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Effects of Agitation on Size Distribution of Particulate Matter in Large-Volume Parenterals

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Abstract □ The particle-size distributions of six types of large-volume parenterals subjected to different degrees of agitation were determined using an automatic particle counter. Data acquired from each solution, which had been maintained in a stored condition, subjected to agitation by inverting 20 times, and then mechanically shaken for 30 min, produced a linear relationship between log $N_{>D}$ and log D. Both the slope (K) and the number of particles per milliliter exceeding 1 μm in diameter ($N_{>1}$) exhibited a dependence on the degree of agitation. Their combined effect indicates that agitation by 20 hand inversions removed particulate matter from the surface of the container, which increased the total number of particles in solution (>1 μm) but did not significantly alter the relative size distribution. Agitation for 30 min, however, disintegrated agglomerates and produced a particle-size distribution with a greatly increased number of particles whose diameters were less than 1 μm and a corresponding decrease in the number of particles exceeding 1 μm in diameter. The particle-size distribution of a parenteral solution determined by this *in situ* instrumental method was, therefore, dependent upon the degree of agitation to which the parenteral was subjected prior to examination.

Keyphrases □ Particle-size distribution—various large-volume parenterals, effect of different degrees of agitation □ Parenterals, large volume—particle-size distribution, effect of different degrees of agitation □ Dosage forms—various large-volume parenterals, particle-size distribution, effect of different degrees of agitation □ Agitation—effect on particle-size distribution of various large-volume parenterals

Particulate matter was defined as "extraneous, mobile, undissolved substances, other than gas bubbles, unintentionally present in parenteral solutions" (1). In a previous report, efforts to compare and contrast the ability of five methods to monitor the levels of particulate matter were described (2). During this investigation, it became apparent that the automatic particle counter was suitable for the rapid determination and sizing of particulate matter. This instrument's ability to provide a reasonably accurate determination of the particle-size distributions present in large-volume parenterals was comparable[1] to the membrane filtration and microscopic examination technique utilized in the recently adopted USP–NF standard (1).

Some difficulties previously were noted in determining the actual levels of particulate matter of a given size present in parenterals. Ernerot *et al.* (3), who utilized a destructive counting procedure, reported that the particle-size distribution in a parenteral solution at a given time was largely dependent upon the degree of agitation the solution had previously received. By its very nature, a destructive counting technique introduces a certain amount of shear force upon the particles in the solution, thereby altering the initial distribution of particles. This shear force introduced by the measuring technique itself would be eliminated by a nondestructive technique, since it would not be necessary to remove the solution from its container. Therefore, a nondestructive *in situ* technique that does not impose any additional shear force on the particles in a solution would be ideally suited for characterizing the effects of agitation on particle-size distributions in parenteral solutions.

Some recent reports gave conflicting accounts of the importance of the effects of agitation. Groves (4) attributed the increase in particle counts observed (3) to the presence of air bubbles generated during the agitation procedure. Ernerot (5) refuted this argument and identified the disintegration and flocculation of particles as the true sources of the variation in particle counts following agitation. Blaug and Sarabia (6) noted that the particle counts recorded using an electronic particle counter were the same for samples taken from intravenous bottles at rest or from the same bottles shaken immediately prior to sampling. Since destructive particle counting techniques were used in these investigations, it is difficult to draw definitive conclusions regarding the actual effects of agitation. Therefore, a major goal of this study was to examine more definitively the effects of agitation on the particle-size distribution in large-volume parenterals utilizing a nondestructive instrumental technique.

