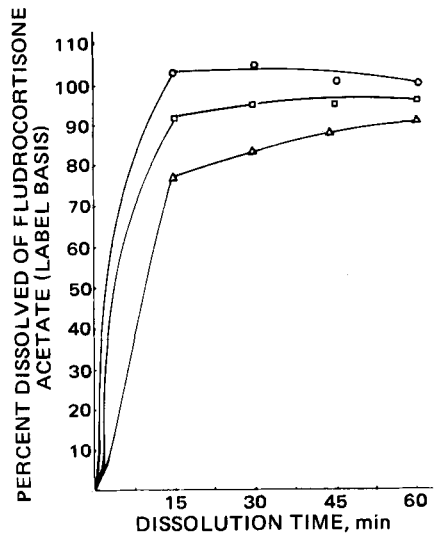

Figure 6—*Comparison between the dissolution rate of different lots of commercial fludrocortisone acetate tablets at 125 rpm in distilled water. Each point is an average of six tablets. Key: △, Lot 1; □, Lot 2; and ○, Lot 3.*

The linearity of the fludrocortisone acetate chromatographic response was checked within a concentration range of 0.8–4.0 μg/ml, equivalent to 25–120% of tablet potency. The response was linear (r = 0.999). The average relative standard deviation for triplicate injections of each standard level varied from 1 to 3%. Figure 3 shows a sample chromatogram from an actual tablet dissolution run. In studying the dissolution rate of fludrocortisone acetate tablets, three different basket rotation speeds were investigated: 50, 100, and 125 rpm. Figure 4 shows that for the same lot the dissolution rate significantly increased with an increase in basket rotation speed.

To compare the dissolution characteristics of different lots, six tablets of each of three different lots were tested in water at 125 rpm. Figure 5 shows the spread in dissolution behavior between different tablets of the same lot, and Fig. 6 compares the dissolution profiles of different lots. In all cases, DT_{50}[12] was less than 15 min while DT_{85}[13] varied between 12 and 37 min. Although the assay values of the three lots were above 95% of the label value, Lot 3 did not reach 90% dissolution even after 2 hr. These tablet-to-tablet and lot-to-lot variations in dissolution characteristics are thought to be due to differences in tablet uniformity.

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[12] DT_{50} is the time required for 50% of the label value of the drug to go into solution.

[13] DT_{85} is the time required for 85% of the label value of the drug to go into solution.

Bioavailability of Sulfonamide Suspensions I: Dissolution Profiles of Sulfamethizole Using Paddle Method

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Received September 19, 1977, from the **Department of Pharmaceutics and the* ‡*Department of Medicinal Chemistry, University of Pittsburgh, Pittsburgh, PA 15261,* and the §*Division of Biopharmaceutics, Food and Drug Administration, Washington, DC 20204.* Accepted for publication February 2, 1978.

Abstract □ A comparative bioavailability study was performed using two commercially available, chemically equivalent brands of sulfamethizole suspension. One gram of each suspension was administered to 12 different subjects following a completely randomized crossover design. Serum levels and derived pharmacokinetic parameters were compared statistically. There were no significant differences in the extent of sulfamethizole absorption from the two suspensions as evidenced by the area under the serum level–time curves. Significant differences ($p < 0.05$) in the mean serum levels at 0.5 and 0.75 hr and differences in C_{max} and t_{max} indicated that the absorption rate differed for the two products. *In vitro* tests including particle-size analysis and dissolution studies were performed. The size-frequency distribution of particles in the suspensions was studied using a resistance particle counter. The dissolution characteristics of the two products were studied using the Food and Drug Administration's paddle method and the spin-filter apparatus. Suspension A had a significantly greater amount of drug dissolved at 15 and 30 min using either method. It also had a greater percentage of particles at the smaller size range, indicating that the greater dissolution rate may be related directly to the decreased particle size. A comparison of the *in vivo* and *in vitro* results demonstrated a definite rank-order correlation between the dissolution performance of the two suspensions and the *in vivo* parameters reflecting the absorption rate. Suspension A had a greater amount of drug dissolved at 15 and 30 min and resulted in higher serum levels at 0.5 and 0.75 hr, a higher C_{max}, and a shorter t_{max}.

