Separation of Multisized Drug Suspensions into Narrow Distributions by Centrifugal Elutriation

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Received April 4, 1977, from the *School of Pharmacy and the ‡Department of Physiology and Biophysics, West Virginia University, Morgantown, WV 26506. Accepted for publication September 8, 1977.

Abstract □ A centrifugal elutriator rotor was used to separate suspensions of micronized hydrocortisone acetate and prednisolone acetate. Up to five distinct particle fractions could be isolated from a single parent suspension, and reasonable reproducibility was exhibited between various elutriator runs. Arithmetic means and associated standard deviations, based on volume, were calculated for each isolated fraction. These data demonstrate the narrowness and uniqueness of each fraction. The particle-size data show an apparent log-normal distribution for each isolate. The uniqueness of each fraction also was demonstrated by dissolution experiments where Fraction 1, the smallest fraction of prednisolone acetate, dissolved much more rapidly than Fraction 4, the largest fraction. The substantial difference in dissolution behavior of these two fractions of prednisolone acetate powder was biopharmaceutically significant.

Keyphrases □ Suspensions—separated into distinct particle-size fractions by centrifugal elutriation □ Particle-size fractions—suspensions separated by centrifugal elutriation □ Centrifugal elutriation—used to separate suspensions into distinct particle-size fractions □ Dosage forms—suspensions, separated into distinct particle-size fractions by centrifugal elutriation

Although it is well established that the particle size of suspended powders influences both the physical stability and dissolution rates of pharmaceutical suspensions, the monographs of only two official suspension products (insulin zinc suspension USP and sterile methylprednisolone acetate suspension NF) mention particle size. The lack of a simple method to obtain narrow populations of suspended material of varying sizes (in the micrometer range) is one major problem in establishing the optimum size requirements of a suspension product. With such a method, it would not be necessary to mill various lots of suspensions with differing particle-size distributions. Only one lot of suspension having a wide particle distribution would be required to separate out those size fractions needed for study. Thus, from a single suspension lot, samples could be obtained from which one could establish optimum product size requirements.

Critical study of dissolution kinetics is another investigational area where a separation technique such as described in this paper would prove invaluable. For example, the dissolution kinetics for multisized particle populations have been discussed (1–7). In some cases (8, 9), the experimental data were treated without calculation of the effect of particle-size distribution. Errors associated with this data treatment may be acceptable, except in cases of critical tests of dissolution rate theory (10). On the other hand, dissolution rate data for multisized methylprednisolone populations were compared with the theoretical expectation from a kinetic model that accounted for the distribution effects (2). The critical test of this dissolution model was influenced by the ability to measure and quantitate the true initial size distribution.

Another report (11) attempted to show that approximate cube root model equations, which incorporate distribution effects, were applicable to dissolution profiles of log-normal populations of oxalic acid particles. The log-normal populations were developed artificially from combined mesh fractions of oxalic acid particles grown under isothermal and nonisothermal conditions (11). An approach was evaluated to demonstrate the application of single-particle dissolution models to explain the dissolution kinetics of the 60–85-mesh fraction of tolbutamide particles (12). This approach permitted the calculation of the intrinsic dissolution profiles with a consideration of both size distribution and particle-size effects. All of these investigations point to the importance of controlling the particle-size distribution to study the dissolution model of interest critically. Therefore, a convenient and dependable method that allows control of particle-size distributions for critical dissolution tests for drug particles is necessary.

The purpose of the present study was to isolate narrow drug particle distributions from a drug suspension by centrifugal elutriation. The technique of centrifugal elutriation has been used in the biological sciences previously to separate cells from various tissues (13–17).

The general principle of elutriation is to counterbalance the sedimentation of particles under centrifugal force with a continuous flow of solution counter to the *g* force (18, 19). Suspended solids of varying sizes sediment at different rates under centrifugal force and may be separated by either varying the *g* force or the flow rate of the buffer, which is counter to the *g* force.

EXPERIMENTAL

Fraction Separation—An elutriation rotor[1] and centrifuge[2] were used. A 100-ml portion of suspension was infused into the elutriation chamber of the rotor in the centrifuge by a large peristaltic pump[3] in series with a smaller pump[4], which was used for fine adjustments of the flow rate. The loading flow rate was set at approximately 30 ml/min with the centrifuge running at approximately 2000 rpm.