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Table I—Effect of Agitation on the Average Size Distribution Characteristics of Large-Volume Parenteral Solutions

Type of Solution	Degree of Agitation	Average[a] Number of Particles per Milliliter >1 μm	Average Slope (K) of log–log Plot ± SE
Dextrose, 5% in half-strength normal saline[b]	Storage	235.5	−3.1445 ± 0.8328
	20 inversions	495.2	−2.9704 ± 0.1111
	30-min shake	336.1	−3.2988 ± 0.8381
Normal saline[c]	Storage	559.5	−3.3050 ± 0.4240
	20 inversions	686.6	−2.7139 ± 0.4143
	30-min shake	454.5	−3.7144 ± 1.0690
Dextrose, 5% in multiple electrolyte solution[d]	Storage	1022.4	−3.1047 ± 0.7970
	20 inversions	1710.1	−2.4779 ± 0.5774
	30-min shake	867.4	−3.1315 ± 0.9748
Dextrose, 5% in normal saline[e]	Storage	838.0	−3.1239 ± 0.1581
	20 inversions	784.2	−3.5116 ± 0.2869
	30-min shake	808.5	−3.8292 ± 0.2384
Dextrose, 5% in water[f]	Storage	869.0	−2.8606 ± 0.4505
	20 inversions	1090.0	−2.8929 ± 0.3545
	30-min shake	1025.2	−3.0351 ± 0.5778
Dextrose, 5% in lactated Ringer's solution[g]	Storage	590.5	−2.7193 ± 0.2445
	20 inversions	813.5	−2.9037 ± 0.2166
	30-min shake	737.8	−2.9229 ± 0.4822

[a] Average of three bottles. [b] Lot A4N265A. [c] Lot A5A130B. [d] Dextrose, 5% in Isolyte M, Lot A5E302C. [e] Lot A5C047C. [f] Lot A5C239B. [g] Lot A5E483B.

EXPERIMENTAL

Large-volume parenteral solutions[2] (1000 ml) were inspected using an automatic particle counter[3]. The six types of commercially available solutions examined were chosen on the basis of their extensive use in clinical practice (Table I).

The particle counter utilized is capable of detecting particles as small as 1 μm in diameter. Solutions were examined by the automatic particle counter in the manner previously described (2). The number of particles per milliliter exceeding the following diameters was determined: 1.000, 1.259, 1.585, 1.995, 2.512, 3.162, 3.981, 5.012, and 6.310 μm. These diameters were chosen to facilitate data analysis since their logarithms are equally spaced. Starting at 1 μm, particle counts were recorded at successively increasing threshold settings until a given setting produced 10 readings whose average was less than 10 particles/ml. This value was chosen to ensure statistical accuracy and to limit the instrumental error resulting from the fact that this instrument displays the truncated, rather than the rounded, version of the number of particles counted (2).

Triplicate samples of each of the six types of large-volume parenterals, stored in an undisturbed condition for 65 days, were gently placed in position on the instrument for counting. After completion of the readings on the stored solutions, each bottle was agitated by being inverted, end-over-end, 20 times. This degree of agitation was chosen to conform with the degree of agitation used in the recently instituted compendial standard technique (1). These inversions were performed by the same individual to obtain a uniform degree of agitation from bottle to bottle.

Upon completion of this set of readings, each bottle was then shaken for 30 min at 140 excursions/min on a mechanical agitator[4]. After each agitation procedure, the bottles sat undisturbed on the measuring platform of the instrument for a *minimum* of 45–60 sec before any readings were recorded. This time period was reported to be adequate to ensure that air bubbles introduced even under "severe" agitation conditions would not be counted by this instrument (7, 8). The present data support these observations.

RESULTS AND DISCUSSION

The particle-size distributions in parenteral solutions often can be described[1] by a straight-line equation of the form:

$$\log N_{>D} = K \log D + \log N_{>1} \qquad \text{(Eq. 1)}$$

where $N_{>D}$ is the number of particles per milliliter with a diameter larger than D, $N_{>1}$ is the number of particles per milliliter with a diameter larger than 1 μm, D is the particle diameter in micrometers, and K is equal to the slope of a plot of log $N_{>D}$ *versus* log D. This equation was used to characterize the particle-size distributions of the parenteral solutions to determine the effects of agitation on particle-size distributions.