Keyphrases □ Sulfamethizole—bioavailability in humans and *in vitro* dissolution, two suspensions compared □ Bioavailability—sulfamethizole, two suspensions compared □ Dissolution, *in vitro*—sulfamethizole, two suspensions compared □ Antibacterials—sulfamethizole, bioavailability in humans and *in vitro* dissolution, two suspensions compared

In vitro dissolution testing can be a valuable tool for predicting or assuring the *in vivo* performance of a dosage form if appropriate correlation has been established. Several methods are employed for measuring the dissolution of the common solid dosage forms, capsules and tablets. For a drug to be absorbed, the solid dosage form must first undergo disintegration, deaggregation, and then dissolution of drug particles. The size of drug particles in the postdisintegration state affects the dissolution rate.

Suspension dosage forms correspond to the postdisin-

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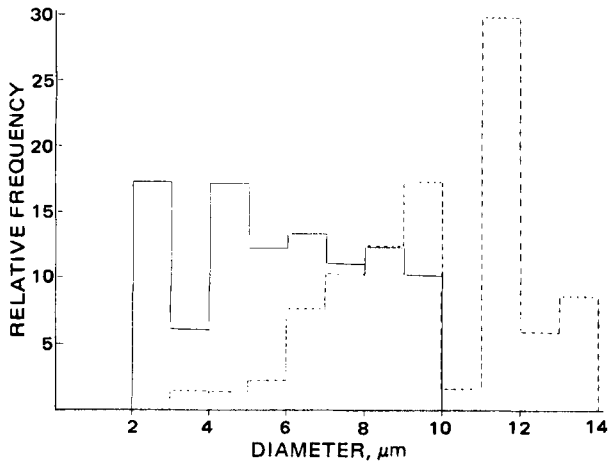

Figure 1—*Particle-size distribution of Suspensions A (—) and B (- - -).*

tegration stage of tablets and capsules, and drug particle dissolution is a prerequisite to bioavailability. Suspensions thus share the dissolution process as an absorption process rate-limiting step with tablets and capsules. Because of the difficulties in handling suspensions by methods primarily designed for tablets or capsules, few research efforts have concentrated on dissolution rate profiles for suspensions. Previous studies (1, 2) emphasized the need to determine the dissolution characteristics of suspension dosage forms. Recently, the dissolution rate profile of steroid suspensions was determined using the spin-filter apparatus (2).

This report presents the methodology for determining the dissolution rate profiles of suspensions using the Food and Drug Administration's (FDA) two-bladed paddle method. The feasibility and reproducibility of the method were demonstrated by determining the dissolution profile of two commercial, chemically equivalent sulfamethizole suspensions. Correlation studies with *in vivo* data and with data generated using the spin-filter method and particle-size measurements also are reported. The paddle method is simple, inexpensive, reproducible, and easily adaptable to the USP basket apparatus.

EXPERIMENTAL

***In Vivo* Study Protocol**—Twelve healthy, normal adult male volunteers, 20–34 years of age and weighing 60–89 kg, each received a 1-g dose of two different commercial chemically equivalent sulfamethizole suspensions, A[1] and B[2], followed by 120 ml of distilled water. The suspensions were administered in a completely randomized crossover design with a 2-week rest between doses.

All subjects fasted for 10 hr before and 3 hr following drug administration.

Blood samples (6 ml) were drawn using evacuated glass containers[3] at 0, 0.25, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 10 hr. The samples were centrifuged[4] at 3500 rpm, and the serum was separated and frozen until analyzed.

Particle-Size Analysis—The particle-size analysis was performed using a resistance particle counter[5] equipped with a triple manometer and a 50-μm aperture tube. The instrument was calibrated with 3.40-μm polystyrene microspheres supplied by the manufacturer. Initial sizing estimates were obtained with a microscope[6] equipped with a calibrated ocular micrometer[6]. Preliminary particle-size studies also were performed using 100- and 200-μm aperture tubes.

