Particle-Size Analysis of Pharmaceutical Powders

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Abstract □ An automated electrolytic sensing zone (electrozone) method was developed to determine the particle-size distribution of milled and micronized pharmaceutical powders. The powdered drugs obeyed log-normal statistics, and the distributions were well defined by their geometric volume mean diameter and the geometric standard deviation. The results show that accurate data can be obtained between 2 and 80 μm with a precision of ~0.5 μm. Pulse-width analyses were performed to determine the feasibility of using a pulse-width discrimination program. However, in this case, the program discriminates against real particles and, therefore, its usefulness is limited. Milled and micronized materials are described adequately by a spherical diameter, and the automated electrozone system described is an excellent method for quality control purposes.

Keyphrases □ *Particle-size distribution*—milled and micronized pharmaceutical powders, geometric volume mean diameter, electrolytic sensing zone method with and without a pulse-width discriminator □ Distribution, particle size—milled and micronized pharmaceutical powders, geometric volume mean diameter, electrolytic sensing zone method with and without a pulse-width discriminator □ Powders—milled and micronized, particle-size distribution, geometric volume mean diameter, electrolytic sensing zone method with and without a pulse-width discriminator.

The physical properties of pharmaceutical powders are as important to an acceptable drug formulation as are its chemical properties (1). For example, the solubility, dissolution rate, and processing characteristics often depend on the particle size of the powders. Furthermore, the particle size can influence the bioavailability of poorly soluble drugs (2).

The growing need for particulate analyses and determination of particulate-size distributions in the quality control of pharmaceutical products emphasizes the need for efficient and reproducible methods. The popular microscopic method has several disadvantages for use in a quality control laboratory operation. It is time consuming, the counting and sizing results are subject to the judgment and experience of the microscopist, and the analyst cannot readily discern particles that are <5 μm long.

Other methods are available for the particle-size analysis of powdered drugs (1, 3). One method that overcomes the disadvantages of the microscopic determinations utilizes the Coulter or electrozone principle (electrolytic sensing). This electrical sensing zone method is capable of detecting and sizing small particles and counting a statistically significant number of particles.

The present study is part of a continuing project to automate the particle-size analysis of powdered drugs used for formulating suspensions, capsules, and tablets. Milled and micronized materials were studied since these particle reduction methods create particles that are well suited for particle sizing by an electrical sensing zone.

EXPERIMENTAL

Materials—The micronized steroids and spectinomycin hydrochloride were commercially available[1]. Research grade sodium chloride, lithium chloride, and 3A ethanol (contains 5% methanol) were used in the electrolytic solutions. Polysorbate 80 (polyoxyethylene 20 sorbitan monooleate) was used as a wetting agent. Polystyrene spheres of 2.0, 3.5, 5.2, 8.0, 9.8, 10.3, 17.7, 20.0, 32.0, 40.0, and 80.0 μm^2 were used to calibrate the instrument. Neomycin sulfate was selected as a mold inhibitor[1].

Equipment—The particle-size analyses were carried out using a multichannel electrozone particle-size analyzer interfaced to a digital minicomputer[3]. The system is depicted graphically in Fig. 1. Orifice tubes with diameters of 48, 76, 120, and 190 μm were used. The depths of the orifices were between 75 and 100% of the diameter.

Electrolyte—The aqueous electrolyte consisted of 0.9% NaCl, 0.1% polysorbate 80, and neomycin sulfate (4 g/liter) in water saturated with the bulk drug to be analyzed. The solution was prepared by stirring the powdered drug into the aqueous solution for 20 min and filtering once through a 0.22-μm filter. The alcoholic electrolyte was made in a similar manner using lithium chloride and 3A ethanol.

Sample Preparation—About 100 mg of the steroid was placed in ~25 ml of the saturated electrolyte. The vial containing the powdered drug and the electrolyte then was immersed in an ultrasonic bath for a time sufficient to disperse the sample and break up the agglomerated particles. The required time varied from 2 to 10 min, depending on the material. No prior sample preparation was required for spectinomycin hydrochloride.