After the drug particles were all loaded into the chamber, a volume of 0.9% NaCl was allowed to flow through the system. Collection of the first sample was then initiated simply by maintaining the same conditions described. After the first fraction appeared to be completely collected (determined by visual observation through the centrifuge window), the pump rate was increased to 53 ml/min and the second sample was collected. Once the second sample was collected, the centrifuge speed was reduced to approximately 1000 rpm and a third sample was removed.

The fourth sample was obtained at a pump rate of approximately 52 ml/min and a centrifuge speed of between 500 and 600 rpm. The fifth sample consisted of the residue left in the chamber after the pump and centrifuge were stopped. This fraction was removed by washing the chamber with 0.9% NaCl and collecting the residue. The exact pump centrifuge speeds used in each separation run are listed in Table I.

Particle-Size Measurement—An automated counter[5] was used for

[1] Model JE 6, Beckman Instrument Co., Fullerton, Calif.
[2] Model J-21B, Beckman Instrument Co., Fullerton, Calif.
[3] Model 1201 multispeed transmission, Harvard Apparatus Co., Millis, Mass.
[4] Model R-L175, Holter Co., Extracorporeal Medical Specialties Inc., King of Prussia, Pa.
[5] Model TA II, Coulter Electronics, Hialeah, Fla.

Table I—Centrifuge Conditions for Separation

Fraction	Pump Speed, ml/min	Centrifuge Speed, rpm			
		Run A[a]	Run B[b]	Run C[b]	Run D[c]
1	30	1830	2160	2030	2000
2	52	1830	2160	2030	2000
3	52	1140	1020	1070	1070
4	52	510	620	620	600
5	—	Residue	Residue	—	—

[a] Prednisolone acetate, 0.3%. [b] Prednisolone acetate, 0.6%. [c] Hydrocortisone acetate, 1%.

all particle-size measurements. This particular apparatus provides particle-size distributions based on equivalent sphere volume, which is converted to weight distribution if all material measured has the same density. The instrument has a 16-channel readout. Doublets and other multiple counts can be considered negligible by maintaining the dilution of the sample in the electrolyte. The instrument has a concentration meter which facilitates this process. Microscopic examination was also used to check for agglomerates.

The electrolyte solution[6] was presaturated with drug and double filtered through a 0.22-μm filter to avoid particle dissolution during measurement.

A population accessory was used to obtain a number count for each channel. Apertures of 200 and 50 μm were used in this study, and polystyrene beads[7] were used as standards to calibrate the channels.

Sample Preparation—The suspensions used were 0.3% prednisolone acetate[8] suspension in normal saline (Run A), 0.6% prednisolone acetate[8] in normal saline (Run B), 0.6% prednisolone acetate[8] in presaturated normal saline (Run C), and hydrocortisone acetate[9] in normal saline (Run D).

Each suspension was prepared using a mortar and pestle with gradual trituration. It was desirable to have as wide a distribution as possible for the initial trials.

Dissolution Determinations—During this study, all reported dissolution data were obtained using a device reported by Shah *et al.* (20) with the sample basket removed. The basic features of this apparatus are a large volume fluid container, a rotating filter assembly, and an external variable speed magnetic stirrer. The rotating filter assembly provides a variable intensity of mild laminar liquid agitation and also functions as an *in situ* nonclogging filter to permit efficient intermittent or continuous filtration of the dissolution fluid samples during dissolution.

One liter of distilled water was utilized as the dissolution medium. The temperature was maintained at 37°, and the stirring speed of the filter assembly was 300 rpm. A strobe lamp was employed to standardize the stirring speed.

Filtered fluid samples were continuously withdrawn at the rate of 100 ml/min and were circulated through a spectrophotometer[10] for assay and then back into the dissolution flask. The prednisolone acetate was assayed at 246 nm.

The dissolution experiments were initiated by injecting a predetermined amount of the desired fraction of prednisolone acetate suspension at a fixed position in the beaker, using a syringe fitted with an extra long needle. The final concentration was kept below 10% of saturation. The chart paper was marked at the exact time the suspension was introduced into the flask. The absorbance of dissolving prednisolone acetate was recorded at 5-sec intervals until 1500 sec from the digital display of the spectrophotometer and then periodically up until approximately 2 hr. A final 24-hr reading was taken to determine an equilibrium concentration.