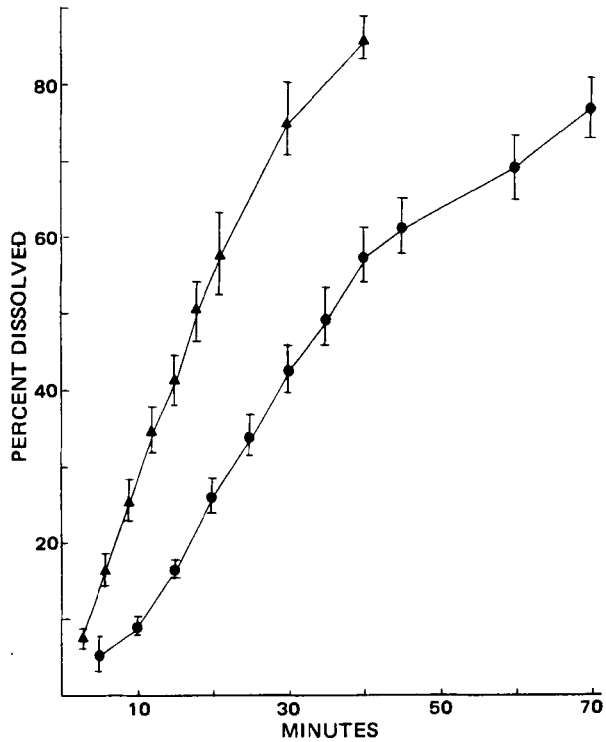

Figure 2—*Mean percent dissolved (±SE) of two commercial sulfamethizole suspensions using the FDA paddle method. Key: ▲, Suspension A; and ●, Suspension B.*

Dilutions of the commercial suspensions (1:50,000) were prepared in a 1% sodium chloride solution saturated with sulfamethizole[7] and containing 0.01% polysorbate 80[8]. The diluent was filtered through a series of membrane filters[9] to eliminate all particles above 0.22 μm in size. To determine the effect of excipient particles on the sizing procedure, an additional set of dilutions (1:50,000) was prepared in the electrolyte–surfactant diluent containing no sulfa drug. All particle counts were corrected for background and coincidence.

Dissolution: Paddle Method—Dissolution profiles of 12 500-mg samples of each suspension were determined at 37° in 900 ml of pH 7.2 phosphate buffer using the FDA paddle method (3) at 25 rpm. Preliminary studies indicated that the dissolution of the suspension samples was too rapid to measure at paddle speeds greater than 25 rpm. The apparatus consists of a cylindrical 1000-ml round-bottom flask[10] secured in a multiple-spindle dissolution drive apparatus[11] and immersed in a controlled temperature bath[12] maintained at 37°. The paddle was positioned to extend to exactly 2.5 cm above the flask bottom.

The suspension was introduced carefully into the flask bottom using a 10-ml glass syringe with an attached 19-cm needle[13]. Paddle rotation was engaged and controlled at a constant 25 rpm using a dissolution stirrer drive[11]. Initial dissolution trials indicated that Suspension A dissolved at a greater rate than Suspension B. On the basis of the data from the preliminary trials, the following sampling schedules were established: Suspension A, 3, 6, 9, 12, 15, 18, 21, 30, and 40 min; and Suspension B, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, and 70 min.

Samples of 2 ml of the dissolution medium were withdrawn (and replaced with an equal volume of drug-free buffer) in a 5-ml glass syringe through an attached 8.9-cm 20-gauge stainless steel needle[14] secured to the cover of the dissolution flask and positioned to extend 5.2 cm into the dissolution medium. Initially, samples were drawn simultaneously from three different needle depths (5.2, 6.7, and 9.0 cm) to determine if the drug was uniformly dissolved in the area of the sampling needle. The

[1] Thiosulfil, lot 1BBW, Ayerst Laboratories, New York, N.Y.
[2] Proklar, lot 107514, Westerfield Division of O'Neal, Jones, & Feldman, Cincinnati, Ohio.
[3] Vacutainers, silicone-coated interior, Becton Dickinson & Co., Rutherford, N.J.
[4] IEC HN-S centrifuge, Damon/IEC Division, Needham Heights, Mass.
[5] Model ZB counter, Coulter Electronics, Hialeah, Fla.
[6] Fisher Scientific Co., Pittsburgh, Pa.
[7] Sulfamethizole, lot R15917B, Ayerst Laboratories, New York, N.Y.
[8] Tween 80, ICI America, Wilmington, Del.
[9] Millipore Corp., Bedford, Mass.
[10] Kimbel Glass No. 33710-51.
[11] Model 53, Spec. 72B, Hanson Research Corp., Northridge, Calif.
[12] Blue M Electric Co., Blue Island, Ill.
[13] Travenol Laboratories, Morton Grove, Ill.
[14] Pitkin spinal needle, Becton Dickinson & Co., Rutherford, N.J.

