption capacity than starch.

Another explanation that may be offered for the slower decomposition of aspirin in formulations containing low silicon dioxide concentrations may be related to the effect of salicylic acid on the system. As pointed out previously, a protective layer of salicylic acid is probably formed on the aspirin surface to control further the access of water vapor to the aspirin. Additionally, the deposition of salicylic acid in the tablet pore system at low silicon dioxide concentrations (Tables II and III) decreases the pore volumes and sizes with longer periods of exposure to water vapor. With formulations containing silicon dioxide levels higher than 3%, the accessibility of water vapor to aspirin is expected to be much greater. This is attributed to the fact that the increased silicon dioxide concentration favors the adsorption of greater quantities of water vapor which recede from the silicon dioxide surface with time as it approaches the liquid state and becomes available for aspirin degradation. The superior adsorptive capacity of these formulations and the favorable pore size may simultaneously operate to facilitate the access of water to unreacted aspirin surfaces. The transfer of salicylic acid also may be enhanced because of the greater pore sizes. This point is evidenced simply by the physical appearance of the tablets, which showed more localized salicylic acid crystals on the external surfaces consistent with increasing silicon dioxide content.

Finally, none of the aforementioned factors, *i.e.*, the superior water vapor adsorptive capacity of silicon dioxide, sublimation of salicylic acid, and the distinct pore size distribution of each formulation, can alone be directly related to the differences in the stability of aspirin at various silicon dioxide levels. However, the present observations along with the foregoing discussion promote additional insight into the stabilizing properties of silicon dioxide in aspirin degradation. It should also be evident that pore-size distribution can be a very useful parameter in interpreting aspirin stability in compressed pharmaceutical systems.

SUMMARY AND CONCLUSIONS

1. The influence of colloidal silicon dioxide on the porosity characteristics of aspirin tablets containing a constant quantity of starch was investigated. The porosity of the samples prepared under constant compression conditions increased linearly with silicon dioxide concentration.

2. Pore-size distribution determinations by mercury intrusion allowed a complete elucidation of the microstructural changes produced by the inclusion of silicon dioxide. At low concentrations, silicon dioxide functioned primarily to reduce the size and volume of coarser pores, presumably representing the spaces between the aggregations of starch and aspirin particles. This effect was optimum at 3%.

3. An assessment of the changes in the pore-size distribution patterns occurring under the influence of water vapor revealed distinct differences in this parameter as functions of the silicon dioxide concentration and the period of exposure to water vapor. These results were explained in terms of the accessibility of water vapor to tablet components.

4. A unique trend in the change of pore-size distribution was noted with tablets containing 3% silicon dioxide. Irrespective of relative changes in the size and volume of pores as a result of swelling of starch grains and the deposition of salicylic acid in the tablet pore system, this formulation consistently exhibited the smallest average pore size among the tablets containing several concentrations of silicon dioxide.

5. The present observations shed considerable light on the interpretation of the stabilizing properties of silicon dioxide in aspirin tablets. It was concluded that pore-size distribution measurements appear to be useful in elucidating aspirin stability in compressed pharmaceutical systems.

REFERENCES

(1) H. Nogami, J. Hasegawa, and M. Miyamoto, *Chem. Pharm. Bull.*, **15**, 279 (1967).

(2) P. Singh, S. J. Desai, A. P. Simonelli, and W. I. Higuchi, *J. Pharm. Sci.*, **57**, 217 (1968).

(3) D. Ganderton, *J. Pharm. Pharmacol.*, **21**, 9S (1969).

(4) D. Ganderton and A. B. Selkirk, *ibid.*, **22**, 345 (1970).

(5) D. Ganderton and D. R. Fraser, *ibid.*, **22**, 95S (1970).

(6) A. B. Selkirk and D. Ganderton, *ibid.*, **22**, 86S (1970).

(7) W. Lowenthal, *J. Pharm. Sci.*, **61**, 1695 (1972).

(8) A. B. Selkirk and D. Ganderton, *J. Pharm. Pharmacol.*, **22**, 79S (1970).

(9) C. A. Kelly, *J. Pharm. Sci.*, **59**, 1053 (1970).

(10) B. K. Martin, in "Advances in Pharmaceutical Sciences," vol. 3, H. S. Bean, A. H. Beckett, and J. E. Carless, Eds., Academic, London, England, 1971, p. 107.

(11) S. Brunauer, P. H. Emmett, and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).

(12) D. S. Carr and B. L. Harris, *Ind. Eng. Chem.*, **41**, 2014 (1949).

(13) E. W. Washburn, *Proc. Nat. Acad. Sci. USA*, **7**, 115 (1921).

(14) M. E. Nicholas, P. A. Joyner, B. M. Tassem, and M. D. Olsen, *J. Phys. Chem.*, **65**, 1373 (1961).

(15) C. Kembell, *Trans. Faraday Soc.*, **42**, 526 (1946).

(16) H. M. Rootare, *Aminco Lab News*, **4**, 4A (1968).