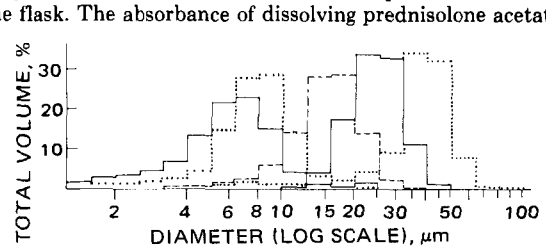

Figure 1—*Particle-size distributions of the five fractions of Run B using prednisolone acetate suspensions. Key (from left to right): —, Fraction 1; . . . , Fraction 2; – – –, Fraction 3; —, Fraction 4; and . . . . . , Fraction 5.*

[6] Isoton, Coulter Diagnostics Inc., Hialeah, Fla.
[7] Latex (2.02 mm diameter) and polystyrene (9.69 mm diameter), Coulter Electronics, Hialeah, Fla.
[8] Schering Laboratories, Bloomfield, N.J.
[9] Micronized, Pfizer Laboratories, Norwich, Conn.
[10] Cary 118, Varian Instrument, Palo Alto, Calif.

Table II—Mean Particle Diameter in Micrometers and Standard Deviation Based on Percent Volume Distribution

Fraction		Prednisolone Acetate			Hydrocortisone Acetate, D
		A	B	C	
1	Mean	5.9[a]	6.5[a]	5.5[a]	5.4[a]
	SD	2.7	3.4	2.4	1.8
2	Mean	7.2[a]	8.0[a]	6.6[a]	7.0[a]
	SD	2.5	2.7	2.4	3.3
3	Mean	11.9[a]	16.3[b]	13.4[b]	7.7[a]
	SD	2.8	5.9	4.8	2.9
4	Mean	25.4[b]	25.3[b]	21.3[b]	9.8[b]
	SD	6.1	6.2	5.6	3.5
5	Mean	33.4[b]	32.7[b]		
	SD	7.7	8.7		

[a] A 50-μm aperture tube was used. [b] A 200-μm aperture tube was used.

RESULTS AND DISCUSSION

As shown in Table I, three elutriation separation experiments were conducted with prednisolone acetate suspensions. In addition, a preliminary experiment was conducted utilizing a suspension of hydrocortisone acetate. A comparison of the means of percent volume particle distribution for these four separation runs can be seen in Table II.

Prednisolone acetate Runs A and B were conducted first, and both used the same lot of raw material. Run C utilized a new lot of prednisolone acetate and was conducted to check the previous two experiments.

The hydrocortisone acetate was the first material tested; although it yielded a good separation, the micronized sample did not contain a wide distribution of particles, especially large particles. Therefore, prednisolone acetate was utilized for most experiments. The result of the hydrocortisone acetate run is included to demonstrate the diversity and reproducibility of the process.

Fraction 1 contained most of the finest particles and was the first fraction collected. As shown in Table II, all mean particle sizes, including the hydrocortisone acetate run, yielded mean particle sizes within 1 μm of each other. The mean particle diameters were calculated from the discrete normalized data where the random variable is the average size per channel.

In the second fraction, the mean particle diameter for each run increased by slightly over 1 μm compared to the data shown for Fraction 1. Once again, the means were close, with no mean more than 1.5 μm apart and all means within 1 *SD* of one another.

Fraction 3 showed more diversification from run to run. The interpretation of these data is complex because the two runs conducted initially, Runs A and D (the hydrocortisone acetate run), were tested using a 50-μm aperture tube while the last two separations of prednisolone acetate were measured with a 200-μm aperture tube. This difference could possibly account for some diversity from run to run. An overlapping aperture tube technique might be more proper for these samples.

In each case, the mean diameter of the prednisolone acetate suspensions increased by approximately 5–8 μm from the next smaller fraction (Fraction 2), thus demonstrating successful separation. Since the micronized hydrocortisone acetate was lacking in larger particles, this fraction and the subsequent fraction (Fraction 4) had a smaller mean than the prednisolone acetate runs.

The prednisolone acetate Fraction 4 yielded mean particle sizes which were approximately 10 μm larger than the previous fraction. The mean particle diameters of each prednisolone acetate run were within 4 μm of one another, well within 1 *SD*.