Particle-Size Determination—For the steriods, a few (1–4) drops of the sonicated suspension were added to the counting cell until the count was between 10 and 100 times greater than the background at the most sensitive current setting. Care also was taken to ensure that the coincident count (*i.e.*, two particles counted as one) was <10%. The background count should be maintained at a level of ~5% of the sample count in the lowest channel. The analysis then was performed under computer control until 2000 counts were accumulated in one of the 128 raw data channels. The particle size in each channel then was calculated by the computer using the calibration tables stored on the flexible disk

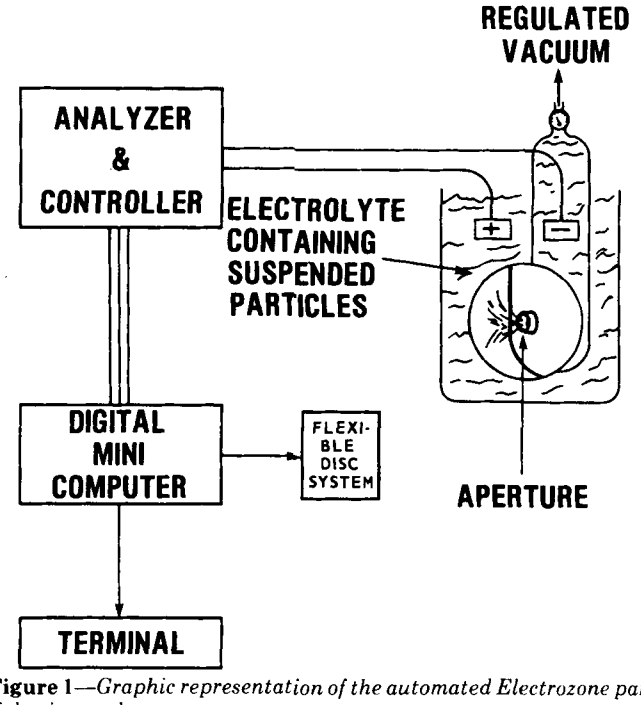

Figure 1—*Graphic representation of the automated Electrozone particle-size analyzer.*

[1] The Upjohn Co.

[2] Duke Standards, Inc.

[3] Celloscope model 112 LTSA/ADCW, Particle Data Inc.

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Table I—Resistivity Measurements

Electrolyte	Resistivity, ohm-cm
0.9% LiCl in 3A ethanol with 0.1% polysorbate 80	316
0.9% LiCl in methanol with 0.1% polysorbate 80	123
0.9% NaCl in water with 0.1% polysorbate 80	62
4% Ammonium thiocyanate in isopropanol with 0.14% polysorbate 80	398
Methanol[a]	>50,000
Distilled water	>50,000
Tap water	1,280

[a] Research grade.

system. This operation was repeated at several current settings until a complete distribution was obtained.

The analyses of spectinomycin hydrochloride were performed in a similar manner, except that a small amount of powder (~9 mg) was introduced directly into the saturated 3A ethanolic electrolyte solution that had been normalized previously. This suspension was stirred vigorously for 15–30 sec before proceeding with the particle-size analysis.

RESULTS AND DISCUSSION

Spectinomycin Hydrochloride—During this investigation, the resistivities of several electrolytes were measured using a conductivity bridge (Table I). Resistivity is important because it limits the maximum

