REVIEW ARTICLE

Particulate Matter in Parenteral Products: A Review

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ABSTRACT: Particulate matter in parenteral products is a complex subject. This article contains a discussion of several aspects of this topic including the use and limitations of the inspection and counting techniques for visible and subvisible particles, the identification of particles, and the elucidation of sources, mechanisms of formation, and particulate reduction techniques. Two significantly different approaches, human and machine inspection, have been used to detect visible particulate matter in parenteral products. A description of both methods is given along with a discussion of their typical performance characteristics. Criteria for comparison of different visual inspection systems are also presented. A variety of methods have been utilized for the measurement of subvisible particulate matter, including microscopic, electrical zone-sensing, light blockage, light scattering, and holographic techniques. Each of these particle counting methods is described. In addition, the factors that affect the measurement of subvisible particulate matter are discussed. An approach to particle identification is outlined. General comments concerning the analysis of particulate matter in parenteral products are discussed along with a description of various particle identification techniques and several examples illustrating how the methods have been applied. In particular, the techniques that are presented include light microscopy, atomic spectroscopic methods (SEM/EDXRA, electron microprobe, ESCA, and Auger electron spectroscopy), molecular spectroscopic techniques (infrared spectroscopy, Raman spectroscopy, and mass spectrometry), and chromatography. Finally, sources of particulate matter including packaging materials, manufacturing variables, formulation components, and miscellaneous factors are reviewed. The different mechanisms of particle formation, namely, direct contamination, precipitation and agglomeration are discussed. Representative examples of particulate reduction steps are presented.

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Introduction

Control of the key features of a product and the processes by which it is manufactured is essential to the assurance of quality for that product. With parenteral formulations there are several important variables including potency, pH, sterility, pyrogenicity, and particulate matter. Of these, control of the particulate quality can represent a significant challenge.

There are at least three reasons for focusing attention on particulate quality of parenteral products. These are

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safety concerns, legal requirements, and process evaluation.

Concerns for patient safety were first addressed in the works of Garvan and Gunner (1, 2). Since their initial reports, numerous papers have been published on this topic and a few review articles have been written concerning the clinical significance of particulate matter (3–5, 234).

Although there is some controversy about this subject, it has been concluded that injectables should not contain an excessive number of particles. The primary evidence for this can be found in the literature on drug abuse (6–12). Injections of crushed tablets, capsules, and other solid dosage products have often resulted in serious consequences. For example, one drug user died after i.v. injection of Darvon capsules (8). In another report, Douglas and coworkers found that three of seven addicts who injected drugs had rontgenographic, pathologic, and functional manifestations of pulmonary foreign body emboli and granulomas; the remaining had abnormal pulmonary function (9).

The results of numerous animal experiments have also been reported (13–28). In many of these studies massive doses of particulate matter were injected into several species including dogs, rabbits, rats, mice, and hamsters. The particles consisted of glass beads, cotton fibers, polystyrene latex spheres, paper fragments, and other insoluble substances. In addition to differences in the type and shape of the particulate matter, the particle sizes ranged from a few microns to several hundred microns. Although these studies provide less direct evidence than drug abuse studies, they do indicate that excessive levels of particulate matter in i.v. solutions can be harmful.

However, the clinical ramifications are much less clear when we are concerned about the use of parenterals containing levels of particles that are typical of current products. As Turco and Davis suggested in a previous review article (3), lack of definition of these effects and, therefore, the significance of particulate matter is caused primarily by the absence of controlled studies on humans. Some studies have been reported (29–38), but their conclusions are qualitative and nonspecific. In particular, control human tissue samples are difficult to obtain because of disease, environmental pollutants, and social habits. Finally, controls are difficult since tissue samples are taken from people who may have received an undetermined number of parenteral solutions (3).

The regulations pertaining to particulate matter in parenterals vary considerably among the different compendia (39–42). For example, the specifications for the British Pharmacopeia, United States Pharmacopeia, and The Pharmacopeia of Japan are shown in Tables I–III.

It is noteworthy that the compendial requirements depend upon the size of the particulate matter and upon whether the injectable is a Large-Volume Parenteral (LVP) or a Small-Volume Parenteral (SVP). In the case of visible particles, there are regulations for both LVP and SVP products. The exact wording varies among the different compendia, but the specifications are very similar. Injectables are supposed to be clear and essentially free of particles that can be seen by the unaided eye. However, the situation is different for subvisible particulate matter. Currently, most compendia have specifications for LVP solutions, but few have requirements for SVP products. Even if one restricts a comparison of the various regulations to LVP solutions, there are significant differences in the size of particles that are measured and the methods by which they are detected. It is not possible to determine which compendial specification is more stringent without making several assumptions concerning the size distribution of particles and their shape (4).

Several rationales have been presented to justify the guidelines concerning particulate matter. Some have ar-

TABLE I. USP XXI

Particle Size	Parenteral	Requirement
Visible	LVP SVP	Good pharmaceutical practice requires that each final container of Injection be subjected individually to a physical inspection, whenever the nature of the container permits, and that every container whose contents show evidence of contamination with visible foreign material be rejected.
Subvisible	LVP	Microscopic: Not more than 50 particles per ml that are equal to or larger than 10 μm and not more than 5 particles per ml that are equal to or larger than 25 μm in effective linear dimension.
Subvisible	SVP	Light-obscuration: Not more than 10,000 particles per container that are equal to or greater than 10 μm in effective spherical diameter and/or 1000 particles per container equal to or greater than 25 μm in effective spherical diameter.

TABLE II. British Pharmacopeia 1980

Particle Size	Parenteral	Requirement
Visible	LVP SVP	Injectable Preparations which are solutions, when examined under suitable conditions of visibility, are clear and practically free of particles.
Subvisible	LVP	Electrical zone-sensing: Does not exceed 1000 per ml greater than 2.0 μm and does not exceed 100 per ml greater than 5.0 μm. or Light blockage: Does not exceed 500 per ml greater than 2.0 μm and does not exceed 80 per ml greater than 5.0 μm.

TABLE III. Japanese Pharmacopeia, Tenth Edition

Particle Size	Parenteral	Requirement
Visible	LVP SVP	When the outer surface of the container is cleaned, injectable solutions or solvents for drugs to be dissolved before use[a] must be clear and free from foreign insoluble matter that is readily noticeable when inspected with unaided eyes at a position of luminous intensity of about 1000 luxes [93 footcandles], right under an incandescent electric bulb. As for injections contained in plastic containers, the inspection is performed with unaided eyes at a position of luminous intensity of 8000 to 10000 luxes [740 to 930 footcandles] with incandescent electric bulb placed at appropriate distances above and below the container.
Subvisible	LVP	Microscopic: The limits are not more than 50 particles per ml that are equal to or larger than 10 μm and not more than 5 particles per ml that are equal to or larger than 25 μm.

[a] There is an analogous requirement for preparations that are to be dissolved before use.

gued that the particle standards are consistent with the capabilities of existing technology and, hence, are a measure of good manufacturing practice (4, 40, 43-45). Others have stated that they can be justified on the basis of cumulative particulate insult the patient receives (40, 46). For example, the differences in LVP and SVP regulations have been rationalized on both accounts (46, 47).

Numerous articles have been published concerning the level and sizes of particulate matter in LVP and SVP injectables (43, 48–78). These studies have utilized a variety of methods for measuring particle counts including microscopic, light blockage, light scattering, and electrical zone-sensing techniques. Furthermore, a wide variety of products, packaging types and dosage forms have been examined.

