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Parameter for Assessing Parenteral Cleanliness Based on Particle-Size Distributions

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Abstract □ A new parameter for assessing the particulate matter content of large-volume parenteral solutions was developed and tested. Some problems and shortcomings associated with previously proposed standards are discussed, together with the potential advantages of employing the proposed parameter. Cleanliness factors were compared with another parameter and were less susceptible to changes resulting from the method of measurement utilized and the premeasurement conditions encountered by the solution. The use of these cleanliness factors in conjunction with an automatic particle counter is proposed as a worthwhile supplement to the USP–NF standard for monitoring the quality of large-volume parenteral solutions.

Keyphrases □ Particle-size distributions—characterized in large-volume parenteral solutions, cleanliness factor developed □ Parenterals, large volume—particle-size distributions characterized, cleanliness factor developed □ Dosage forms—large-volume parenterals, particle-size distributions characterized, cleanliness factor developed

Particulate matter is defined as "extraneous, mobile, undissolved substances other than gas bubbles, unintentionally present in parenteral solutions" (1) and has been long recognized as a problem. Recently, official standards specified the allowable levels of particulate contamination (1, 2).

To aid in the evaluation of the cleanliness[1] of a parenteral solution, an auxiliary parameter that would describe accurately the contamination level is needed. Several desirable characteristics of this parameter were described previously (3, 4); it should provide a true measurement of particulate cleanliness that is not affected by the premeasurement handling conditions, and it should indicate the correct degree of particulate cleanliness using various instrumental techniques. The method also should be rapid, nondestructive, nonsubjective, simple, inexpensive, easy to standardize, and, preferably, not require the use of a clean room.

The present study was undertaken to develop such a parameter for the objective assessment of the relative particulate cleanliness of parenteral solutions.

BACKGROUND

Single-Point Standards—One early standard was proposed in 1966 (5). The initial proposal was revised and formed the basis of the first provisional Australian standard (6), which stated that a parenteral solution should not contain more than 250 particles/ml exceeding 3.5 μm in diameter. In the same year, another suggested single-point standard stated that a parenteral solution should not contain more than 50 particles/ml exceeding 5 μm in diameter (7).

Single-point standards are validly subject to criticism on the basis that the particles counted exceeding a given diameter are not necessarily indicative of the number of particles exceeding another diameter. Single-point standards were based on the observation that log–log plots of $N_{>D}$ (the particle concentration exceeding the diameter, D) *versus* D (the particle diameter) were essentially linear and parallel to one another among the individual solutions examined (5). However the slopes of the log–log plots vary among individual solutions[2] (6, 8), thereby invalidating the use of single-point standards.

Multiple-Point Standards—Recent attempts to establish standards focused on multiple-point determinations. As Kendall (9) noted, a standard should ideally be based upon the determination of the particle-size distribution over a broad size range. This consideration formed the basis for an Australian standard proposed in 1966 (5), which limited the allowable levels of particles exceeding four particle diameters. This and other recently proposed multiple-point standards are shown in Table I.

Multiple-point standards (as well as single-point standards) can be criticized on the grounds that the particle-size distribution may vary with the degree of agitation to which the solution is subjected (8, 10–12). Another potential criticism is that the measurement of particle concentrations may not be obtainable using a single technique (*i.e.*, instrumental, microscopic, *etc.*). If the particle diameters specified in the standard require measurement by more than one technique, the counts provided by each technique would have to correlate extremely well with one another. Besides introducing unnecessary uncertainty, this requirement would necessitate proficiency in more than one technique.

The USP–NF standard (Table I) suggests the use of a membrane filtration and microscopic examination technique. In a practical sense, it is only suitable for counting particles larger than 10 μm in diameter (3, 6). The Australian and British Pharmacopoeia standards do not prescribe a specific measuring technique. However, an electrical resistance counter or a light-scattering or light-blocking device is required, since these standards specify the counting of particles smaller than 10 μm in diameter. A recent version of the Australian standard (Table I), soon to become effective[3], specifies the use of an instrumental particle counter operating

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[1] Throughout this article, the term "cleanliness" is used to denote the level of particulate matter.

[2] J. Blanchard, J. A. Schwartz, and D. M. Byrne, *J. Pharm. Sci.*, in press.