The effects of different degrees of agitation on the measured particle counts in a typical solution are shown in Table II; only the particle counts with diameters greater than 1.000, 1.585, and 2.512 μm are represented. Values for K, $N_{>1}$, and correlation coefficients were calculated from *all* results, provided that at least 10 particles/ml exceeding a given diameter were counted.

The data in Table II for the stored solutions illustrate how the particle-size distribution characteristics varied considerably among individual samples, as previously noted[1]. In addition, the effects of agitation on the measured particle-size distributions varied from sample to sample. For example, 20 inversions of a stored solution caused the particles counted, exceeding 1 μm in diameter, to *decrease* in Solutions C and K and to *increase* in Solution G relative to the stored condition. When these same solutions were subsequently shaken for 30 min, $N_{>1}$ *decreased* for Solutions C and G and *increased* for Solution K relative to the condition in these same solutions following 20 hand inversions.

The effects of these different degrees of agitation on the slopes of the log–log plots also varied between individual samples. For example, 20 inversions of a stored solution *increased* the absolute magnitude of the slopes for Solutions C and K relative to the stored condition, but that for Solution G *decreased*. When these same solutions were subjected to 30 min of shaking, the slopes for Solutions G and K *increased* (*i.e.*, became steeper) while the slope for Solution C *decreased* relative to the condition in these same solutions following 20 hand inversions. A close adherence to Eq. 1 (indicative of a linear relationship between log $N_{>D}$ and log D) is apparent for all three individual solutions shown in Table II regardless of the degree of agitation since the poorest correlation coefficient is −0.9815.

Due to the variable effects of agitation on particle-size distributions among individual solutions (Table II), the data for each solution type were averaged. The averaging of triplicate samples of a given type of solution facilitated the discrimination of the effects of agitation on their particle-size distributions. The data in Table I clearly indicate certain trends. First, the average number of particles per milliliter exceeding 1.000 μm in diameter *increased* upon 20 inversions of a stored solution and then *decreased* when the solution was subsequently shaken for 30 min. Only the 5% dextrose in normal saline solution deviated from this trend. However, individual samples of 5% dextrose in normal saline exhibited some of these trends (Table II). Second, the average slopes of the log–log plots varied throughout the six solution types. Upon 20 inversions of the stored solutions, the slopes for three solution types *increased* while three decreased. However, the subsequent 30 min of shaking resulted in an *increase* in all slopes of the log–log plots.

Finally, the correlation coefficients between log $N_{>D}$ and log D for any type of solution were very high, regardless of the degree of agitation, and ranged from −0.9257 to −0.9998. These high correlation coefficients show that neither the type of solution nor the degree of agitation had any significant effect upon the linearity of the log–log plots. These observations differ from those of Ernerot (5) who reported a bend in the log–log plots of the particle-size distributions of stored parenterals.

Since these results and those in another study[1] indicated that the effects of agitation upon the particle-size distributions and the particle-size distributions themselves were independent of the type of solution, the data were analyzed without regard to solution type. When the 18 stored

[2] McGaw Laboratories, Glendale, CA 91201.
[3] Prototron, model ILI 1000, Spectrex Corp., Redwood City, CA 94063.
[4] Eberbach model 6000, Eberbach Corp., Ann Arbor, MI 48106.

Table II—Effect of Agitation on the Size Distribution Characteristics of Individual Large-Volume Parenteral Solutions

Dextrose, 5% in Normal Saline Solutions[a]	Degree of Agitation	Number[b] of Particles per Milliliter			Slope (K) of log–log Plot	Correlation Coefficient between log $N_{>D}$ and log D
		>1.000 μm	>1.585 μm	>2.512 μm		
Sample C	Storage	599.3	122.9	<10	−3.2137	−0.9998
	20 inversions	524.5	147.9	<10	−4.0791	−0.9939
	30-min shake	444.8	141.9	<10	−3.4268	−0.9963
Sample G	Storage	1105.1	150.6	65.5	−3.3415	−0.9815
	20 inversions	1158.5	274.9	<10	−3.3020	−0.9946
	30-min shake	1123.3	121.3	23.4	−4.2520	−0.9948
Sample K	Storage	968.6	300.5	55.6	−2.8165	−0.9985
	20 inversions	828.7	165.9	43.7	−3.1538	−0.9981
	30-min shake	1126.9	151.6	41.6	−3.8088	−0.9982