There has also been a considerable interest in particulate matter for the purpose of process evaluation. Others have suggested that a significant increase in the level of particles for a parenteral could be used as an indication that the product or the process by which it is manufactured may not be well-controlled (4, 43, 46, 49, 65, 79).

The levels of both visible and subvisible particles have been considered as useful measures for process control requirements. For example, Brownley reported on the use of process control charts for rejection rates in visual inspection (79). In addition to data for the total number of rejects, he discussed the use of charts for specific types of visible particulate matter such as lint and glass. Brownley argued that every parenteral manufacturer could benefit from this type of information in assessing the process capabilities of an operation to produce a high quality product.

As an index of quality, others have suggested that the data on the level of subvisible particles could be even more helpful than the results of visual inspection (4, 43, 46, 49). Particle size distributions have been reported for numerous parenterals (49–52, 54, 59, 63, 64, 67, 73). In the case of subvisible particles, it has been observed that there is a log–log relationship between the size and the number of particles (4, 43, 46, 49) and most workers have decided to summarize their data using the following equation

$$\ln N = \ln N_{1.0} - M \ln D$$

where N is the cumulative number of particles at the threshold corresponding to diameter D, $N_{1.0}$ is the value of N for $D = 1.0$ μm and M is the slope of the log–log plot. Based on the results of these size distributions, a variety of limits have been suggested for both LVP and SVP solutions (4, 43, 46, 49, 65).

The detection and quantitation, identification and ultimate reduction of particulate matter in parenteral products represent a complex subject. This paper addresses particulate matter in parenteral products in three sections: (*1*) inspection and counting techniques—visible and subvisible particles; (*2*) identification of particulate matter; and (*3*) sources of particles, mechanisms of their formation, and particulate reduction steps.

1. Inspection and Counting Techniques—Visible and Subvisible Particles

Inspection for visible particulate matter and the enumeration of subvisible particles provide a quantitative assessment of product quality. This section is separated into two parts: (*A*) Visible Particles and (*B*) Subvisible Particulate Matter. Each part will contain a description of the various techniques that are utilized as well as a discussion of their performance capabilities and limitations.

(*A*) Visible Particles: As shown in Table I, the USP stipulates 100% inspection of injectables for visible foreign material. Not all compendia require 100% inspection of parenterals, but most state that the injectables are supposed to be practically free of particles which can be seen by the unaided eye. Two significantly different methods have been used to detect the presence of visible particles. One utilizes people and the other uses machine detection. For each method a general description will be followed by a discussion of the typical performance characteristics of the various techniques. Next, criteria that can be used to compare different inspection systems will be presented. Finally, in view of the current knowledge of visual inspection methods some general comments will be presented.

Human Visual Inspection: A review of the literature indicates that human inspections have been carried out in a variety of ways (80–86). General guidelines for this process were developed by a Parenteral Drug Association (PDA) Task Force (85). In particular, the normal inspection apparatus is comprised of a box containing a lamp

with sufficient light intensity and suitable lighting conditions. The lighting may be fluorescent, incandescent, spot, and/or polarized. Also, a combination of light sources may be employed and the light source(s) may be positioned above, below or behind the units to be inspected. Magnification (2×–3×) is used by some but not all manufacturers. In general, the background consists of both black and white sections, permitting inspection under both conditions. In addition, pacing methodology is often utilized in order to provide an effective rate of inspection while maintaining acceptable quality levels. Finally, those factors which can affect the human component such as training, visual acuity, and operator fatigue are usually controlled.

Besides manual inspection systems, numerous semi-automated machines have been developed which also use people for the detection of particles (80, 82–84, 87–92). These systems have a significantly higher throughput than manual processes because they perform most of the mechanical manipulations normally done by humans. These include such operations as swirling containers, inverting samples, stopping containers, and removing defects. Several people have claimed that these machines reduce eye strain for the operators and provide improved inspection quality by using significantly better imaging capabilities than exist for manual systems (80, 82–84, 87–91).

Whether one uses a completely manual system or one of the semi-automated processes, the decision to accept or reject a container is still made by a person. Therefore, it is important to review what is known about the human visual inspection process. From the broad range of literature on the subject (80–106), the articles published by Knapp and coworkers (80, 93–97) stand out as key references.

The USP specifications for visible particles suggest that human visual inspection is a deterministic process. For a deterministic process, if the same set of containers is examined under the same inspection conditions several times, then the same containers will always be rejected. The rejection probability can be only one of two values, 0 for good and 1 for bad containers. In contrast, for a probabilistic process, each container has a rejection probability associated with it, and the rejection probability can be any value between 0 and 1.

Knapp and Kushner carried out some experiments to determine if human visual inspection is deterministic or probabilistic (80). In their studies a set of 1000 uninspected vials was examined by each of five inspectors ten times each for a total of fifty inspections. Rejection records were maintained for each vial; any rejection score from 0 to 50 was possible. A summary of these results, shown in Table IV, indicates that containers were found in every rejection probability group. Only 2 samples were rejected all the time and approximately 20% of the containers were rejected at least 10% of the time. These experiments confirmed that the inspection process is probabilistic.

In addition, Knapp and coworkers found there is a relationship between rejection probability and the size of the particle (80, 96–97). They observed that the vials with the smallest particulate matter were in the lowest rejection probability groups and the samples with the largest particles were in the highest rejection probability groups. They concluded that the larger the particle (all else constant) the more certain its detection. Therefore, it is no longer adequate to state that particles have been observed in a parenteral, but the probability with which they can be detected is also essential information (80, 93–97).

TABLE IV. Results of Knapp and Kushner Experiments (Data Taken from Ref. 80)

Rejection Probability	Number of Vials
0.0	805
0.1	98
0.2	33
0.3	17
0.4	11
0.5	10
0.6	8
0.7	6
0.8	5
0.9	5
1.0	2

The findings summarized above are consistent with the biophysical literature on human vision (96, 107–109). An objective description of visual inspection contains several essential elements, including the capability of the viewer, the size of the target, the total background illumination, and the contrast of the target against its background.

The concept of rejection probability zones, introduced by Knapp and coworkers, is very useful for assessing visual inspection systems (80, 93–97). The range of the rejection probability, p, can be conveniently divided into three regions:

$0.0 \leq p < 0.3$	Accept Zone
$0.3 \leq p < 0.7$	Gray Zone
$0.7 \leq p \leq 1.0$	Reject Zone

The region of low rejection probability, to which most containers in a well-controlled process will belong, is termed the "Accept Zone." The region of moderate rejection probability, the "Gray Zone," is most sensitive to any changes in the visual inspection process. This zone is a buffer region between the truly bad containers, which should be rejected, and good containers that should be accepted. The remaining region of high rejection probability is termed the "Reject Zone." This group of samples is especially interesting from a quality assurance standpoint and the inspection process should be very efficient in rejecting these containers.

A more thorough understanding of this subject can be obtained by reviewing the references cited above. However, for the purposes of this article it will be sufficient to discuss several general observations concerning human visual inspection.

First, one of the most important characteristics of any visual inspection system is its detection limit. Several workers have reported that particles larger than 50 μm are usually detected by the naked eye (87, 110–113). Although these claims are not necessarily inaccurate, they

can be very misleading if they are applied without qualification to human visual inspection processes.

