Experimental Method for Studying Growth Rates of Particles in Aqueous Media

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A simple procedure for following growth of suspension particles has been developed. It is suitable for studying mechanisms of growth, for evaluation of potential growth retarding agents, and possibly for accelerated stability studies in dispersed systems. It involves the determination of the particle concentration and size distribution as a function of time with the Coulter counter. The sensitivity of the method is better than 0.1 μ for particles in the micron size range. Prior removal of the particles from the suspension is unnecessary. The technique has been applied to growth rate studies in the methylprednisolone system.

THERE ARE A NUMBER of situations in which an understanding of the factors determining the rate of crystal growth is helpful to the pharmacist. In connection with stability, the coarsening of suspensions by growth of the larger particles at the expense of the smaller ones is a good example. The problem of caking, the cementing together of particles in a sediment, is also well known. More recently, attention (1) has been given to the possible utilization of more energetic crystal forms of drugs in pharmaceutical preparations. The problem of preventing the conversion of these crystals may be frequently one of inhibiting the growth of more stable and less active forms. Thus, a systematic investigation of the growth rates of crystals may greatly extend our knowledge in these areas.

To study situations mentioned above, the experimental method should be one in which the following requirements are met: The method should be highly sensitive, capable of detecting growth in the order of one micron. The method should be reproducible or, alternatively, statistically significant. This requirement is most conveniently met if the growth of a large number of crystals could be followed at the same time. Finally, the method should be convenient and rapid if reasonable progress is to be expected.

The present report describes a method which meets these requirements. It is highly sensitive, reproducible, and simple. In its present form it might be used, for example, to perform accelerated stability studies and to screen and study potential growth inhibiting agents.

EXPERIMENTAL

General Consideration.—It was decided to select a method which involves following the changes in the distribution of suspension particle sizes with time. A solution suitably supersaturated with a drug could be seeded with crystals of the drug and the size distribution of the crystals determined from time to time with the Coulter counter.[1] If, during the experiment, sedimentation or aggregation of the crystals remains negligible, the changes in the particle sizes may be attributed entirely to growth by molecular transport and deposition.

The steroid, methylprednisolone, was chosen for this study because the polymorphism of this compound has been investigated (2). In the present studies growth from solution of the most stable form of methylprednisolone was followed.

Procedure.—Methylprednisolone[2] was recrystallized from a 50% ethanol-water solution and dried. The crystals were then finely ground by means of a mortar and pestle. An amount of the powder equal to about four times that necessary for saturation was placed in 500 to 1000 ml. of redistilled water in a round-bottom flask. The suspension was stirred vigorously for at least 2 hours at some temperature higher than the 25° employed for the growth studies. In most of the experiments this saturation temperature was around 50° where the solubilities are about twice that at room temperature. After saturation, the suspension was filtered through a 0.45 μ pore-size Millipore[3] filter and the filtrate was placed in a 1 L. glass-stoppered round-bottom flask, ready for seeding.

The seed crystals were prepared and added to the supersaturated solution in the following manner: About 10 ml. of the above prepared solution was placed in a 25-ml. glass-stoppered graduated cylinder. To this was added about 25 mg. of the finely ground drug powder. To promote dispersion, surfactant was added, and the seed suspension was subjected to about 5 minutes of ultrasonic irradiation in a cleaning unit.[4] Then it was allowed to stand undisturbed for about 30 minutes to 2 hours to insure removal of large crystals by sedimentation. After this period, a few tenths of a ml. was pipetted from the uppermost regions of this suspension and added to the supersaturated solution.

The flask containing the seeded solution was then placed in a water bath thermostated at 25.0 ± 0.05°. A Teflon-coated magnetic stirrer activated from below the bath provided gentle agitation of the suspension. Periodically, 50 ml. of the growing

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[1] Marketed by Coulter Industrial Sales, Chicago, Ill.
[2] Kindly supplied to us by Dr. J. W. Shell, The Upjohn Co., Kalamazoo, Mich.
[3] Marketed by Millipore Filter Corporation, Bedford, Mass.
[4] Model DR-125AH, Acoustica Associates, Mineola, N. Y

suspension was pipetted out and mixed with an equal volume of 0.9% sodium chloride solution which had been previously saturated at 25° with methylprednisolone and filtered through the 0.45 μ Millipore filter. This mixing with an electrolyte was necessary for the Coulter counter operation. It also reduced the supersaturation, thus retarding growth during the actual counting.