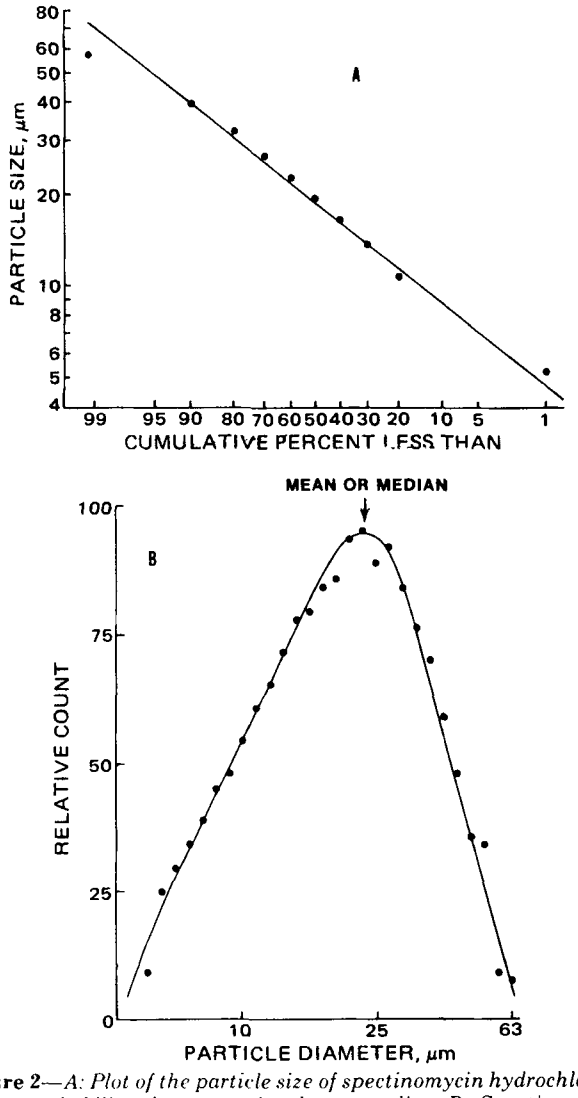

Figure 2—*A: Plot of the particle size of spectinomycin hydrochloride versus probability demonstrating log-normality. B: Spectinomycin hydrochloride particle-size distribution on a volume or mass basis.*

Table II—Calibration and Linearity Data for the Orifice Tubes

Tube Size, μm	Observed Count Diameter, μm	Microscopic Count Diameter, μm[a]
76	1.9	2.0
48	4.2	3.5
120	5.2	5.2
48, 190	8.9, 7.9	8.0
76	9.8	10.3
120	10.5	10.4
48, 76	18.2, 17.7	17.7
120, 190	19.9, 19.7	20.0
76, 120	31.3, 32.3	32.0
120	40.4	40.0
190	83.0	80.0

[a] The relative standard deviation as listed by Duke Standards was 10%.

usable current through the appearance of thermal noise. Thermal noise occurs due to power dissipation at the orifice (*e.g.*, released bubbles of dissolved gases). This effect is observed as ragged baseline grass on the display scope or is viewed microscopically as a grayish stream line trailing from the orifice.

Power dissipation and hence thermal noise are inversely proportional to the orifice size. Consequently, an electrolyte of low resistivity is required when a small orifice is used. A resistivity in the range of 10–100 ohm-cm is considered optimum, although solutions having a resistivity between 1 and 1000 ohm-cm are usable. Spectinomycin hydrochloride is an electrical nonconductor that is soluble in water and methanol but is only slightly soluble in 3A ethanol. Therefore, even though the lithium chloride–3A solution has a conductivity that is outside the optimum range, it was the electrolyte of choice because of the low solubility of spectinomycin hydrochloride in this solution.

The measured primary spectinomycin particles obeyed log-normal statistics when the data were converted to a volume or mass basis as shown in Fig. 2A (4). A typical volume distribution is depicted in Fig. 2B. For a log-normal distribution, only the geometric mean and the standard deviation are needed to define the whole distribution function. These values can be calculated from a log-normal plot of the cumulative data. A computer program was used to calculate the geometric volume mean (mass median) diameter ($\overline{M}v$) and the geometric standard deviation (σ) defined by:

$$\overline{M}v = \exp\left(\frac{\sum_{i=1}^{n} N_i \ln M_i}{\sum N_i}\right) \qquad \text{(Eq. 1)}$$

and:

$$\sigma = \exp\left(\sqrt{\frac{\sum_{i=1}^{n} N_i(\ln M_i - \ln \overline{M}v)}{\sum_{i=1}^{n} N_i}}\right) \qquad \text{(Eq. 2)}$$

where M is the particle diameter on the volume or mass basis, and N is the number of particles. The geometric count mean diameter, $\overline{M}c$, was derived from the geometric volume mean diameter by the relation:

$$\overline{M}c = \exp\left[\ln \overline{M}v - 3.0\,(\ln \sigma)^2\right] \qquad \text{(Eq. 3)}$$