In any analytical measurement there are several factors (sample matrix, experimental conditions, signal/noise, etc.) which must be specified in order to determine the detection limit (114). For example, it is usually significantly more difficult to measure low levels of a species in a solution containing many components than it is in a medium with only a few species. The actual experimental conditions are defined because methods of concentrating samples, time-averaging, and other procedures can dramatically affect the detection limit. Finally, the ratio of the measured signal to the response in the absence of the species of interest is specified at the limit of detection.

An analogous situation exists for a human visual inspection process. The set of samples used to determine the detection limit should be well-characterized. In particular, one needs to specify the fraction of that set which is defective, the solution volume, and the nature of the defects, including the number of particles per container, their size, their shape, and their reflectivity. Also, the conditions under which the inspections are carried out should be described. The inspection rate, the amount of magnification, the visual acuity of the inspectors, and the type of illumination and background that are used can have a significant effect on the detection of particulate matter. Finally, the rejection probability at the detection limit should be defined.

The work of Knapp and coworkers provides useful information concerning detection limits for human visual inspection (97). The ampoules that were inspected were thoroughly characterized using a nondestructive technique, transmission holography. The inspection conditions were also well-defined. The Schering standard 10-sec paced inspection (two ampoules) with a 3× magnifying lens, a diffuse light source, and a white/black background were utilized. The light intensity at the position of the samples was approximately 225 foot-candles. In addition, the inspectors chosen for the study were selected on the basis of measurements of their visual acuity, and the results of 70 inspections provided an accurate estimate of the rejection probability for each of the ampoules. In these studies a 70% detection probability was obtained for a spherical particle with a diameter of 65 μm. The equivalent rejection probability using the same conditions without magnification would be seen for a spherical particle approximately 100 μm in diameter.

Using a slightly different protocol than that reported by Knapp et al. (97), we have also studied the visual inspection process. In our experiments a set of 1000 ten-ml ampoules having the composition shown in Table V was used. The defectives were randomly distributed and all the ampoules had been thoroughly characterized by nondestructive techniques. The particles were fluorescent-dyed polystyrene divinylbenzene spheres and the sizes of these beads were measured in-situ using an inverted microscope procedure. Each inspector examined the entire group without magnification in an inspection booth with typical lighting and background conditions. Paced inspection was utilized with a clip of 10 ampoules being examined every 38 sec. The people chosen for the studies included both quality assurance and production inspectors at a few of our manufacturing sites. The results of one study with 14 inspectors at one facility is shown in Table VI. Based on these data, the 70% rejection probability would occur for a spherical particle with a diameter between 100 and 165 μm. In view of the differences in inspection rates, magnification and other conditions, these results are comparable with the findings of Knapp and coworkers (97).

TABLE V. Ampoule Particulate Set

Number of Ampoules	Number of Particles/Ampoule	Size of Particles (μm)
50	1	165
75	1	100
875	—	—

TABLE VI. Average Results for 14 Inspectors at One Facility

Category	Mean Rejection Probability (%)
Good	1.1
One 100-μm particle per 10-ml ampoule	59
One 165-μm particle per 10-ml ampoule	82

Most of the above discussion has assumed that there is one visible nonreflecting particle per container. As expected, for the same type and size of particle the detection probability increases as the number of particles increases. Also, the human rejection probability is strongly affected by the optical characteristics of the particulate matter (97).

A second important characteristic of a visual inspection system is its reproducibility (84, 93, 94, 97, 106). If the same set of samples is examined several times by several people under identical inspection conditions, one would like to know the consistency of both the rejection rate and the defectives for an individual inspector as well as for the entire group of inspectors. Moreover, it would be desirable to have this information as a function of time. Although a number of articles have been published on this subject (84, 93, 94, 97, 106), it is difficult to summarize the observations. In particular, this topic is similar to the previous subject since an informative discussion cannot be given without defining the specific range of rejection probabilities for the samples of interest.

In general, the performance of a human visual inspection system is only moderately reproducible. There is a wide variability in the capabilities of individual inspectors and the performance of each inspector can change significantly over the course of time. For example, when the set of ampoules described in Table V was examined by inspectors at one facility, the results in Table VII were observed. Among these inspectors the rejection probability varied from 19 to 84% for a 100-μm sized particle and from 64 to 96% for a 165-μm sized particle. Similar results

TABLE VII. Rejection Probability

Inspector	Reject Rate (%)	Good (%)	One 100-μm Particle/Ampoule (%)	One 165-μm Particle/Ampoule (%)
1	10.3	0.6	71	94
2	8.0	2.1	40	66
3	6.9	1.3	19	88
4	8.4	0.1	53	90
5	9.9	1.5	60	84
6	8.4	0.2	58	82
7	6.0	0.3	35	64
8	9.4	0.8	59	86
9	12.1	2.5	81	78
10	10.0	1.4	68	78
11	12.1	1.4	84	96
12	12.1	1.7	80	94
13	8.3	0.2	58	78
14	8.7	0.8	56	76

have been seen by Knapp et al. (93) when they studied the inspector variability in 10 inspections using a reject-seeded test batch. They found that among 23 inspectors the Reject Zone Efficiency varied from 57 to 99% and the reject rate varied from 13.7 to 49.3%. Also, the variation in the performance of individual inspectors over time can be seen by the data shown in Table VIII. For this study several inspectors at a different site examined the set described in Table V two or three times during a 24-month period.

Finally, human visual inspection systems usually have a small, but detectable false reject rate. Several factors can lead to the rejection of good containers, i.e., those which do not contain any particles of sufficient size to be detected by an inspector. A very small fraction of the containers in a batch are removed by people because the inspectors occasionally interpret an air bubble as a particle (88). Also, when several samples are examined at the same time and particulate matter is observed in one of the containers, human error could result in the removal of a good sample instead of the defective (88). In practice, these containers are included in the group of samples having a very low rejection probability (80, 93–97). Nevertheless, because all measurement techniques have noise, it is probably more accurate to classify them as good containers which are rejected as a consequence of noise in the inspection system. The results in Tables VII and VIII for the good samples suggests that the false reject rate for human visual inspection systems is approximately 0.5–1.0%.

Machine Visual Inspection: An alternative to human visual methods is machine inspection. Several machines, using a variety of particle detection methods, are commercially available (93, 94, 96, 97, 99–102, 105, 106).

Although the machines are based on different principles, there are several common features which all of the systems have. First, the containers are spun at a high rate of speed and the movement of the container is stopped just prior to the time the sample is viewed by the detector. The primary purpose of this step is to place the particulate matter in motion. On most systems the spin speed can be varied (93, 94, 99–101). In the case of the Eisai system, this parameter can be varied from 800 to 3500 rpm (Table IX).

Second, the interval between the deceleration of the container and the observation time is usually very small. This is done in order to achieve reliable detection of heavy particles such as glass fragments (93). On the Eisai system a brake is used to stop the containers, and the location

TABLE VIII. Rejection Probability (%)[a]

Inspector	Good			One 100-μm Particle/Container			One 165-μm Particle/Container		
	0 mo.	12 mo.	24 mo.	0 mo.	12 mo.	24 mo.	0 mo.	12 mo.	24 mo.
1	0.2	0.3	0.1	25	33	45	67	70	88
2	—	1.3	3.4	—	13	16	—	48	41
3	0.2	0.1	0.0	27	41	35	55	54	44
4	0.2	0.0	0.2	21	26	36	80	82	64
5	0.4	0.0	0.1	31	26	44	69	84	82
6	1.8	2.9	—	30	58	—	69	92	—
7	0.0	0.0	0.1	48	82	92	79	94	96
8	—	0.0	0.1	—	39	47	—	62	86
9	—	0.2	0.7	—	51	27	—	80	80
10	—	0.2	0.7	—	39	39	—	56	60

[a] Each inspector examined the set described in Table V two or three times during a 24-month period.