In all of the present experiments, the 50- and the 100-μ aperture tubes were employed. With these, the 1- to 20-μ diameter (equivalent volume sphere) range of particle sizes could be conveniently studied. The 100-μ aperture tube was calibrated with a suspension of partially aggregated 1.83 μ polystyrene latex particles which had been sized with the 50-μ aperture tube. The latter was calibrated earlier (3, 4) with nonaggregated 1.17 μ and 1.83 μ polystyrene latex particles.

There were some important variations in the above procedure. In most experiments a surfactant was added to the growth solution either during the high temperature saturation period or after filtering of the suspension. In some experiments the suspension equilibrated at high temperatures was not filtered through the Millipore filter but instead through a medium-grade sintered glass filter. In these runs, the solutions were not seeded since it was expected that much of the fine crystals would pass through the filter. However, this procedure was unsatisfactory in that the crystallite population varied greatly from run to run. In another modification of the procedure, about 0.1% of Tween 80 was added to the 0.9% sodium chloride solut on which was used to mix with the aliquot of the growing suspension for counting. The presence of this surfactant apparently helped to further reduce growth during the counting.

Along with the Coulter counter determinations, the solution concentrations of the drug were determined as a function of time. Samples were removed from the growth flask by means of a syringe fitted with a Swinny adapter[5] for a Millipore filter. The solutions were then spectrophotometrically assayed after appropriate dilution in 95% ethanol.

DISCUSSION OF THEORY AND EXPERIMENTS

Change of Particle Size with Time (Theory).—Before we proceed with the presentation of data, let us briefly consider the mathematical relationships which apply to the various models for particle growth. These will be helpful in interpreting the experimental results.

For the growth rate, G_i, of a given particle of radius (equivalent volume sphere), a_i, n the distribution we may write

$$G_i = 4\pi\rho a_i^2 \frac{da_i}{dt} \qquad \text{(Eq. 1)}$$

where t is time and ρ is the density of the solid drug. This may be combined with various rate expressions presented below to give a relationship between a_i and t.

If the growth rate is proportional to the particle radius, then

$$G_i = K_1 a_i \qquad \text{(Eq. 2)}$$

where K_1 is a constant if everything else but a_i is constant. This rate law applies to the important case of the diffusion-controlled growth of fine isometric particles ($\lesssim 10$ μ) under zero to moderate agitation (5). For this special case, K_1 may be expressed as

$$K_1 = 4\pi D\Delta C \qquad \text{(Eq. 3)}$$

where D is the diffusion coefficient of the drug molecule and ΔC is the supersaturation defined as $\Delta C = C - C_o$ where C is the solution concentration of the drug and C_o is the solubility. If Eq. 2 and 1 are combined, we obtain

$$\frac{da_i}{dt} = \frac{K_1}{4\pi\rho a_i} \qquad \text{(Eq. 4)}$$

Thus, according to this equation, the rate of change of the particle radius (or diameter) with time is proportional to the reciprocal of the radius.

Consider now the case in which the growth rate is proportional to the square of the particle radius, *viz.*

$$G_i = K_2 a_i^2 \qquad \text{(Eq. 5)}$$

where again K_2 is a constant if everything but a_i is constant. Equation 5 may apply to the growth of isometric particles in which the rate of growth is controlled by the particle surface area and the probability of growth is the same for all surfaces of the particle. If Eq. 5 is combined with Eq. 1 we obtain

$$\frac{da_i}{dt} = \frac{K_2}{4\pi\rho} \qquad \text{(Eq. 6)}$$

This relationship predicts that the rate of change of a_i with time is constant. As will be seen later, most of the data appear to fit Eq. 6 the best.

The last case we shall present is that in which the growth rate is proportional to the cube of the radius of the equivalent volume sphere. We may write

$$G_i = K_3 a_1^3 \qquad \text{(Eq. 7)}$$

where again K_3 is a constant if a_i is the only variable. To the authors' knowledge, no simple physical counterpart exists for this case. The following case, however, might approximate the cube law. If the particles in the initial distribution are composed of aggregates of relatively uniformly sized primary particles, and if the growth rate is surface-area controlled, then at constant t Eq. 7 might apply since the true surface area would be proportional to the mass in this instance. If Eq. 7 is combined with Eq. 1, we obtain

$$\frac{da_i}{dt} = \frac{K_3 a_i}{4\pi\rho} \qquad \text{(Eq. 8)}$$

Thus, for this case the rate of change in a_i is proportional to a_i.