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Table I—Some Standards for Particulate Contamination Levels

Standard Test	Maximum Allowable Number of Particles per Milliliter of Various Sizes					
	2.0 μm	3.5 μm	5 μm	10 μm	20 μm	25 μm
USP-NF	—	—	—	50	—	5
Australian I[a]	1000	250	100	25	—	—
Australian II[b]	—	—	100	—	2	—
BP$_1$[c]	2000	—	200	—	—	—
BP$_5$[c]	100	—	100	—	—	—

[a] See Ref. 3. [b] See footnote 2. [c] The BP$_1$ test refers to readings taken on one container, whereas the BP$_5$ test refers to the average reading of five containers.

on the light-blockage principle and the counting of particles exceeding two diameters.

Complex Standards—Ernerot (13) suggested the silting index determination as an alternative to particle counting for evaluating particulate contamination in large-volume parenterals. However, this method is not simple, either in theory or practice. While it offers certain advantages over other methods, it appears to be far from ideal because of the numerous assumptions involved. Further definitive studies might resolve some of these uncertainties.

To alleviate some shortcomings of the single- and multiple-point standards, the particle-size distribution of the contaminants should be examined over as broad a size range as practical to ascertain the correct relationship describing the particle-size distribution. In previous studies[2] (3, 12), many samples (both individually and when averaged) closely adhered to a linear 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 the plot of log $N_{>D}$ versus log D. This equation previously was utilized to describe the particle-size distribution of contaminants in parenterals (6). Recently (12), the effects of agitation on the particle-size distribution of particulate contamination of parenterals were determined. An unequivocal characterization of the effects of agitation on the particle-size distribution of a parenteral solution must be obtained by simultaneously examining both parameters that describe the distribution (i.e., the slope and the intercept of the log $N_{>D}$ versus log D plot). A single parameter based upon the slope and the intercept of the log–log plot would specify accurately the degree of cleanliness of a parenteral solution.

EXPERIMENTAL

The particle-size distribution data of large-volume parenteral solutions[4] (1000 ml) were obtained by two methods: a nondestructive instrumental technique, using an automatic particle counter[5], and a membrane filtration and microscopic method similar to the USP–NF technique, which is a destructive method. The exact procedures followed and the method of data analysis are described elsewhere[2] (3, 12).

The six types of parenteral solutions examined were: (a) 5% dextrose in 0.45% sodium chloride, (b) normal saline, (c) 5% dextrose in multiple-electrolyte solution[6], (d) 5% dextrose in normal saline, (e) 5% dextrose in water, and (f) 5% dextrose in lactated Ringer's solution. These solutions were chosen on the basis of their extensive clinical use.

RESULTS AND DISCUSSION

Groves (6) attempted to relate the slopes and intercepts of the log $N_{>D}$ versus log D plots, which characterize the particle-size distribution of parenteral solutions, to their relative cleanliness. The form of Groves' postulated relationship is:

$$\frac{\log N_{>1} - 2.5}{-K} = \text{specific value } (S) \qquad \text{(Eq. 2)}$$

The S value was based upon the empirical observation that most parenteral solutions examined that passed a provisional Australian
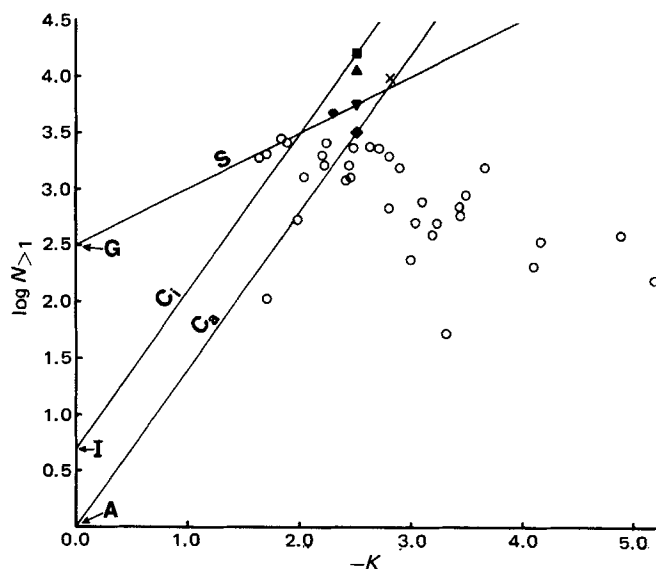
Figure 1—Relationship between cleanliness factors and official standards. Key: ■, USP–NF standard; ♦, fivefold reduction of USP–NF standard; ▲, BP$_1$ standard; ▼, BP$_5$ standard; ♥, Australian I standard; ×, Australian II standard; and ○, individual parenteral solutions. Points represent values of the slopes and intercepts of the particle-size distributions determined by a least-squares fitting procedure of the log–log plots of N$_{>D}$ versus D.

standard ($+$250 particles/ml > 3.5 μm) would lie below an arbitrarily drawn line (S) having a slope of 0.5 and a y intercept (point G) of 2.5 when log $N_{>1}$ was plotted versus $-K$. Conversely, most solutions that failed this standard would lie above the line. This line (S) in Fig. 1 represents a family of straight lines on the log–log plot of $N_{>D}$ versus D (Fig. 2), all passing through the common point S' corresponding to 316 particles/ml >3.16 μm. Due to the arbitrary manner in which Groves admittedly developed the S value and because some solutions examined in this study would have failed the Australian standard and yet passed the S value, and vice versa, the suitability of using another parameter to assess parenteral cleanliness was investigated.