The orifice tubes were calibrated using polystyrene spheres of known

Table III—Precision of Spectinomycin Hydrochloride Particle-Size Analyses

Sample	Geometric Mean Diameter, μm	
	Volume	Count
1	18.5	4.9
2	18.2	4.9
3	18.9	5.4
4	18.7	5.7
5	18.9	5.4
6	19.2	6.1
7	19.5	5.8
8	18.8	5.5
9	18.2	4.9
Mean	18.8	5.4
SD	0.4	0.4

Table IV—Results of Milling Effects

Sample	Millfeed Rate	Geometric Volume Mean Diameter, μm	Geometric *SD*
1	Unmilled	47.0	1.88
2	Fast feed	18.5	1.85
3	Slow feed	16.6	1.86
4	Blocked feed	13.0	1.62

Table V—Pulse-Width Analyses

Sample	Current, mamp[a]	Mode, μsec	*SD*[b], μsec
Spectinomycin	1/16	33	10
Spectinomycin	$\sqrt{2}$	31	13
Spectinomycin	1/16	34	10
Spectinomycin	$\sqrt{2}$	29	13
32-μm Polystyrene	1/16	63	7
32-μm Polystyrene	1/2	116	13

[a] Log = 8 and gain = 2⅛ for all analyses. [b] *SD* = 0.425 × (full width at half maximum).

diameter. The data (Table II) show that there was good agreement between the present results and the microscopic results of the manufacturer. A linear regression of the data for the observed size *versus* the microscopic size showed that the method was linear with little or no bias (slope 0.97, intercept 0.3, and correlation coefficient 0.999). The precision (Table III) of the method was excellent for the volume mean diameter (*RSD* 2.3%) and adequate for calculating the count mean diameter (*RSD* 8.0%).

Spectinomycin hydrochloride is formulated as a powder for resuspension. It is injected into muscle tissue as a saturated aqueous suspension, and the particle size must be controlled for patient comfort and good injection properties (*i.e.*, the suspension should not block the injection needle). The results in Table IV show that different size particles were created by different milling speeds. This observation indicates that the method is capable of determining the effectiveness of particle reduction during the milling operation and, therefore, makes an excellent quality control method.

Pulse-Width Analysis—There is a direct relationship between the basic transit time of small spheres flowing through the center of the orifice tube aperture and the width of the electrical pulse produced (5). The pulse-width overlay for the analysis program allows the analyzer to provide data on typical transit times for solid particles. The results of the pulse-width analysis are listed in Table V, and typical distribution curves are illustrated in Figs. 3 and 4. It took a normal particle of spectinomycin hydrochloride 32 μsec to travel through an orifice that was 90–120 μm deep. Prolonged transit times result if the particles are near the aperture wall where the fluid velocity is less or if they are prolate or oblate ellipsoids or flexible structures (5). The results indicate that particle size also has an important effect. This effect is well illustrated by the fact that the apparent transit time was almost twice as long (63 μsec) for the 32-μm

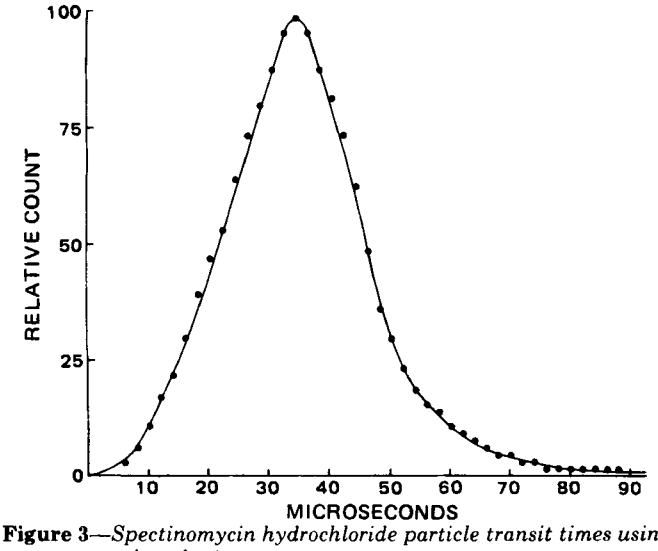

Figure 3—*Spectinomycin hydrochloride particle transit times using a current setting of 1.414 mamp.*