Experimental Results.—The results of experiments under a number of different conditions are presented in Figs. 1–5. These are illustrative of the method. In each, both the Coulter counter data and the solution concentrations of the drug are given as a function of time. The counter data are plotted as the cumulative count *vs.* particle size. Note that the derivatives of these plots would be the usual particle-size distribution curves.

[5] Marketed by Millipore Filter Corporation, Bedford, Mass.

In all of these results the time decrease in drug concentration of the solutions agreed with the amount of growth calculated from the counter data. The calculations here involve the use of the following equation for the conservation of matter

$$\int_{a=o}^{\infty} {}^4/_3\pi\rho\, a^3 n(a)\, da = \text{constant} - M$$

where M is the solution concentration of the drug and n (a) is the distribution function and equal to minus twice the slope of the curves in the plots. The integral may be easily evaluated at various times by means of conventional numerical methods (6) and compared with the right side of this equation. The data given in Fig. 1 may be used to best illustrate this, since in this instance, appreciable solution depletion occurred. This mass balance is a confirmation of the validity of the method.

In cases where all of the initial seed particles had grown beyond the lower size limit of the counter, plateaus or near-plateaus appeared in the plots of counts *vs.* size (see Figs. 1 and 5). This again demonstrated that growth is the main contribution to the time change in the particle size distribution. If, say, aggregation were to be important in the early stages of the experiment, the development of a plateau would be highly unlikely. Instead, the slope of the plots would be expected to steadily increase with decreasing particle diameter.

In those runs in which plateaus appeared, the levels of the plateaus remained constant for an appreciable length of time. For example, in the 0.025% Tergitol 4 case (Fig. 1) the level of the plateau remained constant to a few per cent for about 2 hours during which rapid growth took place. However, after this period, the plateau level began to fall slowly, then more rapidly. This suggested that when the number of relatively large particles reached some level, aggregation or wall losses became important. This drop-off of the plateau level occurred in all experiments whenever the number of large particles became great.

Negligible aggregation and wall losses in the absence of large particles was also demonstrated by an experiment identical to that shown in Fig. 1, except that the supersaturation was about 0.03

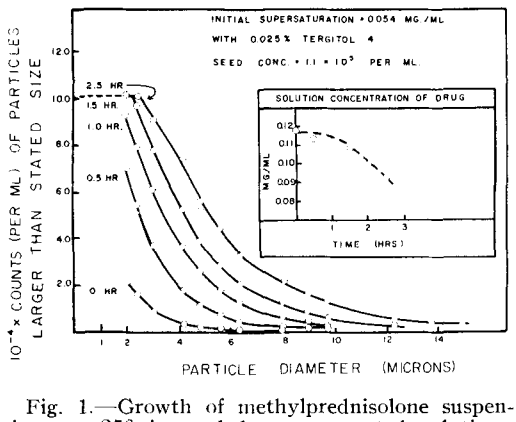

Fig. 1.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

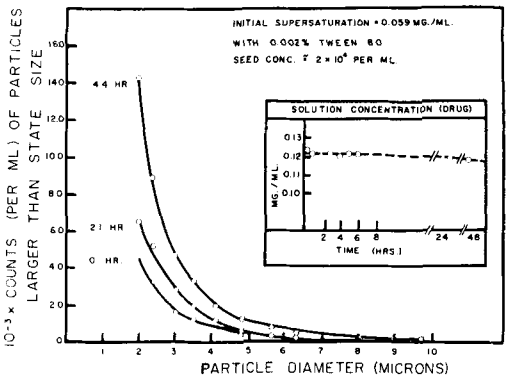

Fig. 2.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

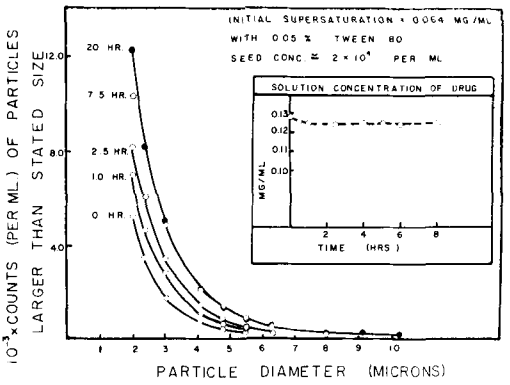

Fig. 3.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

mg./ml. The growth rate was found to be negligible in this case and both the particle concentration and the distribution of sizes remained unchanged for the duration of the experiment (about 5 hr.). Thus, aggregation or wall losses was absent when large particles were absent.