[a] Lot A5C047C. [b] Average of 10 readings.

samples were inverted 20 times, the slopes of the log–log plots of nine of the samples *increased* while the other nine *decreased*. The values of the individual slopes were analyzed by the Student t-test, and no significant difference ($p > 0.5$) was observed between the slopes corresponding to these two degrees of agitation. When these bottles that had been inverted 20 times were subjected to an additional 30 min of shaking, 13 of the 18 individual slopes *increased*. This increase was significant ($p < 0.01$) by the Student t-test.

A similar analysis of the number of particles exceeding 1 μm in diameter indicated that, when a stored solution was inverted 20 times, 15 of the 18 individual intercepts (log $N_{>1}$) *increased;* the subsequent 30 min of shaking caused 12 of the 18 individual intercepts to *decrease*. A Student t-test revealed that these trends were significant at the $p < 0.005$ and $p < 0.05$ levels, respectively.

Figure 1 illustrates the effects of agitation on K and $N_{>1}$. Since the particle-size distribution was independent of the type of solution, the data in Fig. 1 represent the average values of these two parameters for the 18 solutions.

When a stored solution was inverted 20 times, two effects were evident (Fig. 1). As previously noted, there was no statistically significant change in the slope of the log–log plot whereas the intercept *increased* significantly. These simultaneous effects can be interpreted to mean that there is an increase in the number of particles at all size ranges measured. The origin of these additional particles is most likely particles that are dislodged from the inner surface of the container (9) by the relatively mild agitation produced by 20 inversions (10).

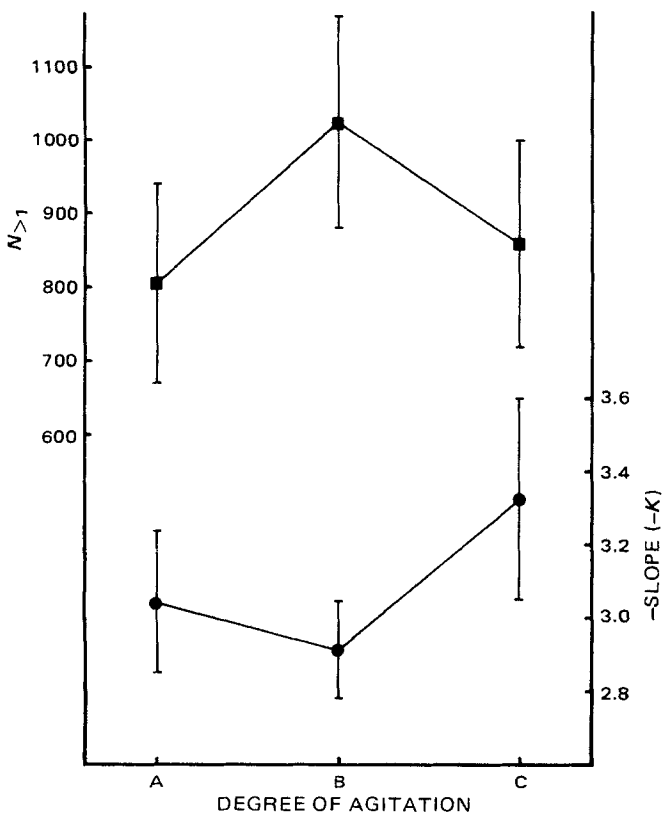

Figure 1—*Effects of degree of agitation on the slopes and intercepts of the log–log plots. Key: A, storage for 65 days; B, 20 hand inversions; and C, 30-min shake.*