Table I—*In Vitro* Dissolution Performance (Mean Percent Dissolved ± *SD*) of Two Sulfamethizole Suspensions

Minutes	Dissolution Method	Suspension A	Suspension B	Statistical Evaluation
15	Paddle[a]	41.67 ± 10.72	16.92 ± 4.64	$p < 0.05$
30	Paddle[a]	75.68 ± 16.07	43.29 ± 11.27	$p < 0.05$
15	Spin filter[b]	72.38 ± 4.27	54.39 ± 6.69	$p < 0.05$
30	Spin filter[b]	92.11 ± 3.05	76.57 ± 7.39	$p < 0.05$

[a] Twelve trials. [b] Six trials.

samples were immediately filtered through a 13-mm, 0.2-μm membrane filter[15] and analyzed for sulfamethizole using the Bratton and Marshall (4) colorimetric method.

Dissolution: Spin Filter—The dissolution profiles of six 500-mg samples of each suspension were determined at 37° in 900 ml of pH 7.2 phosphate buffer at 100 rpm using the rotating filter–stationary basket apparatus described by Shah *et al.* (5). Initial studies indicated that filter speeds greater than 100 rpm resulted in a dissolution rate that was too rapid to measure. The basket was removed for all dissolution trials. The sampling schedule was established on the basis of preliminary trials as described for the paddle method. Samples of 2 ml were withdrawn through the pilot tube into 5-ml glass syringes and analyzed for sulfamethizole using the Bratton and Marshall (4) colorimetric method.

Analysis of *In Vivo* Samples—All *in vivo* serum samples were analyzed for sulfamethizole content by high-pressure liquid chromatography (HPLC). The serum sample (0.2 ml) was transferred to a 1-ml vial[16] containing 5 μl of an internal standard, and 0.1 ml of 14% trichloroacetic acid was added. The samples were mixed[17] and then centrifuged[18] at 2500 rpm for 15 min. A 5-μl aliquot of the clear supernate then was injected onto the column. A linear calibration curve was observed in the 1–90-μg/ml range with an estimated precision of ±2% (*RSD*).

The modular high-pressure liquid chromatograph consisted of a constant-flow pump[19], a valve-type injector[20], a fixed wavelength (254 nm) UV detector[21], and a strip-chart recorder[22]. The stainless steel column (3.9 nm × 30 cm) was packed with fully porous 10-μm silica particles to which was chemically bonded a monomolecular layer of octadecylsilane[23].

Adequate separation of the serum components and unmetabolized drug was achieved using a mobile phase of acetonitrile–1% acetic acid (22:78) and a flow rate of 1.5 ml/min. The column temperature was maintained at 32° by inserting the column into a glass sleeve immersed in a constant-temperature water bath[24].

A stock solution of sulfamethizole[25] was prepared by dissolving 100 mg of sulfamethizole in 0.1 *N* NaOH and adjusting to volume in a 100-ml volumetric flask. Plasma standards were prepared by transferring the appropriate volume of the stock solution to control plasma. Calibration curves were obtained daily. The internal standard stock solution was prepared by transferring 300 mg of sulfamethoxazole[26] to a 100-ml volumetric flask and diluting to volume with 0.1 *N* NaOH.

Data Analysis—The number of drug particles for each size range was determined by subtracting the particle count due to excipients from the total particle count. The number distribution was then converted to a weight distribution (6), and the percent oversize at each size interval was calculated and plotted against the midpoint of the size interval on log probability paper. The mean diameter was determined from the equation for the fitted linear regression line.

The dissolution data for the two suspensions were treated by converting observed drug concentrations at each sampling time to amounts dissolved and, in turn, to percents dissolved. The mean percent dissolved and standard deviation were calculated for each sampling time for the two brands. Values at common sampling times were compared statistically using the Student *t* test.