TABLE IX. Eisai Visual Inspection: Model 578SD

Parameter	Range
Spin speed	800–3500 rpm
Time delay	0.86–1.72 sec
Rejection threshold voltage	0.08–1.80 V

of the brake determines the time delay that the vials or ampoules experience before they are viewed by the detector. The time delay is an adjustable parameter for most visual inspection machines. On the Eisai system it can be varied from approximately 0.9 to 1.7 sec. It should be noted that there is a practical minimum limit to the time delay imposed by cavitation. Cavitation creates bubbles which, during short inspection times, cannot be distinguished from particulate contamination, thus resulting in false rejects (93).

Third, the systems detect moving images and are moderately blind to stationary images. The latter characteristic precludes response to container markings and flaws. Although the detectors are based on a common principle, most of the significant differences in the systems lie in the manner in which particles are detected. In the case of the Eisai machine a lamp shines through the containers and illuminates a detector, which consists of a narrow array of photodiodes. If there is a moving particle, a shadow will be cast on one or more of these optical elements. This generates a signal which is amplified and compared to a threshold voltage. If the signal is greater than the threshold voltage, the sample is rejected. Besides this method of detection, TV technology and light scattering have been utilized (93, 94, 99–101). For example, on the Autoskan machine a video image of the solution is first recorded with a TV camera. This image, which is projected onto a master frame, is digitized in memory. The master is then compared electronically to four successive frames of digitized video data. If any of the four pictures are not identical to the master, an error signal results. Rejection of the container occurs when the number of error signals reaches a predetermined setting.

Fourth, although their detection principles so differ, the sensitivity is an adjustable parameter on all machines (93, 94, 99–101). On the Eisai machine the rejection threshold voltage is the sensitivity parameter and, as shown in Table IX, it can be varied between 0.08 and 1.80 V.

Finally, since the systems detect moving particles, their detection of a particle is dependent both on the movement of the particle normal to the observation axis and on the orientation of the particle during the viewing time. To increase detection probability, some machines make more than one measurement for each container. For example, the Eisai machine carries out two inspections per container; if a particle is detected during either inspection, the container is rejected. In contrast, the Schering system uses only one observation interval, but utilizes two orthogonal viewing axes (93, 94).

As was true for human visual-inspection processes, each of the machines can be evaluated in terms of critical characteristics, including detection limit, reproducibility,

TABLE X. Rejection Probabilities as a Function of Particle Size and Rejection Threshold Voltage

Rejection Threshold Voltage (V)		Particle Size (μm)[a] 40	50	70	80	100	115
0.33	Mean	0	10	90	95	99	98
	Std. Dev.		7	6	3	2	3
0.85	Mean	0	1	3	32	76	96
	Std. Dev.		2	4	6	10	4

[a] Each defective sample contained one polystyrene divinylbenzene sphere per vial; 10-ml containers were used.

and false reject rate. In general, the detection limit of the various machines is as low or lower than the human process (97, 99). For any particular automated system the detection limit depends upon the setting of the sensitivity parameter and the type of defect. For example, the results in Table X were obtained at two different rejection threshold voltages on an Eisai machine using 10-ml vials that contained only one fluorescent-dyed polystyrene sphere per container. For this system the 70% detection probability for a single particle was seen for a sphere between 50 and 70 μm in diameter at a sensitivity of 0.33 V. If a rejection threshold voltage lower than 0.33 V had been used or if the samples had contained more than one particle per container, then a smaller size detection limit would have been observed.

Visual-inspection machines are more reproducible than their human counterparts (93, 94, 96, 97, 99–101). The results in Tables XI and XII were obtained on an Eisai machine for time intervals of 2 weeks and 30 months, respectively. Assuming that these data are typical of the precision capabilities of machines, the results indicate that such systems are consistent over even long periods of time.

Finally, the false reject rates for machines can be very low, as evidenced by the data for the good containers in Tables XI and XII. Also, the false reject rates for machines are probably significantly lower than the rates typically observed for human processes.

Comparison Criteria: Several workers have discussed different criteria for comparing two inspection processes such as a human system and a machine (80, 93–96, 98, 103–104, 106). Some evaluations have simply compared the rejection rates for both processes. However, as the discussion above emphasizes, comparisons which use only rejection-rate information are not sufficient. Others have used parameters such as bias and consistency for comparing manual and automated systems. Although this approach is probably better than one concerned solely with reject rates, it is not based on our present understanding of visual inspection processes. In our opinion the most useful criteria are those proposed by Knapp and coworkers (80, 93–96).

Knapp et al. presented a model that uses three parameters to describe the comparative performance of a human process and alternative methodologies (95). The first variable, the Reject Zone Efficiency (RZE), summarizes the relative security of the inspection; it is the average proba-

TABLE XI. Rejection Probability for 10-ml Vials as a Function of Particle Size and Rejection Threshold Voltage

Rejection Threshold Voltage (V)		Good Vials 10 to 40 inspections of 500 vials (%)	One 80-μm Sphere/Vial 10 inspections of 250 vials (%)	One 115-μm Sphere/Vial 10 inspections of 250 vials (%)
0.68	Mean	0.16	66	98
	Std. Dev.	0.16	3	1
0.85	Mean	0.08	32	97
	Std. Dev.	0.13	6	2
1.03	Mean	0.02	13	93
	Std. Dev.	0.06	4	2
1.20	Mean	0.04	6	85
	Std. Dev.	0.08	2	3

TABLE XII. Rejection Probability at 0.85 V for 102 Inspections of Each Group During a 30-Month Time Interval[a]

	Good Vials (%)	One 80-μm Sphere/Vial (%)	One 115-μm Sphere/Vial (%)
Mean	0.04	42	96.4
Std. Dev.	0.13	8	1.4

[a] Each category contained 250 ten-ml vials.

bility of rejection for containers in the Reject Zone. RZE for a human porcess is between 70 and 100% by definition. RZE for any alternative can take on any value between 0 and 100%. The second parameter, the rejection rate of Accept and Gray Zone containers (RAG), can provide a measure of a system's discrimination between good and bad samples. The third variable is the estimated reject rate. They recommended that the first two parameters be determined using multiple inspections of a reject-seeded test group, which included a relatively uniform distribution of representative particulate matter in and near the Reject Zone. In the case of the third parameter, they suggested that it be evaluated using multiple inspections of a test group that is typical of standard production.

Use of Inspection Systems: First, the most important group of samples is that in the Reject Zone. In particular, Knapp and coworkers used the RZE to measure the security of an inspection (80, 93–97), and it is the value of RZE for the visual inspection system which determines if there is adequate assurance that the process is very efficient in rejecting these containers. In addition, changes in the Reject Zone Efficiency with time are also important. Reproducibility and the ability to monitor a system's RZE are desirable, and can be achieved for either a machine or a human process by using a standard particulate set that contains Reject Zone samples (115). Although we have used a standard set to evaluate the performance of inspectors, it should be noted that it is more accurate and much easier to measure a machine's performance than that of a human system.