However, Ernerot *et al.* (3) previously noted that one effect of agitation on a parenteral solution was a disintegration of large agglomerates into smaller sized particles. This effect would result in an increase in the intercept and a steeper slope. Since no significant increase in the slope was observed at this degree of agitation, the predominant effect appeared to be the dislodging of particles from the surface of the container whose size distribution was similar to those already present in solution. An alternative possibility is that only the larger particles were resuspended by the 20 hand inversions and that the process, in addition to resuspending these particles, also supplied sufficient shear force to disintegrate them into smaller particles whose resulting distribution was similar to the existing distribution.

When the solutions previously subjected to 20 inversions were subsequently shaken for 30 min, there was a significant *increase* in the slope of the log–log plot coupled with a significant *decrease* in the intercept. This decrease in the intercept is obviously indicative of the reduction of the total number of particles greater than 1 μm in diameter. The increased slope of the log–log plot represents a greater reduction in the number of larger sized, relative to smaller sized, particles following 30 min of shaking. A logical explanation for these concurrent effects is that the reduction in the number of larger sized particles is due to their disintegration caused by the high degree of agitation. This agitation, in turn, reduces these smaller particles to a size less than 1 μm in diameter, which is below the minimum size detectable by the automatic particle counter, causing a reduction in the observed intercept value. Since the disintegration of particles is the predominant event at this degree of agitation, the slope increases and the intercept decreases concurrently.

The health-related implications of these observations are difficult to state unequivocally, since the clinical significance of the very presence of particulate matter in a parenteral solution has not been proven conclusively. There is some controversy as to the most harmful size of particles (9), although one would intuitively suspect that a given number of larger sized particles would be potentially more harmful than a like number of smaller particles. The comment by Groves (11) that a container of parenteral fluid should not be subjected to violent shaking prior to injection is therefore somewhat difficult to reconcile. One point that is clear is that particle counting procedures should be carried out under well-controlled and reproducible conditions utilizing measuring techniques that do not impose a shear force upon the particles.

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Actions of Mescaline on Isolated Rat Atria

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Abstract □ Mescaline, in concentrations of 5×10^{-4} and 1×10^{-3} *M*, produced negative chronotropic and positive inotropic responses in isolated, spontaneously beating rat atria. In tissues driven at a constant rate, the inotropic response was diminished greatly, indicating that the increment in the force of contraction was secondary to the reduction in rate. These chronotropic and inotropic responses were not altered consistently by pretreatment with the histamine antagonists chlorpheniramine and metiamide.

Keyphrases □ Mescaline—chronotropic and inotropic effects on isolated rat atria □ Chronotropic effects—mescaline on isolated rat atria □ Inotropic effects—mescaline on isolated rat atria □ Phenethylamines—mescaline, chronotropic and inotropic effects on isolated rat atria □ Cardiovascular effects—mescaline on isolated rat atria □ Psychotomimetics—mescaline, chronotropic and inotropic effects on isolated rat atria

In this study, the effects of mescaline (3,4,5-trimethoxyphenethylamine) on isolated rat atria were examined to reveal its possible actions directly on the pacemaker and atrial tissue. By the use of histamine antagonists, the involvement of histamine receptors (both H_1 and H_2) was examined as a possible mechanism of the *in vitro* cardiac action of mescaline.

BACKGROUND

The cardiovascular actions of the psychotomimetic amine mescaline were reported previously (1–8). Mescaline consistently evoked bradycardia in several species of animals. Mescaline-induced slowing of the frog heart was first observed by Dixon (1); others confirmed this action in the dog (2), rabbit, and cat (3). The bradycardia was inhibited by vagotomy or pretreatment with atropine (3); however, other published data (1, 2, 4) challenged these findings. Most evidence favors the conclusion that mescaline does not produce an effect on the heart by a vagal reflex or cholinergic stimulation.