The peak serum concentration, C_{max}, and time of the peak concentration, t_{max}, were obtained from the individual serum level *versus* time curves. The area under the curve (*AUC*) values were calculated according to the trapezoidal rule and corrected for the area beyond the last data point. The individual *AUC* values were then "normalized" (7) by multiplying the calculated *AUC* by the K_E value obtained from the terminal linear portion of the serum level *versus* time curves. The *in vivo* performance of the two suspensions was statistically compared by analyzing the individual values for C_{max}, t_{max}, K_E, normalized *AUC*, and serum levels at each sampling time using a *t* test for matched pairs.

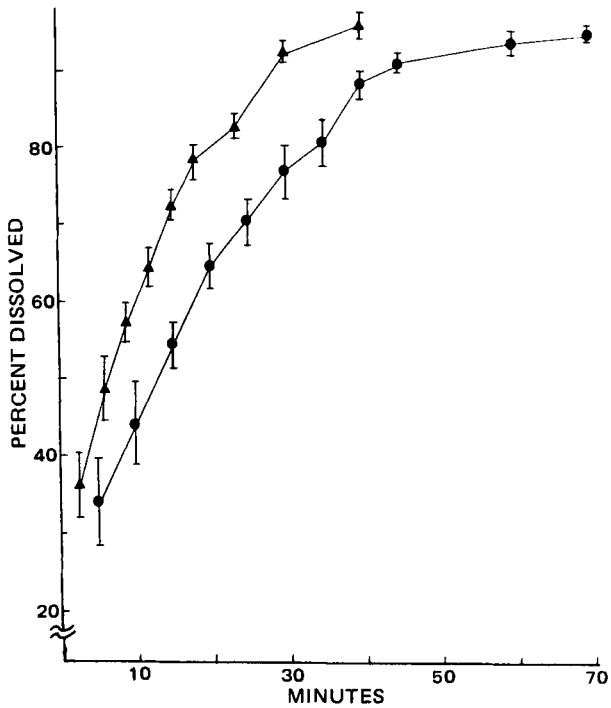

Figure 3—*Mean percent dissolved (±SE) of two commercial sulfamethizole suspensions using the spin-filter method. Key: ▲, Suspension A; and ●, Suspension B.*

RESULTS

An initial microscopic examination of the suspensions indicated that most particles had diameters of 10 μm or less, with no particle diameter exceeding 24 μm (Fig. 1). Data obtained using the 100- and 200-μm aperture tubes indicated that the particles were distributed almost entirely between 2 and 15 μm, with only an insignificant number of counts occurring beyond 15 μm. The particle distribution according to number was converted to a weight distribution to provide an appropriate relationship with the weight of suspension dissolved or absorbed. Particles in Suspension B had a geometric mean diameter of 8.69 ± 1.37 μm, while particles in Suspension A had a geometric mean diameter of 4.90 ± 1.5 μm.

Analysis of the dissolution data obtained using both the paddle method and the spin-filter apparatus demonstrated a substantial difference in the dissolution characteristics of Suspensions A and B. A statistically significant difference ($p < 0.05$) existed between the two suspensions for the percent of drug dissolved at the 15- and 30-min sampling times using both methods (Table I). The entire dissolution profiles of both products are shown in Figs. 2 and 3 for the two methods. An analysis of the amount of drug dissolved at the three different sampling depths indicated that there was no statistically significant difference in drug concentration at those depths.

Figure 4 illustrates the mean serum values for both suspensions at each of the 14 sampling times. A summary of the *in vivo* results and statistical evaluation appears in Table II. Statistically significant differences between the two suspensions were noted when individual values for C_{max}, t_{max}, and serum concentrations at 0.5 and 0.75 hr were analyzed. The results indicated that Suspension A administration resulted in significantly higher serum levels at 0.5 and 0.75 hr and a higher peak concentration, C_{max}, at an earlier time, t_{max}. There were no significant differences in the values for *AUC*, K_E, and serum concentrations at the remaining sampling times. These results indicated that the differences were related to the rate rather than the extent of absorption.