(17) C. Orr, Jr., *Powder Technol.*, **3**, 117 (1969/70).
(18) H. Gucluyildiz, G. E. Peck, and G. S. Banker, *J. Chem. Ed.*, **49**, 440 (1972).
(19) A. Y. Gore, K. B. Naik, D. O. Kildsig, G. E. Peck, V. F. Smolen, and G. S. Banker, *J. Pharm. Sci.*, **57**, 1850 (1968).

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Analysis of Sulindac and Metabolites by Combined Isotope Dilution–Radioimmunoassay

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Abstract □ Sulindac, a new anti-inflammatory agent, and its sulfone and sulfide metabolites were conjugated to bovine serum albumin by the *N*-hydroxysuccinimide active ester procedure. Antiserum from rabbits immunized with each of these haptens exhibited extensive cross-reactivity, precluding differential analyses of the three species by displacement assay without prior separation. Therefore, an analytical method based on a combination of isotope dilution and radioimmunoassay was devised. A known mixture of the three chemical species, each labeled with tritium, was equilibrated with plasma or urine samples, reisolated chromatographically, and quantitated by binding to an appropriate immunoglobulin. The radiolabeled materials thus served as recovery standards as well as labeled antigens for each displacement assay. Sulindac and each of its metabolites in plasma or urine at concentrations as low as 500 ng/sample were differentially determined by this procedure. However, since an extraction is required, several milliliters of plasma can be used for each sample, thus increasing the actual sensitivity of the assay.

Keyphrases □ Sulindac—and metabolites, combined isotope dilution–radioimmunoassay, human plasma and urine □ Radioimmunoassay—combined with isotope dilution, analysis of sulindac and metabolites, human plasma and urine □ Isotope dilution–radioimmunoassay—analysis of sulindac and metabolites, human plasma and urine □ Anti-inflammatory agents—sulindac and metabolites, combined isotope dilution–radioimmunoassay, human plasma and urine

Sulindac, *cis*-5-fluoro-2-methyl-1-[*p*-(methylsulfinyl)benzylidenyl]indene-3-acetic acid (I), is a new anti-inflammatory agent (1, 2) currently in clinical trials. Its two principal metabolites, the sulfone (II) and sulfide (III), differ from the parent drug only with respect to the oxidation state of the sulfur moiety (3). Metabolic reduction of sulindac to the sulfide is reversible, but oxidation to the sulfone is irreversible; thus, disposition patterns of this drug are complex. Furthermore, the sulfide metabolite has been proposed as the pharmacologically active species[1]. Thus, any analytical procedure should ideally be capable of distinguishing among these closely related compounds.

GC analysis of sulindac and metabolites was attempted without success[2]. The principal difficulties encountered were the lack of sensitivity for sulfide and the insufficient separation of sulindac and its sulfone metabolite. Another potentially useful method is mass fragmentography, and such an assay for sulindac is in the final stages of development[3].

A third possible method is radioimmunoassay, provided that specific antiserums to each compound are available. Early results suggested that specific antibodies to sulindac and metabolites could not be obtained. One solution to the problem of poor selectivity is to separate the compounds chromatographically prior to radioimmunoassay. This approach has been applied to assays of steroids and prostaglandins (4–6). A slightly different technique utilizing isotope dilution in combination with radioimmunoassay has been devised for the differential determination of sulindac and its metabolites and is the subject of this report.

EXPERIMENTAL

Materials—Bovine serum albumin[4], *N*-hydroxysuccinimide[5], dicyclohexylcarbodiimide[5], neutral charcoal[6], and dextran[7] were used. Dextran-coated charcoal was prepared by suspending 6.25 g of prewashed charcoal and 0.625 g of dextran in 100 ml of 0.05 *M* phosphate buffer, pH 7.5.

Preparation of Labeled Antigens—*[^{3}H-Methylene]sulindac Sulfide*—A mixture of sulindac sulfide (0.5 g), potassium *tert*-butoxide (0.6 g), and tritiated water (2 ml, 25 Ci) was heated at 90° for 2 hr. Water was then added, and the mixture was acidified. The labeled sulfide that precipitated was collected and dissolved in methanol. The solution was evaporated (several times) to remove labile tritium. Several recrystallizations from benzene afforded pure tritiated sulfide (350 mg), mp 186–187°, specific activity 172 μCi/mg.

[^{3}H-Methylene]sulindac—Sodium metaperiodate (144 mg) in water (2 ml) was added to a solution of tritiated sulindac sulfide (102 mg) in methanol (8 ml) and acetone (2 ml), and the mixture was stirred at 25° for 16 hr. The mixture was concentrated, diluted with water, acidified, and extracted with ethyl acetate. The solid obtained by evaporating the ethyl acetate was recrystallized several times from ethyl acetate to yield 53 mg of pure sulindac, mp 181–183°, specific activity 167 μCi/mg.

[1] C. G. Van Arman, Merck Sharp & Dohme Research Laboratories, unpublished data.
[2] H. B. Hucker and G. O. Breault, Merck Sharp & Dohme Research Laboratories, personal communication.
[3] W. J. A. VandenHeuvel, Merck Sharp & Dohme Research Laboratories, personal communication.
[4] Nutritional Biochemical Co.
[5] Pierce Chemical Co.
[6] Norit A, Amend Drug and Chemical Co.
[7] T-70, Pharmacia Labs.