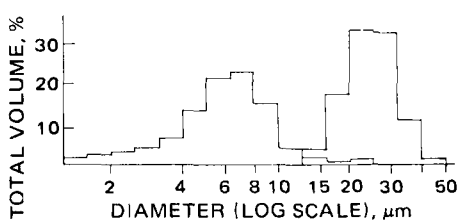

Figure 2—*Particle-size distributions of Fractions 1 (left) and 4 (right) of Run B, prednisolone acetate.*

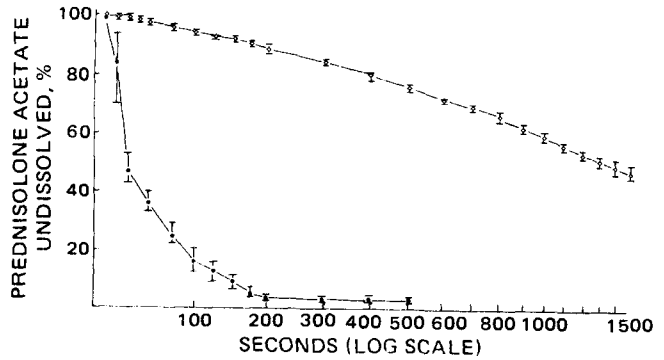

Figure 3—*Aqueous dissolution profiles of Fractions 1 (◇) and 4 (●) of prednisolone acetate suspensions from Run B. The points are the average of three experiments, and the bars are extremes of the three experiments.*

Fraction 5 consisted of washings from the elutriator chamber. Run C, which was conducted on a different lot of prednisolone acetate than Runs A and B, had insufficient washings to be collected. Hydrocortisone acetate, which was micronized and had few larger particles, also contained no particles within this fraction. All data for these two runs were extremely close, with a 7- or 8-μm increase in mean particle size from the previous fraction.

To demonstrate the nature of the shift of particle distribution from fraction to fraction, the particle distribution for Run B was plotted (Fig. 1). Five fractions were obtained, each with its own distinctive mean particle size. The only two fractions with heavy overlapping of particles were Fractions 1 and 2, which did show a shift of one channel. The other fractions all showed a shift of two or more channels.

Each fraction was distributed according to an apparent log-normal distribution. This fact is significant because of the widespread use of log-normal distributions in dissolution modeling (1–7, 11, 21, 22). Veng Pedersen (22), for example, assumed a log-normal distribution in successfully characterizing the dissolution of micronized glyburide using the cube root law.

To demonstrate the potential biological importance of the fraction differences, the aqueous dissolution rates of Fractions 1 and 4 of Run B were determined (Fig. 2). These two fractions provided two distinct particle-size distributions with essentially no overlapping of particle sizes.

The particle-size differences of these two fractions are dramatically demonstrated in the dissolution results (Fig. 3). These data indicate that 50% of Fraction 1 dissolved in less than 30 sec while 1400 sec elapsed before 50% of the large Fraction 4 dissolved.

The mixing of these fractions and the dissolution testing of other fractions would seem to be valuable tools in testing theories designed to predict dissolution kinetics from particle size. The design of sustained- or controlled-release suspensions from a mixing of fractions also may be another attractive possibility.

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Liquid Chromatography in Pharmaceutical Analysis IX: Determination of Muscle Relaxant–Analgesic Mixtures Using Normal Phase Chromatography

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Received June 1, 1977, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication September 6, 1977.

Abstract □ High-pressure liquid chromatography was used to optimize the resolution of eight widely prescribed therapeutic agents commonly found in muscle relaxant–analgesic mixtures. The compounds were chromatographed on normal phase porous silica or cyanopropylsilane columns, using various solvent systems paired on the basis of Snyder's solvent selectivity scheme to give a polarity index for each system of 3.3. A carisoprodol, phenacetin, and caffeine mixture was selected to demonstrate the utility of the separation and quantification method. The mixture was chromatographed on a porous silica column, using tetrahydrofuran–toluene (50:50) at a flow rate of 2.0 ml/min. Each determination can be achieved in approximately 8 min with an accuracy of 3–5%.

Keyphrases □ Muscle relaxants, various—high-pressure liquid chromatographic analyses in mixtures with analgesics □ Analgesics, various—high-pressure liquid chromatographic analyses in mixtures with muscle relaxants □ High-pressure liquid chromatography—analyses, various muscle relaxants and analgesics in pharmaceutical mixtures

Muscle relaxant–analgesic mixtures are widely prescribed drugs. The continued interest in these laboratories in the use of high-pressure liquid chromatography (HPLC) for the separation and quantitation of multicomponent