[15] Gelman Instrument Co., Ann Arbor, Mich.
[16] Reacti-Vials, No. 13261, Pierce, Inc., Rockford, Ill.
[17] Vortex-Genie, Fisher Scientific Industries, Springfield, Mass.
[18] IEC EXD centrifuge 460G, Damon/IEC Division, Needham Heights, Mass.
[19] Model M-6000A chromatography pump, Waters Associates, Milford, Mass.
[20] Model U6K universal injector, Waters Associates, Milford, Mass.
[21] Model 440 absorbance detector, Waters Associates, Milford, Mass.
[22] Varian A-25 dual-channel strip-chart recorder, Varian Associates, Walnut Creek, Calif.
[23] Prepacked μBondapak C_{18}, Waters Associates, Milford, Mass.
[24] Braun Thermonix II, Bronwill Scientific Co., Rochester, N.Y.
[25] Powder lot R15917B, Ayerst Laboratories, New York, N.Y.
[26] Powder lot 708115, Roche Chemical Division, Nutley, N.J.

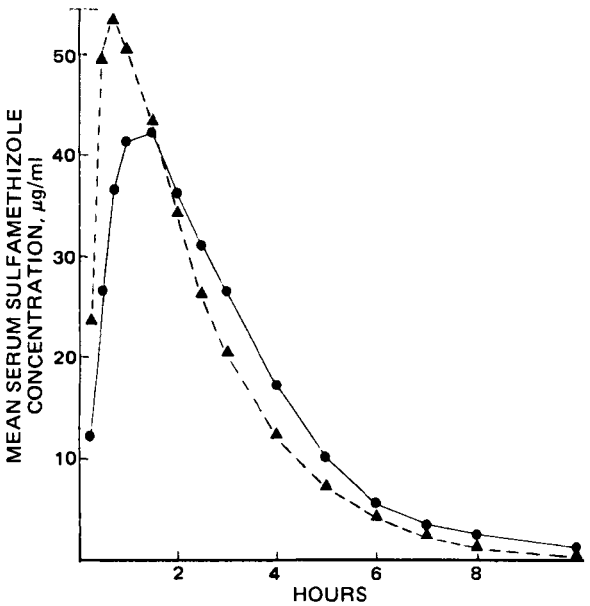

Figure 4—*Mean serum concentrations for 12 subjects for two different brands of sulfamethizole suspension. Key: ▲, Suspension A; and ●, Suspension B.*

DISCUSSION

A statistical evaluation of the results indicated that the absorption rate for Suspension A was greater than that for Suspension B. There were no statistical differences in the extent of drug absorption from the two suspensions.

Suspension A had a significantly greater amount of drug dissolved at 15 and 30 min using either the FDA paddle method or the spin-filter apparatus. Suspension A also had a greater percentage of particles at the smaller size range, indicating that the greater dissolution rate may be directly related to the decreased particle size.

A comparison of the *in vivo* and *in vitro* results demonstrated a definite rank-order correlation between the dissolution performance of the two suspensions and the *in vivo* parameters reflecting the absorption rate. The product with the greater amount of drug dissolved at 15 and 30 min (Suspension A) also resulted in higher serum levels at 0.5 and 0.75 hr, a higher C_{max}, and a shorter t_{max}. This result indicated that the absorption rates of these two suspensions were directly related to the dissolution process.

Table II—*In Vivo* Parameters (Mean ± *SD*) Derived from Serum Levels of Unchanged Drug

Parameter	Treatments		Statistical Evaluation
	Suspension A	Suspension B	
Serum level at 0.5 hr, μg/ml	49.55 ± 25.13	26.95 ± 16.20	$p < 0.05$
Serum level at 0.75 hr, μg/ml	53.60 ± 19.90	36.88 ± 15.24	$p < 0.05$
C_{max}, μg/ml	62.88 ± 14.94	48.34 ± 11.27	$p < 0.05$
t_{max}, hr	0.958 ± 0.520	1.67 ± 0.778	$p < 0.05$
Normalized *AUC*, μg/ml	74.76 ± 10.50	71.98 ± 16.72	$p > 0.05$[a]
K_E, hr^{-1}	0.4997 ± 0.0843	0.4814 ± 0.0812	$p > 0.05$[a]

[a] Not statistically significant.

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