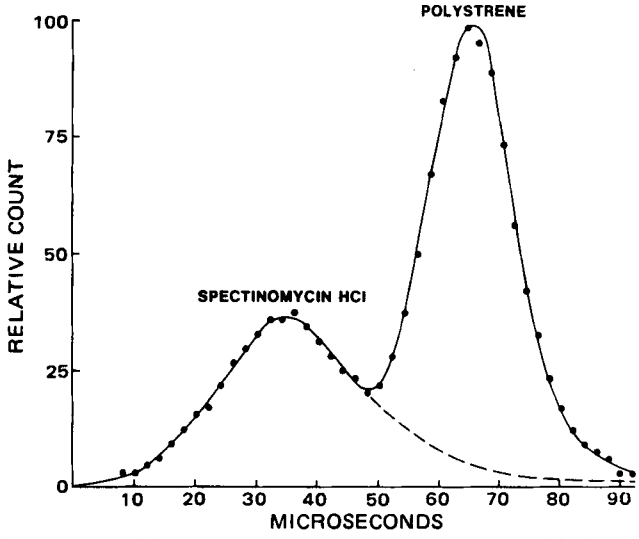

Figure 4—*Particle transit times of spectinomycin hydrochloride and 32-μm polystyrene spheres using a current setting of 0.062 mamp.*

reference than for the spectinomycin whose average particle size was 19 μm (Table III).

It has been suggested that since pulses produced by particles traveling along the orifice walls have correspondingly longer durations than pulses produced by particles passing along the orifice axis, the undesirable pulses may be eliminated by pulse-width discrimination. However, as noted earlier, this effect also would discriminate against the larger particles present. The effect of using a pulse-width discrimination of 79 μsec (pulses greater than 79 μsec are not counted) can be demonstrated by comparing the results listed in Tables III and VI. These data were obtained on the same sample of material. The apparent smaller geometric volume mean diameter (12.8 *versus* 18.8 μm) can only be the result of the computer ignoring the larger particles. It is suggested that pulse-width discrimination is a viable technique only if all of the particles are much smaller (≤10%) than the orifice length and diameter.

Steroids—The aqueous saline solution was chosen as the electrolyte and saturated with bulk drug even though the steroids are rather insoluble in water. This precaution controls the nonproportional dissolution of the smaller particles relative to the larger ones. As a further check on dissolution, the number of particles in 100 μl of prepared sample was counted before and after the determination at a fixed current. Comparison of these counts indicated that sample dissolution (or decomposition) was negligible.

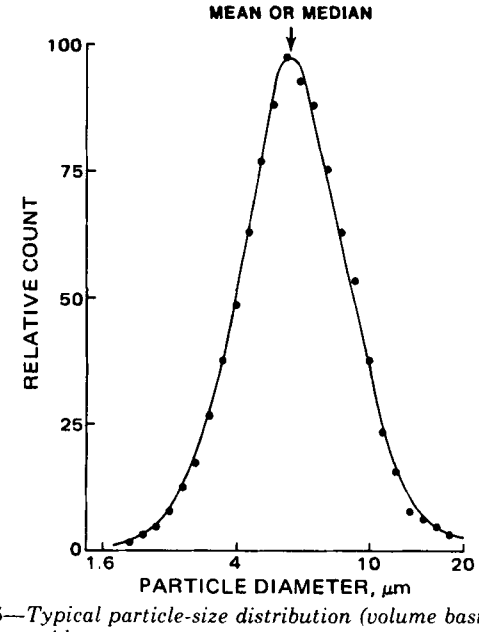

Figure 5—*Typical particle-size distribution (volume basis) of a micronized steroid.*