Second, none of the machines or human visual-inspection systems are 100% efficient in removing Reject Zone containers. Consequently, there is a limit to the capabilities of any process to produce an acceptable product after a single inspection (101). The probabilistic nature of particulate detection enforces a direct relationship between the RZE and the actual quality of the product. Therefore, if the initial rejection rate exceeds a certain value, a second inspection might be necessary in order to achieve an acceptable overall RZE. Inspection systems are designed to remove the infrequent occurrence of visible particulate matter, but they are not designed to inspect quality into a product.

Third, results of visual inspection can be used for process evaluation. Brownley used reject rates and other information concerning defectives to assess the process capabilities for producing a high quality product (79). Since the rejection rate is determined by the rejection of Accept Zone and Gray Zone samples as well as Reject Zone containers, knowledge of the performance of a visual-inspection process in all rejection probability zones is essential for the application of visual inspection results to process evaluation. Consequently, estimates of parameters such as RAG and the false reject rate are desirable. These estimates can be obtained by periodic monitoring with a standard particulate set containing both Accept and Gray Zone samples (115, 116). In fact, as a diagnostic tool for the reproducibility of a visual inspection system, one needs to monitor Gray Zone containers because this is the region which is most sensitive to changes in the process (see Table XI). Since the machine can have a very low false reject rate and can be significantly more reproducible than a human system, smaller changes in the particulate quality of products can be detected by using a machine inspection instead of a human process. This is particularly useful for measuring the effect that a manufacturing change, storage time or product revision has on the level of visible particulate matter. For example, Knapp and coworkers were able to observe changes in the particulate quality when different rubber stoppers were used (116).

For purposes of process evaluation, visual inspection results can be very helpful. However, one should be aware that consistent levels of visible particulate matter in a product does not necessarily mean consistent particulate

quality since the sources of visible and subvisible particles can be different.

Finally, acceptability of an inspected lot is frequently determined in an audit inspection using a statistical sampling plan such as the Military Standard 105D (85). Generally, the audit utilizes human inspectors instead of a machine. A PDA task force (85) has recommended that care be exercised to assure that the inspection procedures established for auditing product quality are equivalent to those of the production inspection process. In particular, the task force stated that the procedures for auditing the quality of manually inspected lots should use the same manual techniques, relative dwell times and equipment as used in the original inspection process. Also, the procedure for auditing the quality of a lot inspected with a machine should use the same manual procedure as used to initially establish the proper machine operating parameters.

As noted by Knapp and Kushner, the probabilistic nature of visual inspection does have implications for the audit inspection (80). If some of the audit inspectors have a significant false reject rate or reject a significant fraction of Accept and Gray Zone containers, then the results of the audit inspection may not be indicative of the level of Reject Zone samples remaining in the inspected lot. A major benefit of auditing a machine inspected lot by human methods is the potential for detecting the occasional lot that has a significant amount of an atypical Reject Zone defective which is not easily detected by the machine.

(*B*) **Subvisible Particulate Matter:** In addition to visible particles, the levels of subvisible particulate matter can be important. Besides the legal requirements which may pertain to products, the enumeration of subvisible particles has been considered by several workers to be useful for purposes of process evaluation. Whatever the reason, numerous articles have been published concerning the level and sizes of subvisible particulate matter in LVP and SVP solutions (43, 48–78, 113, 117–119). Since a variety of methods can be used for the measurement of particle counts, a description of the different techniques will be presented first and followed by a discussion of their characteristics and general comments.

Particle Counting Techniques: A variety of methods have been utilized for measuring subvisible particulate matter including microscopic, electrical zone-sensing, light blockage, light scattering and holographic techniques. One of the most common procedures for enumerating particles is the microscopic method. It is the official test in the USP and JP for LVP solutions (39, 42). Detailed protocols are contained in these compendia and several people have discussed the specific aspects of microscopic procedures (5, 112, 120, 121, 123). In general, this method involves filtration of the sample through a color contrast filter membrane and counting the particles collected on the membrane at a hundredfold magnification with oblique illumination. Particles are usually counted in two different size ranges such as ≥ 10 μm and ≥ 25 μm. The size of each particle is defined by the longest effective linear dimension, which is the diameter of the largest circle that would circumscribe the particle.

Modifications to the basic microscopic protocol have also been described (61, 122). Changes in the sizing and counting steps have been recommended to minimize both the tedium and lengthy analysis time associated with these parts of the procedure. One modification used a microprojector to project the image of the entire effective filtering area of the membrane onto a special screen (122). A person was still used to count and size the particles, but to facilitate this process the screen provided individual calibration reference grids for each particle. With other changes the manual counting has been replaced by an electronic scanning procedure such as that used by the π MC system (61).

As noted in the BP, the Limit Test for Particulate Matter in LVP solutions can be carried out using an automated particle counting instrument such as the Coulter Counter, which operates on the electrical zone-sensing principle (41). Although a protocol is contained in this compendium, more detailed descriptions of the technique have been published elsewhere (5, 72, 112, 121, 124–126). This method detects particles by a change in resistance and requires the presence of an electrolyte. The sample, drawn by vacuum, flows at a constant rate through an aperture having immersed electrodes on either side. As each particle passes between the electrodes, it displaces a volume of the electrolyte equal to the particle volume. This causes a temporary change in the electrical resistance which is proportional to the particle volume. The resultant series of pulses is electronically amplified, scaled, and counted. For each particle the size is registered as the diameter of a sphere having an equivalent volume.

Light blockage instrumentation such as the HIAC/Royco Automatic Particle Counter can also be used to count subvisible particles. Carver has provided a thorough analysis of the HIAC system (127), although other descriptions of the technique and some compendial protocols are available in the literature (5, 39–41, 64, 70, 72, 121, 128). In a light blockage method, the sample fluid passes through a small rectangular passage and past a window. The light from a lamp is formed into a parallel beam by the window to an exact size and directed through the liquid stream and onto a photomultiplier tube. As long as the number of particles does not exceed the specified concentration, the particles will pass the window one by one. Whenever a particle traverses the light beam, the intensity of light reaching the phototube is reduced. The amplitude of the resulting signal pulse is proportional to the projected area of the particle in a plane normal to the light beam, and the particle size is registered as the diameter of a circle having an equivalent projected area. Pulses are counted within ranges determined by amplitude thresholds in order to measure particle size distributions.

Subvisible particles have also been counted using instruments that operate on the principle of light scattering. Detailed descriptions of several systems have been reported (5, 59, 64, 112, 128–132). Some of the instruments have a system of lenses that focus a sharply defined beam of light onto a "sensitive volume" of the sample cell and all

the fluid under test is forced to pass through this sensitive volume area. If the liquid is particle free, then the light beam passes through the fluid and is absorbed by a light trap. On the other hand the presence of particles causes diffraction of the light beam onto a photomultiplier tube by means of a series of lenses, and a current pulse is generated. The pulse amplitude is a function of the amount of light received, which is proportional to the particle size of the contaminant. By utilizing electronic gates, both the number and size of particles passing through the sensing zone can be ascertained. These instruments can be designed to detect light scattering at forward or right angles and usually report particle size as the diameter of a circle having an equivalent cross section. The basic difference between these systems and the light blockage instrumentation is that they measure diffracted light instead of the amount of light that is blocked by the particle as it travels through the sensing cell.