Competition for epinephrine receptors by mescaline was proposed (5) because both mescaline-induced bradycardia and hypoglycemia in rats were reduced upon pretreatment with epinephrine. In another study (6), mescaline showed no propranolol-like antagonism of isoproterenol-induced relaxation of spontaneously contracting rat uterus. Mescaline failed to antagonize isoproterenol-induced positive chronotropism and hypotension (4) or to reverse an ethylnorepinephrine depressor response (6) in anesthetized dogs. From these data, β-adrenergic receptor blockade by mescaline or one of its metabolites was an unlikely explanation.

Histamine release or direct stimulation of histamine receptors by mescaline might account for the observed bradycardia. Potentiation of the hypotensive response to histamine in rats (7) was attributed to inhibition of the histaminolytic action of diamine oxidase by mescaline. Dogs receiving an intravenous infusion of mescaline showed (4) increased plasma histamine levels and a hypotensive response similar to that produced by compound 48/80, a known histamine-liberating substance (9). Alteration of respiratory dynamics and elevated right ventricular pressure, resembling responses to histamine, were observed in the guinea pig (8). Attempts to block these effects of mescaline with the antihistamine diphenhydramine were unsuccessful (4, 8).

More recent evidence suggested that histamine produced bradycardia by a stimulation of histamine receptor subtypes (H_2-receptors), which were not blocked by conventional antihistaminic agents such as diphenhydramine (10). The possible interaction of mescaline or mescaline-released histamine with H_2-receptors in the myocardium could be evaluated with an H_2-blocker, such as metiamide, in an attempt to confirm or refute this hypothesis.

EXPERIMENTAL

Sprague–Dawley rats of both sexes, 250–400 g, were sacrificed by cervical fracture and the hearts were rapidly removed. Paired atria were sectioned from the ventricles with a bridge of tissue joining the two atria. For examination of inotropic responses, left atria were electrically driven by paired platinum electrodes. Monophasic square wave pulses of 1-msec duration were delivered by a stimulator[1]. Tissues were suspended in a 10-ml glass tissue bath filled with Krebs–Henseleit solution (11) at a constant temperature of 35° and equilibrated with 95% O_2–5% CO_2. The solution pH was adjusted to 7.3.

A constant resting tension of 1.0 g was maintained throughout each experiment. All preparations were allowed to equilibrate for a minimum of 50 min with washes at 10-min intervals prior to drug challenge. Isometric contractions were measured with a force-displacement transducer[2] and recorded on a polygraph[3]. A linear tachometer[4] was used to record changes in heart rate. All drug solutions were prepared daily in Krebs–Henseleit solution and expressed as molar concentration of base.

Dose–Response Curves with Mescaline—To observe chronotropic and inotropic responses to mescaline, concentrations of 1×10^{-4}, 5×10^{-4}, and 1×10^{-3} *M* mescaline base were generated in a tissue bath containing spontaneously beating, paired atria. Heart rate and developed tension were recorded as maximum responses within 5 min. To minimize possible tachyphylaxis, only two drug challenges per preparation were employed. The challenges were separated by three washes and a 15-min reequilibration period. Sixteen atrial preparations were tested in this manner. All data were reported as the percent change from the pretreatment period and analyzed by Duncan's New Multiple Range Test (12).

The effects of mescaline were studied on separated left and right atria of the same heart to determine differences in inotropic responses in both the presence and absence of chronotropic responses. Right atria were allowed to contract spontaneously, while left atria were electrically driven (150% threshold voltage). After a 1-hr equilibration period, the rate of electrical stimulation was adjusted to equal the heart rate of the spontaneously contracting right atrium. At 5 min after the addition of mescaline to the tissue bath, changes in the developed tension for both atria

[1] Grass model S48.
[2] Grass model FT-03.
[3] Grass model 7B.
[4] Grass model 7P4-D.