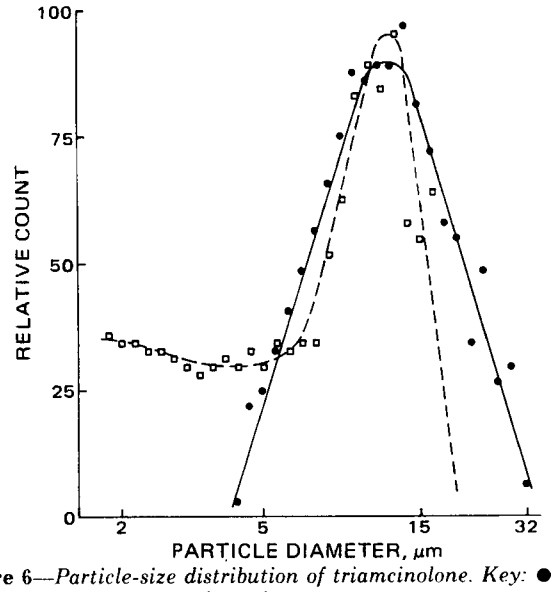

Figure 6—*Particle-size distribution of triamcinolone. Key: ●, nonsonicated; and □, sonicated 10 min.*

One basic objective of particle-size determinations is to measure the size of primary particles rather than the size of agglomerates or fractured primary particles. In general, samples routinely are sonicated to break up agglomerates into primary particles prior to obtaining particle-size distributions. Since extended sonication can reduce the size of polyethylene spheres (6), the effects of sonication on steroid particle size was examined.

The effect of sonication time on the agglomerate and primary particle size indicated that optimum times exist for each of these steroids. Samples were prepared as described under *Experimental*, except that the time of sample sonication was varied. All steroids displayed a rapid initial decrease in their volume mean and in the cumulative percent of observed particles with a diameter greater than 10 or 20 μm. This apparent decrease in size with sonication leveled off within the optimum times listed in Table VII. The appearance of the size distribution remained smooth and log-normal for all of the micronized steroids (Fig. 5). However, for triamcinolone (not micronized), the ratio of small to large particles increased with sonication time (Fig. 6).

The effect of sample dispersion also was studied using microscopic methods. The results (Fig. 7) confirm that sample sonication disperses the agglomerates into single crystals for counting and sizing. This observation was true for all of the steroids except triamcinolone. This steroid exists as individual crystals with very few agglomerates. The deterioration (Fig. 6) of the triamcinolone particle-size distribution upon sonication observed with the celloscope and the fact that sonication is known to fracture particles (6) suggest that sonication results in primary particle breakdown for this steroid.

The case of triamcinolone is important because it points out that, although dispersion of samples by ultrasonication works well, it should not be used without examining the effects on the material to be dispersed. This consideration is important for any analytical measurement made directly on the solid material. The distributions depicted in Fig. 6 show that a significant number of small particles are created by sonication. It is clear that a sample sonicated for 10 min does not represent the actual particle size of triamcinolone. As shown by the unsonicated sample in Fig. 6 and the results listed in Table VIII, it is possible to obtain a rea-

Table VI—Particle Size of Spectinomycin Hydrochloride Determined Using Pulse-Width Discrimination [a]

	Geometric Mean, μm	
	Volume	Derived Count
	14.5	5.0
	14.5	4.9
	14.8	5.1
	10.8	5.2
	11.6	5.4
	10.3	5.2
Mean	12.8	5.1
RSD, %	16.4	3.9

[a] Pulse-width discrimination set at 79 μsec.

Table VII—Optimum Sample Dispersion Time Using Ultrasonic Mixing

Steroid	Sonication Time, min
Hydrocortisone	1–2
Hydrocortisone acetate	1–2
Methylprednisolone	1–2
Methylprednisolone acetate	1–2
Medroxyprogesterone acetate	2–5
Prednisolone anhydrous	1–2
Prednisolone acetate	1–2
Prednisone	2–5
Prednisone acetate	5–10
Progesterone	5–10