Let us now direct our attention to the actual rates of growth and the rather interesting effects of small amounts of surfactants. When no surfactant was employed (see Fig. 5) the rate of growth was most rapid. In fact, from the data given in Fig. 5, the rate was found to be within a factor of approximately three of the diffusion controlled rate predicted by Eqs. 3 and 4 and with $D = 5 \times 10^{-6}$ cm.^{2} sec.$^{-1}$ for methylprednisolone. The addition of one drop of Tween 80 (see Fig. 2) reduced the rate of growth by about 500-fold. At somewhat higher Tween 80 concentrations (see Fig. 3) there appeared to be an initial period of growth; this was followed by a great reduction in the rate. In the presence of 0.05% Triton X-100, similar effects were observed (Fig. 4). Tergitol 4, an anionic surfactant, did not reduce the rate (Fig. 1) as markedly as the nonionics. Comparable amounts of sodium lauryl sul-

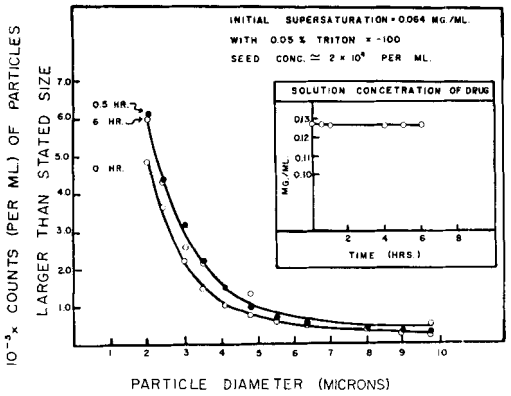

Fig. 4.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

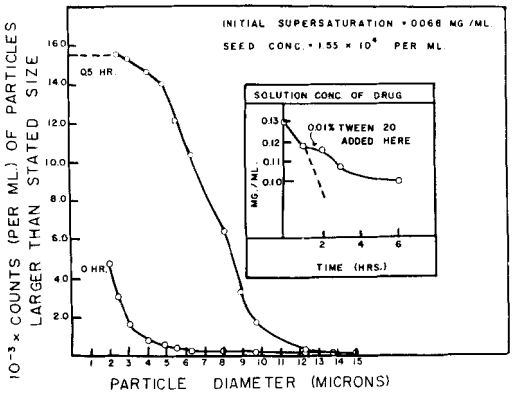

Fig. 5.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

fate elicited effects similar to Tergitol 4. Solubility studies carried out showed that none of these surfactants increased the solubility of methylprednisolone by more than 0.01 mg./ml. at the concentrations used in these studies. Thus, these effects are not due to reduction in the driving force for growth but probably due to the creation of some kind of an interfacial barrier.

In most instances, particularly for those cases in which the growth rates were relatively small, the rate law given by Eq. 6 appeared to be approximately obeyed. In terms of the plots of the cumulative counts *vs.* particle size, conformation of the data to Eq. 6 would mean that the curves would be displaced to the right at a constant rate with respect to time. The results shown in Figs. 2 and 3 appear to do just that. This suggests strongly that in these cases the growth rates are proportional to the surface area of the particles.

In the experiments with Tergitol 4, it was observed that, while a given ordinate value moved constantly with time to the right, the upper portions of the curves moved more slowly than the lower. For reasons mentioned earlier, this was probably not due to aggregation of the particles. This type of behavior appears to be analogous to the theoretical case leading to Eq. 8. At any given time t the smaller particles appear to be growing more slowly than the larger ones. Further studies are necessary to establish the situation unambiguously for this type of growth behavior.

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Control of Urine pH and Its Effect on Sulfaethidole Excretion in Humans

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A technique is described whereby urine can be excreted by human subjects, at a pH near 5.0 or 8.0, with a variation of not more than 0.1 or 0.2 pH unit over periods of at least 15 hours. The significance of controlled urinary pH in humans with respect to the excretion kinetics of a weak acid, sulfaethidole, has been determined. The harmonic mean $t_{1/2}$ for SETD at a controlled urinary pH of 5.0 is 11.4 hours; the $t_{1/2}$ for this drug at a controlled urinary pH of 8.0 is 4.2 hours.

THIS STUDY was undertaken to establish a technique whereby urine would be voided at a constant pH over a protracted time. Using this technique, the elimination of sulfaethidole (SETD) from human subjects was studied to determine the influence of controlled urinary pH on its excretion kinetics. That the elimination processes of some drugs are influenced by administration of agents which affect the acidity or alkalinity of the urine is well known. For example, Smith, Gleason, Stoll, and Ogorzalek (1) and others (2–6) have demonstrated that renal

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