Not all light scattering systems pass a flowing liquid through a sensing zone. For example, the Prototron counts particles by registering pulses of light from a scanning laser beam scattered by suspended particles in a stationary solution (131). Even among instruments that examine stationary liquids, not all are based on the principle of static light scattering. Some, such as the Coulter Nanosizer and the Malvern system, detect the presence of subvisible particles via dynamic light scattering, also termed quasielastic light scattering or photon correlation spectroscopy (132).

Finally, Knapp and coworkers have described an instrument that uses transmission holography to measure subvisible particulate matter in sealed ampoules (97). In this system, coherent light from a laser passes through a collimator/spatial filter assembly before entering a liquid cell and being projected onto a holographic plane. The hologram is monitored with a vidicon, whose output is displayed on a TV monitor. Inside the liquid cell the ampoule is held, spun and quickly stopped in order to generate particulate movement. The cell contains optically flat end windows and matches the refractive index of the ampoule to minimize container optical effects.

Characteristics: Each of the techniques for measuring subvisible particles has its own set of unique characteristics. Numerous comparisons of the different methods have been published and the specific advantages and disadvantages of each of the techniques have been addressed (44, 55, 56, 58, 59, 61, 65, 72, 77, 113, 120, 123, 133–137). The following discussion considers several factors which affect particle counting and which can be used to compare and contrast different techniques.

First, the number of particles of a particular size which are counted for a given sample depends upon the technique used to make the measurement. Several observations of count differences, not all of which are statistically significant, among identical samples analyzed by different particle counting techniques have been reported (44, 55, 56, 58, 59, 61, 65, 72, 77, 136). For example, in studies using both the Coulter Counter and HIAC instrumentation workers have observed that the electric zone sensing instrument recorded significantly greater counts than the light blockage method for particles ≥ 1 and ≥ 2 μm. They also found that, for larger particles such as ≥ 10 and ≥ 20 μm, the Coulter Counter yielded fewer counts than the HIAC instrument. The crossover point for these results occurred at approximately 6 μm (77, 136). In fact, the BP regulations for subvisible particles acknowledges the above facts (41, Table II).

Since the particle counting methods are each based on different principles, the aforementioned observations are not surprising. The theoretical bases for each technique have been thoroughly reviewed by Schroeder and DeLuca (121). Their treatment predicted the influence of shape on particle counts by comparing counts that would be obtained using the manual microscopic method, an automated microscopic technique, and methods based on light blockage and electrolyte displacement instrumentation. Schroeder and DeLuca considered seven different shapes, including a sphere, a cube, an equant-shaped particle, a prolate ellipsoid, a flake, a rod, and a fiber. Each technique was assumed to be calibrated with and, therefore, give identical results for spherical particles. As the shape of the particle deviated from sphericity, the size measured by the microscopic method was greater than the sizes obtained by the other techniques. For example, a fiber 25 μm long and 2.5 μm in diameter would be sized by the microscope at 25 μm, by the horizontal projection method at 16 μm, by the light blockage technique at 9 μm and by the electric zone-sensing method at 6 μm (121).

Most particles in parenterals are not spheres. Consequently, one should expect the results for a sample to depend upon the technique used to measure the particles. Closer agreement between the techniques could be obtained if a calibrant was chosen to be more representative of common contaminants. However, use of a nonspherical calibrant, such as AC fine test dust, can lead to practical problems (121, 136). In general, spherical particles can provide a more precise calibration than nonspherical particles.

Second, the precision of different counting techniques can vary considerably. In general, the instrumental methods are more reproducible than the microscopic technique (61, 65, 123, 127, 138). For example, tests of HIAC instruments are repeatable to within 5% for particles ≥ 10 μm (127), whereas replicate measurements using the microscopic method can yield variations of >10% (123).

Besides technique-dependent variations, significant differences in particle counts are observed among containers of the same lot of product from the same manufacturer (43, 53, 123). Using a Coulter Counter, Groves measured particle counts in forty-five containers of sodium chloride injection from seven different batches and concluded that the number of particles in each container could be regarded as unique to that container (43). Container-to-container variability has been addressed by some of the compendia. For example, the USP specifications for particulate matter in injectables state that "the results obtained in examining a discrete unit or group of units for particulate matter cannot be extrapolated with certainty to other units that remain untested. Statistically sound sampling plans [are to be used] ..... if valid inferences are to be

drawn from the observed data to characterize the level of particulate matter in a large group of units (39)."

The detection limit, or smallest observable particle size, also varies considerably among the different techniques. Electrical zone-sensing, light blockage, and light scattering instrumentation can detect particles larger than 1–2 μm, whereas some of the techniques such as the microscopic method and transmission holography normally have greater minimum size requirements (46, 97, 131). Most of the techniques cannot measure submicron particles. The instruments based on dynamic light scattering are an exception and can routinely monitor submicron particulate matter (46, 132).

A fourth factor that can affect the results of a particle counting measurement is the detection of nonparticulate matter such as air bubbles, silicone oil droplets, and other immiscible liquids. The counting of air bubbles can be legitimately classified as false responses. As expected, the microscopic method does not count air bubbles as particles. However, other techniques including electrical zone-sensing, light blockage, and light scattering instrumentation will record air bubbles as particles (64, 70–72, 112, 129). To minimize this problem, several people have discussed precautions that should be observed with these techniques (64, 70–72, 129).

In the case of liquid droplets that are not miscible with the parenteral solution, it is not clear if their detection should be regarded as false responses. Some of these such as droplets of pump oil or a PVC plasticizer should probably be categorized as foreign contaminants (55, 139). In studies reported by Horioka and coworkers, they found that when some PVC bags containing infusion fluids were shaken, di(2-ethylhexyl) phthalate was liberated and dispersed as microdroplets. The latter were recorded as particles by a HIAC instrument, but were not observed on a membrane filter under a microscope (55). In general, immiscible liquid droplets will be detected by all the particle counting techniques with the exception of the microscopic method.

Another important feature of particle counting measurements pertains to sample preparation, especially the extent of the sample manipulation and its effects on the results. Most of the techniques perform destructive analyses, although some of the instruments based on light scattering or transmission holography are capable of nondestructive measurements on parenteral solutions (54, 59, 71, 97, 113, 131, 132). Particle counting of lyophilized injectables necessarily requires reconstitution, and none of the techniques can perform an in-situ analysis of a freeze-dried product.

In the case of destructive analyses, each of the techniques requires scrupulous attention to certain details, for example, a laminar air flow hood is usually recommended. Cleanliness of equipment, operating surfaces, and the environment is important in order to obtain accurate and reproducible results (112, 123, 138). It is noteworthy that a mandatory step in any destructive measurement is the analysis of blanks. It is important to duplicate during the blank analysis precisely each step which is performed during the sample analysis. Furthermore, extremely low blank counts should be observed when operating in a clean environment and using proper technique (112, 138).

Proper sampling of the container contents is critical for accurate counting, particularly with ampoules. In practice, ampoules are designed to be snapped open at the constricted neck, and this inevitably introduces glass particles (46, 65, 76, 117, 118). To minimize these effects, most people use a version of the melt-open technique which involves placing the upper widest part, above the breakline, of an ampoule into an oxygen-gas flame and then melting off the residual container above the opening (65, 76, 117, 118). This method of opening ampoules introduces very little particulate contamination. In contrast, snap opening of the ampoules increases the number of counts in all the size ranges, but it has the most dramatic effect on the number of larger particles (76).