The recently developed USP–NF standard (Table I) was examined in this context. If the values listed in this standard exhibit the linear relationship described by Eq. 1, then the slope and the y intercept of this line shown in Fig. 2 are -2.5126 and 16,280 (log $N_{>1}$ = 4.2116), respectively. These values are the coordinates of the point shown as the filled square (■) in Fig. 1. This point (■) was plotted and connected to point I, which represents a hypothetical particle-size distribution such that the log–log plot of $N_{>D}$ versus D (Fig. 2) has a slope of zero and a y intercept of 5 (log $N_{>1}$ = 0.6990). The resultant line, C_i, in Fig. 1 can be described by:

$$C_i = \frac{\log N_{>1} - 0.6990}{-K} \qquad \text{(Eq. 3)}$$

This line represents a family of straight lines on the log–log plot of $N_{>D}$ versus D, all passing through the common point C_i' in Fig. 2 corresponding to five particles/ml >25 μm. This point was selected since it is representative of the USP–NF standard.

Practically all commercially manufactured solutions examined would have passed a more stringent standard (3). Since the most contaminated of these solutions contained approximately one-fifth of the level of particulate matter allowed by the USP–NF standard, the possibility of using a cleanliness factor based upon a fivefold reduction of this standard was investigated. A more stringent standard for evaluating parenteral cleanliness based upon averaged data is currently used in the BP (Table I and Fig. 2). This standard specifies a twofold reduction in allowable particles when averaging data from five solutions in contrast to data from individual solutions. Increased stringency for evaluating parenteral cleanliness when averaging data is reasonable, since the mean of a set of samples exhibits much less variability than individual samples.

When this fivefold reduction of the USP–NF standard was plotted in Fig. 2, the slope and the y intercept of the line were -2.5126 and 3255 (log $N_{>1}$ = 3.5126), respectively. These values are the coordinates of the point (♦) plotted in Fig. 1 and connected to point A, which represents a hypothetical particle-size distribution in Fig. 2 with a slope of zero and a

Table II—Effect of Method of Measurement on the Cleanliness Factors

Cleanliness Factor $(CF) \pm SE^a$	Critical Value	Method of Measurement		Differences in Cleanliness Factor, %
		Microscope	Prototron	
$\dfrac{\log N_{>1}}{-K} = C_a$	1.3979	0.8905 ± 0.0456	1.1354 ± 0.0742	27.5
$\dfrac{\log N_{>1} - 0.6990}{-K} = C_i$	1.3979	0.5582 ± 0.0571	0.8694 ± 0.0619	55.8
$\dfrac{\log N_{>1} - 2.5}{-K} = S$	0.5000	-0.2978 ± 0.0913	0.1842 ± 0.0349	161.9

^{a} Although the parameters C_i and S were developed to evaluate individual solutions, the data shown were based upon an average of 36 individually calculated cleanliness factors to demonstrate clearly the overall effects of the method of measurement.

y intercept of 1 ($\log N_{>1} = 0$). Line C_a in Fig. 1 can be described by:

$$C_a = \frac{\log N_{>1}}{-K} \qquad \text{(Eq. 4)}$$

This line represents a family of straight lines on the log–log plot of $N_{>D}$ versus D, all passing through the common point C_a' in Fig. 2 corresponding to one particle/ml >25 μm. It is proposed that this cleanliness factor, C_a, be used to evaluate the cleanliness of parenterals based upon averaged data for several solutions.