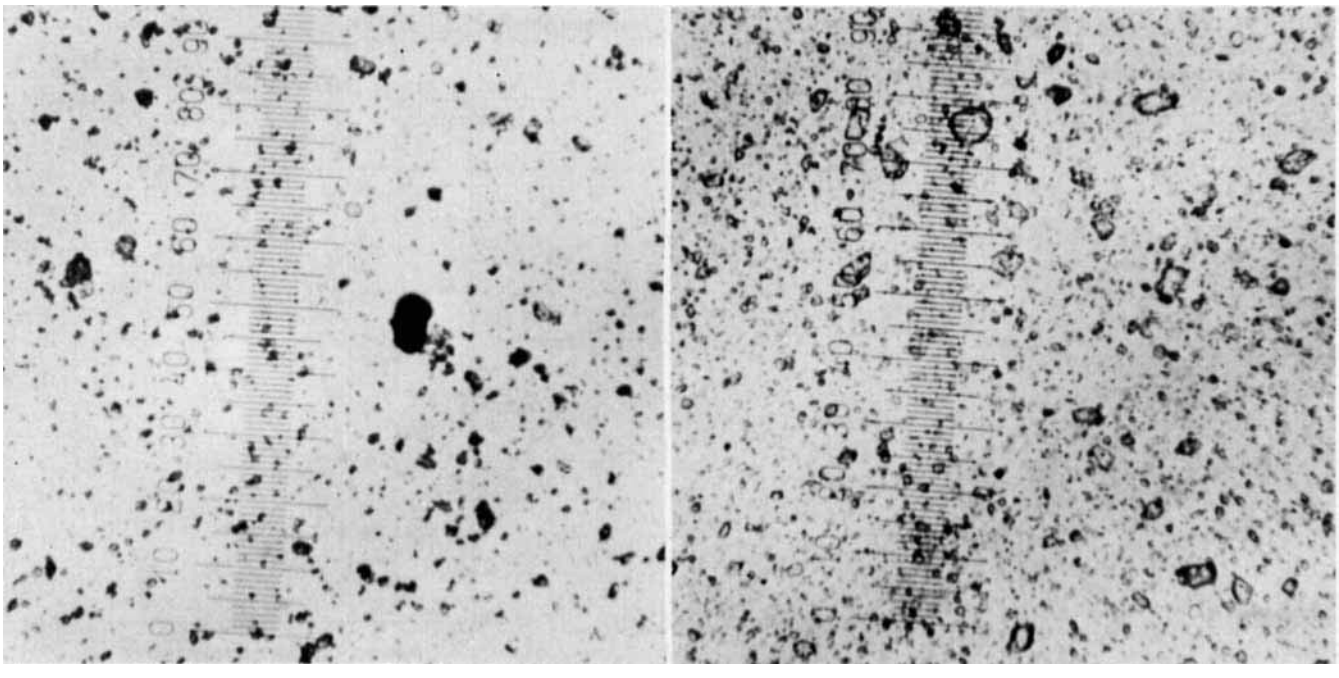

Figure 7—*Photomicrographs of hydrocortisone acetate; magnification is 125×. Key: left, sample not sonicated; and right, sample sonicated 10 min.*

Table VIII—Particle-Size Results for Triamcinolone

Sample	Geometric Volume Mean Diameter, μm	Geometric *SD*
A	9.5	1.7
B	8.5	1.8
C	9.2	1.7
D	10.3	1.8
E	10.8	1.8
F	9.3	1.9
G	8.3	1.8
H	8.7	1.8

sonable measure of the particle size for triamcinolone by shaking the sample vial gently to disperse the powder. The results presented for triamcinolone indicate that the method of sampling and dispersion is as important to the particle-size measurement as the parameters relating directly to the measurement. In fact, it was found previously that the error in counting particles by the electrozone method is less than the error due to sampling (3).

CONCLUSIONS

Experience has shown that, for pharmaceutical powders, the volume or mass basis (identical to volume if the measured particles have equal densities) is the most useful way of representing particle diameters for milled and micronized powders. These powders usually have log-normal particle distributions (7) that can be described completely by the geometric median or mean diameter and the geometric standard deviation. In addition, milled and micronized materials are described adequately by a spherical diameter; therefore, the automated electrozone system described in this paper is excellent for quality control purposes.