Often, sample preparation procedures required for a specific technique can affect particle counting results. For example, before measurements with an electrical zone-sensing instrument can be performed for a nonconducting parenteral, it is necessary to add an electrolyte. This can lead to several problems including the difficulty of homogeneously mixing the sample (77). On the other hand, it is well known that, with microscopic measurements, the choice of filter and the use of clearing solvents can affect the detection of particles (123, 133–135). In the case of light blockage instrumentation, if a sample has so many particles that the number of counts is in the coincidence range of the instrument, one is usually forced to dilute the sample. Dilution can have an effect on the results, particularly if some of the particles dissolve when the sample is diluted. Actually, any addition of a solvent or change in the nature of the medium can influence the measurement of particles (55, 119). Illum, Jensen, and Moller found that placing a parenteral solution in blood plasma increased the deagglomeration process and led to a particle size distribution significantly different from that in normal saline (119).

A sixth factor that can influence the results of particle counting analyses concerns the handling and storage conditions that a sample may experience prior to the preparation and analysis steps. For example, people have observed changes in the number of counts in various size ranges due to the effects of transportation, mechanical agitation, or ultrasonic treatment (51, 53–55, 63, 76, 140). In most cases, the effects of shaking and other forms of agitation have resulted in a significant increase in the total number of particle counts. However, an increase is usually not found for every size range. The number of particles in some of the size ranges, especially the larger ones, decreased after the treatment. A variety of explanations have been put forth to explain these results. Some have argued that the agitation dislodges particulate matter adhering to the surface of the container and/or closure, while others have stated that the situation is more complicated since agitation can break up agglomerates into smaller particles (51, 53–55, 63, 76, 140).

Besides agitation, storage time and/or temperature can influence the particle count results (53, 140). Ernerot and coworkers found that as the age of a solution increased

there was a marked reduction in the particle counts, which they attributed to adsorption to the container surface (140). On the other hand, Whitlow, Needham, and Luzzi observed an initial increase in the number of particles with increasing storage time followed by a decrease for significantly longer storage times. The latter workers also noted differences in the particle counts as a function of storage temperature (53).

Finally, ease of use, the amount of operator training and the degree of automation are other noteworthy characteristics of particle counting techniques. The microscopic method is significantly more time-consuming and requires more operator training than some of the other methods (45, 59, 61, 70, 121, 129, 137, 138). Although most of the techniques can be automated to a certain extent, automatic microscopic methods are at best cumbersome and still relatively time-consuming.

Use of Detection Methods: The levels of both visible and subvisible particles are useful measures of product quality. The use of visual inspection results for these purposes has already been discussed. Importantly, a consistent level of visible particulate matter in a product does not necessarily imply consistent quality since the sources of visible and subvisible particles can be different. Even if the sources are the same, enumeration of subvisible particles provides a more quantitative assessment than visual inspection of product quality. The population of subvisible particulate matter tends to be more reproducible than the number of visible particles.

Particle size distributions have been reported for many injectables and most people have found that there is a log-log relationship between the size and the number of particles (4, 43, 46, 49, 50, 53, 54, 59, 63, 119). Consequently, most workers have summarized their data using the following equation,

$$\ln N = \ln N_{1.0} - M \ln D$$

which was described earlier.

Based on the results of these size distributions, a variety of limits have been proposed for both LVP and SVP solutions (4, 43, 46, 65, 136). For example, Hayashi fit the size distributions of 150 Small-Volume Parenterals to the above equation and, using a 99% confidence interval for the regression line, proposed the following as a target for quality control (49).

$$(\log N_{1.0} - 1.53)/M < 0.65$$

On the other hand, Groves and Wana have taken the British Pharmacopeia limit test for LVP products as a guideline and proposed the following equations for LVP solutions

$$C = (\ln N_{1.0} - 4.147)/M$$

where C was defined as the contamination index and C must be less than 1.792 in order to pass the BP test (136). They recommended monitoring C for purposes of process evaluation since it can be readily calculated from size analyses by either a light blockage or electrical zone-sensing instrument, and it is sensitive to changes in the particulate quality (136).

Monitoring indices such as those mentioned above can serve as a useful guide for assessing the particulate quality of parenterals. Nevertheless, these equations should be used with caution. This is especially true if one monitors only two particle size ranges. In particular, Spence has pointed out that if the size distribution of the particles is unimodal, limits for two size ranges can adequately control the particulate quality. However, he further mentioned that the size distribution can oftentimes be multimodal, owing to contamination from several sources and, hence, monitoring several size ranges may be necessary (46).

Finally, a comparison of particle results for different products may enable one to determine the key variables that impact on particulate quality. For instance, Green et al. discussed the container size/volume relationship. As they noted, since the containers and the closures are the prime contributors to the particulate load, this relationship is one of the dominant factors that ultimately determines the particulate quality found in products (47).

2. Identification of Particulate Matter

Successful attempts to reduce particulate content normally require identification of the particles. The many analytical techniques which are applicable to particle identification should be considered in the context of some general approach to solving particle problems. One approach to particle handling and identification, developed by McCrone et al. (141–146), has been successfully applied to problems in many fields, including forensic science, mineralogy, and environmental science. The following discussion outlines a similar approach.

Selection of appropriate samples and isolation of the particulate matter of interest are the first and most important steps. Next, the particles are examined by light microscopy. If the identification is not complete, elemental techniques are used to gather atomic information concerning the particulate matter. Molecular information is then obtained if further characterization is required. Finally, if additional separation is required, chromatographic techniques are employed in conjunction with various atomic and molecular spectroscopic methods.

This section will contain some general comments concerning the analysis of particulate matter in parenteral samples, a description of various particle identification techniques, and several examples illustrating how the methods have been applied. Table XIII contains a summary of the characteristics of the techniques that will be discussed. For convenience, this section will be separated into the following parts: (*A*) General Comments, Sample Selection and Sample Preparation; (*B*) Microscopy; (*C*) Atomic Spectroscopic Techniques; (*D*) Molecular Spectroscopic Methods; and (*E*) Chromatography.

(*A*) General Comments, Sample Selection and Sample Preparation: In spite of the considerable sensitivity and selectivity offered by the analytical techniques described below, the identification of particulate matter in injectable solutions is a nontrivial problem. The data of Table XIV emphasize the fact that particle analysis invariably requires measurements on trace quantities of material.