Equations 3 and 4 describe the two lines shown in Fig. 1 denoted by C_i and C_a, respectively, whose slopes are both numerically equal to 1.3979. This number represents the critical value, i.e., the maximum allowable value of the cleanliness factors. Therefore, any individual solution whose C_i exceeds 1.3979 or a representative sample of a batch of solutions whose C_a exceeds this critical value would fail the respective standard. Effectively, this approach means that any line joining point I in Fig. 1 with a point depicting an individual parenteral solution, or point A with a point depicting the average of several parenteral solutions, whose slope exceeds that of the C_i or C_a lines would fail the respective standard. Since the relative distribution of particles is largely independent of the composition of the solution (3, 10), the cleanliness factors for the solutions were averaged without regard to solution type.
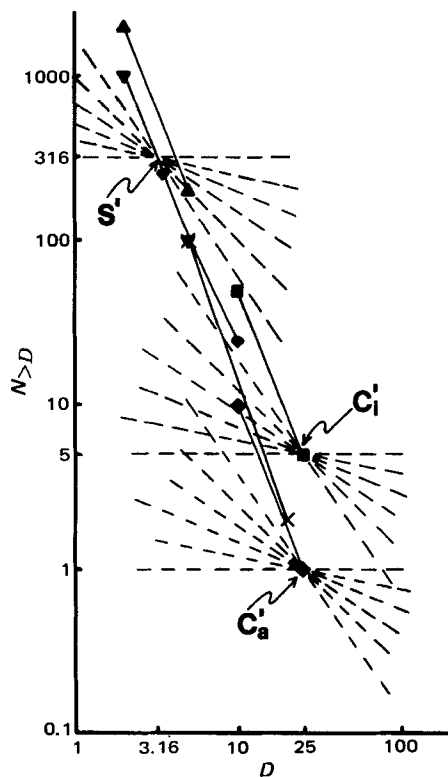

Figure 2—*Particle-size distributions of the official standards and of the critical values of the cleanliness factors. Key: -■—■-, USP–NF standard; -♦—♦-, fivefold reduction of USP–NF standard; -▲—▲-, BP₁ standard; -▼—▼-, BP₅ standard; -♥—♥-, Australian I standard; -✕—✕-, Australian II standard; and - - -, particle-size distribution of the critical value of the respective cleanliness factor.*

The cleanliness factors were calculated as follows. A particle-size distribution for a given solution was obtained by measuring the particle counts exceeding several diameters (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. Equation 1 was fitted to the data points by means of a least-squares procedure. The slope (K) and the intercept ($\log N_{>1}$) were determined and used to calculate the cleanliness factors described by Eqs. 2–4. The cleanliness factors were then compared with the critical values of the respective standards to determine whether the solution(s) examined passed or failed.

As previously noted, one desirable feature of a parameter for assessing parenteral cleanliness is that it should be relatively independent of the method of measurement. Values for the two cleanliness factors, CF, proposed here (i.e., C_i and C_a) were calculated using both microscopic and instrumental counting data based upon an average of 36 samples (Table II). The percentage differences in the CF values due to the measuring techniques employed varied considerably and were calculated as follows:

$$\% \text{ difference} = \left| \frac{CF^I - CF^M}{CF^M} \right| \times 100 \qquad \text{(Eq. 5)}$$

where CF^I and CF^M refer to the CF values based upon data obtained using the instrumental and microscopic methods of measurement, respectively. This method of calculation was used since it was desirable to compare differences resulting from the method of measurement with the accepted compendial (microscopic) technique (1). Both C_i and C_a clearly exhibited much lower percentage differences resulting from a change in the method of measurement than did Groves' S value.

These observations, together with the standard error values reported in Table II, clearly indicate that the cleanliness factor utilized by Groves is much more susceptible to variations resulting from different methods of measurement than either C_i or C_a. Also, all cleanliness factors calculated for the average of 36 solutions were less than their respective critical value. Thus, the data shown in Table II are indicative of solutions whose average cleanliness factor value would have been passed by all three parameters (S, C_i, and C_a) using either microscopic or instrumental counting techniques.

Another consideration in selecting a cleanliness factor is that it should be relatively independent of the conditions to which the parenteral is subjected prior to measurement (8, 10, 12). Table III shows the effect of various degrees of agitation on the three cleanliness factors based upon averaged data for 18 parenteral solutions. The percentage change in the three CF values resulting from different degrees of agitation also varied considerably and was calculated as follows:

$$\% \text{ change} = \left| \frac{CF^A - CF^B}{CF^B} \right| \times 100 \qquad \text{(Eq. 6a)}$$

or:

$$\% \text{ change} = \left| \frac{CF^C - CF^B}{CF^B} \right| \times 100 \qquad \text{(Eq. 6b)}$$

where A refers to solutions stored in an undisturbed condition for 65 days, B refers to readings taken after these same solutions were inverted 20 times by hand, and C refers to the same solutions subsequently shaken for 30 min at 140 excursions/min on a mechanical agitator[7]. This method of calculation was used since it was desirable to compare the differences resulting from the effects of agitation to the degree of agitation recommended in the compendial standard technique (i.e., 20 hand inversions).