The electrolytic sensing zone method does have some limitations. The particles must be relatively insoluble in a solvent that has a moderate dielectric constant. Unusually shaped particles (*i.e.*, long rods or platelets) are converted to a mean diameter that is considerably less than the longest dimension of the actual particle. For example, a sphere of 4-μm diameter would have approximately the same volume as a cylinder of diameter 2.5 μm and length 7.5 μm. Thus, the method is relatively insensitive to particle shape. However, the advantages clearly outnumber the disadvantages. The method is highly sensitive, fast, and generally applicable. Sample preparation and data reduction are simple. The operator-fatigue factor is much less than for microscopy.

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Simultaneous Partitioning and Hydrolysis Kinetics of Amoxicillin and Ampicillin

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Abstract □ The kinetics of ampicillin and amoxicillin partitioning with simultaneous acid-catalyzed hydrolysis were studied in a stirred transfer cell containing isobutanol as the extract and aqueous hydrochloric acid (0.1–0.5 *N*) as the raffinate at 37°. Biexponential data for the concentration in both the raffinate (C_1) and the extract (C_2) as a function of time were analyzed simultaneously by nonlinear regression to estimate the apparent first-order rate constant for transfer from hydrochloric acid to isobutanol (k'_{12}), the reverse transfer constant (k'_{21}), and the hydrolysis rate constant (k). Agreement between k values determined in the presence of simultaneous partitioning and those determined in the absence of partitioning (k_{app}) verified the nonlinear estimates. Apparent partition coefficients, which represent the values that would be obtained in the absence of hydrolysis ($K'_D = C_1^\infty/C_2^\infty$), were estimated from $K'_D = k'_{12}/k'_{21}$. During terminal monoexponential loss, where $C_1 \simeq Y'e^{-\beta t}$ and $C_2 \simeq Z'e^{-\beta t}$, the kinetically controlled C_2/C_1 ratio (r) is described by $[k'_{12}/(k'_{21} - \beta)]$, which decreases with decreasing k values until r approaches K'_D. The difference between the terminal concentration ratio, r, and its corresponding partition coefficient, K'_D, is a measure of the degree to which kinetic processes control distribution. Both ampicillin and amoxicillin showed kinetic control of the distribution ratios in 0.5 *N* HCl, where the hydrolysis rate constant was significant relative to the distribution rate constants. Ampicillin had $r \simeq 1.74$ and $K'_D \simeq 0.92$; amoxicillin had $r \simeq 0.95$ and $K'_D \simeq 0.65$. As the $(k'_{12} + k'_{21})/k$ ratio increased, the r values approached K'_D so that in 0.1 *N* HCl, $r \approx K'_D = 0.33$ for amoxicillin and $r \approx 0.6$ and $K'_D \approx 0.56$ for ampicillin. In general, amoxicillin distribution rate constants ($k'_{12} + k'_{21}$) were roughly twice those of ampicillin, whereas ampicillin K'_D and r values were nearly double those of amoxicillin. Thus, the kinetic and thermodynamic rank orders are opposite. This result may have implications in drug design *via* molecular modification.

Keyphrases □ Amoxicillin—kinetics of simultaneous partitioning and hydrolysis □ Ampicillin—kinetics of simultaneous partitioning and hydrolysis □ Hydrolysis kinetics—amoxicillin and ampicillin, simultaneous partitioning □ Partitioning kinetics—amoxicillin and ampicillin, simultaneous hydrolysis □ Kinetics—amoxicillin and ampicillin, simultaneous partitioning and hydrolysis

A stirred transfer cell can be used to determine the rate constants for distribution between partially miscible phases with simultaneous degradation in the aqueous phase (1). This technique is potentially useful for comparing the partitioning of closely related drugs under conditions where they are not stable. The method allows calculation of the apparent partition coefficient, K'_D, that would be observed if the drug were stable. It also allows estimation of the concentration ratio, r, of the two phases at a time sufficiently long for r to approach a constant value. Thus, the calculated equilibrium value, K'_D, and the kinetic value, r, can be compared. If these values vary

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