[7] Model 6000, Eberbach Corp., Ann Arbor, MI 48106.

Table III—Effect of Agitation on the Cleanliness Factors

| Cleanliness Factor (CF) ± SE[a] | Degree of Agitation | | | Change of Cleanliness Factor, % | |
	Stored (A)	20 Inversions (B)	30-min Shake (C)	Stored (A) versus 20 Inversions (B)	30-min Shake (C) versus 20 Inversions (B)
$\dfrac{\log N_{>1}}{-K} = C_a$	0.9829 ± 0.0715	1.0566 ± 0.0685	0.9647 ± 0.0918	7.0	8.7
$\dfrac{\log N_{>1} - 0.6990}{-K} = C_i$	0.7402 ± 0.0588	0.7722 ± 0.0500	0.7285 ± 0.0735	4.1	6.3
$\dfrac{\log N_{>1} - 2.5}{-K} = S$	0.0981 ± 0.0447	0.1635 ± 0.0295	0.1040 ± 0.0407	40.0	36.4

[a] Although the parameters C_i and S were developed to evaluate individual solutions, the data shown were based upon an average of 18 individually calculated cleanliness factors to demonstrate clearly the overall effects of agitation.

Once again, the two cleanliness factors proposed here (i.e., C_i and C_a) exhibited less variability (as indicated by the standard error values shown in Table III) and significantly lower percentage changes due to agitation effects than did Groves' S value. This finding indicates that the S value is also much more affected by the degree of agitation (i.e., the manner in which the solution is handled prior to measurement) than either C_i or C_a. The CF values calculated for the average of 18 solutions were all less than the critical values dictated by the respective standard, i.e., 1.3979 for C_i and C_a and 0.5 for S. Thus, the values shown in Table III are indicative of solutions whose average cleanliness factor value would have been passed by all three parameters when subjected to any of the three agitation conditions. These findings indicate that the large-volume parenterals tested here contained considerably fewer particles than the USP–NF standard allows.

For the 179 tests performed on parenteral solutions, perfect agreement was observed between the ability of a fivefold reduction of the USP–NF standard and the cleanliness factor for averaged data (C_a) to pass or fail parenteral solutions correctly. A perfect agreement was also observed between the USP–NF standard and the cleanliness factor for individual solutions (C_i). In contrast, five solutions failed the provisional Australian standard from which Groves' S value was developed, whereas the S value failed only one of these solutions. Furthermore, the S value failed one solution that would have passed this standard. This apparent inadequacy of the S value was likely related to the arbitrary manner in which Groves selected it. The C_i and C_a values suggested here were based upon the USP–NF standard and a fivefold reduction of it, respectively. Thus, the correlation (observed here) between the cleanliness factors and the standard would be anticipated.

The ability to obtain nondestructive readings in a short time would be advantageous when monitoring the cleanliness of batches of parenteral solutions, since a relatively large number of individual bottles could be averaged to obtain a representative sampling of the batch. However, the appropriate number of samples that should be averaged to ensure statistical accuracy is difficult to state unequivocally, since the operational factors involved in the manufacturing process may vary from batch to batch.

SUMMARY

To characterize a particle-size distribution as accurately as possible, it is advantageous to obtain counts over a broad range of particle sizes. This method enables the particle-size distribution to be determined by statistical weighting procedures so that any unusual variation in counts at one particle diameter can be effectively "smoothed out" by using a least-squares fitting procedure of the entire particle-size distribution. When utilizing these techniques, a particle-size distribution can best be described by specifying both the slope and the intercept of the $\log N_{>D}$ versus $\log D$ plot (12). A parameter based upon both of these particle-size distribution characteristics was developed and is referred to as the cleanliness factor. The cleanliness factor for individual solutions (C_i) was based upon the present USP–NF standard; the cleanliness factor for an average of solutions (C_a) was based upon a fivefold reduction of this standard.

An ideal cleanliness factor would not be affected by differences in the measuring technique employed or by the degree of agitation experienced by the solution prior to measurement. When the cleanliness factors developed here were compared to Groves' S value, C_i and C_a were indeed affected much less by both the degree of agitation and the measuring technique.

A parameter based upon the total distribution of the particulate matter would reflect more accurately the true nature of a parenteral solution's cleanliness than mere statements of the allowable concentration of particles exceeding certain sizes. Since C_i and C_a appear to be relatively independent of the measuring technique utilized, it is desirable that these cleanliness factors be used in conjunction with a rapid, nondestructive, and easy to operate instrument such as the one described here. The proposed cleanliness factors can be regarded as complementary, more effective, means of applying the USP–NF standard.

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