TABLE XIII. Characteristics of Techniques

Technique	Minimum Sample (g)	Spatial Resolution (μm)	Minimum Thickness (μm)	Elemental Information	Molecular Information
Light Microscopy	10^{-10}-10^{-12}	1	0.6	$z \geq 4$; Qualitative using element-specific spot tests.	Functional group using spot tests; several interfering reactions may be encountered.
SEM/EDXRA	10^{-10}-10^{-12}	0.1	1	$z \geq 9$; Semiquantitative; Detection Limit = 1-2%.	N/A
Electron Microprobe	10^{-10}-10^{-12}	1	1	$z \geq 4$; Semiquantitative to Quantitative; Detection Limit = 0.1%.	N/A
ESCA	10^{-6}-10^{-7}	4000	0.005	$z \geq 3$; Semiquantitative to Quantitative; Detection Limit = 0.01-0.1%.	Limited; primarily oxidation states.
Auger Spectroscopy	10^{-9}-10^{-10}	1	0.005	$z \geq 3$; Semiquantitative to Quantitative; Detection Limit = 0.01-0.1%.	Limited; primarily oxidation states.
Infrared Spectroscopy	10^{-10}	10-20	0.5-2	N/A	Functional group; spectral matching can provide identification.
Raman Spectroscopy	10^{-9}-10^{-12}	1	0.5-2	N/A	Functional group; spectral matching can provide identification.
Mass Spectrometry	10^{-10}	0.2-10	N/A	Can provide elemental composition.	Molecular weight and fragmentation pattern can provide identification.

N/A = not acceptable.
z = atomic number.

TABLE XIV. Levels of Spherical Particles Having a Density of 1 g/cm^3 for Various Limits

LVP	Limit	Particle Mass (μg) (per container)	Particle Concentration (ppm)
Subvisible	1000 2-μm particles per ml	0.0042	0.004 ppm
Subvisible	100 5-μm particles per ml	0.0065	0.006
Subvisible	50 10-μm particles per ml	0.0262	0.026
Subvisible	5 25-μm particles per ml	0.0409	0.041
SVP	**Limit (Assume 10-ml Container)**	**Particle Mass (μg) (per container)**	**Particle Concentration (ppm)**
Subvisible	10,000 10-μm particles per container	5.2	0.52
Subvisible	1000 25-μm particles per container	8.2	0.82
Visible	One 100-μm particle per container	0.5	0.05

For example, a typical 100-μm particle, which can easily be detected by machine inspection in a ten-ml container, corresponds to a concentration of approximately 0.05 ppm.

This characteristic of parenteral particulate matter has often been the explanation for the occurrence of apparently contradictory results (4, 147). For instance, solutions stored in polyvinyl chloride intravenous bags were observed to have very low particulate counts prior to shaking. After severe shaking, a Coulter Counter measured nearly 20,000 counts per ml for particles greater than or equal to 2 μm in size. Almost all the counts were due to the release of diethyl hexyl phthalate (DEHP), a plasticizer, from the bag (4, 147). However, when the shaken solutions were assayed for DEHP, none was detected. The above results were not contradictory, since the limit of detection of the assay method for DEHP was 0.24 ppm whereas a particle count of twenty thousand 2-μm particles per ml corresponded to a concentration of approximately 0.08 ppm.

Since parenteral particles are usually present in only trace levels, this necessarily requires that a concentration step be carried out prior to analysis. This isolation, handling, and preparation of the particulate matter for analysis is one of the reasons why the identification of particles is oftentimes nontrivial.

Heterogeneity, both within the total particulate population and within individual particles, also poses analytical problems. As noted earlier, particulate contamination can come from several sources. Categorizing and quantitating each type of particle according to the fraction of the particulate population would provide a better description of the problem (71). As a result, careful selection of samples and careful analysis of many particles is necessary before one can generalize about the identity of the primary particles.

For the aforementioned reasons, the first steps, problem definition, sample selection, and particle isolation, are the most important parts of the analysis. Proper definition of the problem, i.e., "a few large particles," "haze," or "particles only appear after agitation," and close communication between the person observing the particles and the person characterizing them are prerequisite to solving the "right" problem.

The samples selected for analysis must be representative with respect to both type and level of particles, and appropriate control ("good") samples should be examined as well. Ancillary information, such as sample history and results of previous investigations, is also useful. When feasible, it is also important to verify the status of samples at the time that they are selected and prepared. For instance, the presence or absence of visible particles can be confirmed using an inspection hood. For smaller particulate matter one can check for the presence of particles while the container is still intact by using an inverted microscope (148, 149).

Once the samples have been selected, the particles need to be isolated from the solutions. This can be accomplished in at least three ways (148–150). Very small particles can be separated from the parenteral solution by filtration (149, 150). Although the choice of filter is dependent upon the types of analyses and any subsequent sample handling that may be carried out, the Nuclepore® membrane filter, which traps the particles on its flat surface, is well-suited for most purposes. Other isolation procedures are more appropriate for larger particles. In one method particulate matter can be drawn up via capillary action into a microcapillary pipet, deposited onto a glass slide, washed, and mounted for examination (150). A third procedure, which employs centrifugation, is not generally applicable even though it has also been used (148, 149).

Whatever method of sample preparation is used, further sample manipulation is often necessary, which is dependent upon the type of analysis to be performed. Finally, a cautionary note must be mentioned. As with all isolation procedures, there is a real danger of either introducing artifacts via contamination or losing particles (71, 149). If artifacts are occurring reproducibly during this step, analysis of control or blank samples can reveal their presence and, hence, the latter should be performed whenever possible.

(*B*) **Microscopy:** For several reasons light microscopy is the identification technique initially used. First, it has excellent sensitivity. Analysis of subnanogram amounts is straightforward and the detection limit is approximately one picogram (141). Second, it is well-suited for determining whether or not the particles are homogeneous. The diameter of a particle large enough to be identified with the light microscope varies somewhat with the detection method, but it is usually about 1 μm (141). Third, since microscopes are often used during sample selection and sample preparation, one can begin the characterization while the particulate matter is still in the field of view. Fourth, the analysis is quick and reliable in the eyes of an experienced operator, and several types of particles can be identified using light microscopy alone. Finally, even if the particulate matter cannot be identified using light microscopy, the analysis is particularly helpful in directing further characterization steps.

A wide range of information can be obtained from a light microscopy analysis (71, 141, 142, 145, 149, 151–153, 211). In general, the information can be divided into two categories based on the morphological and optical properties of particulate matter. Morphological properties include size, shape, and surface texture, whereas optical properties include color (transmitted and reflected), luster, refractive indices, and birefringence.

Besides the morphology and optical properties, solubility tests and various microchemical assays provide additional information (145, 157, 158, 212–218). The solubility tests, listed in Table XV, are based on the procedures proposed by Shriner et al. (212). The particulate matter is subjected to a series of solvents in order to categorize the particles into one or more of the solubility classes shown in Table XVI. Functional group tests or specific elemental analyses can then be performed. Microchemical tests can be extremely sensitive and give both elemental and molecular information. However, there are some drawbacks. Not all elements can be determined simultaneously as with many instrumental methods, and false positives can be observed for functional group tests.

The literature contains many reports of the identification of particles using light microscopy (5, 71, 148, 154–156). In particular, many different types of particles can be identified solely on the basis of the light microscopy data. For example, biological particles such as pollens, diatoms, paper fibers, and human hairs can be recognized by their morphological characteristics (Figs. 1 and 2).

TABLE XV. Light Microscopy: Solubility Tests (3 g/100 ml = Soluble)

• H_2O	• Methanol
pH by Litmus	• Acetone
• 5% NaOH	• $CHCl_3$
• 5% $NaHCO_3$	• DMF
• 5% HCl	• DMSO
• [H_2SO_4]	• Acetonitrile
• [H_3PO_4]	• Iso-octane

TABLE XVI. Solubility Classes[a]

1	Salts of organic acids (RCO_2Na, RSO_3Na), amine hydrochlorides (RNH_3Cl); amino acids ($R-\underset{\displaystyle NH_3^+}{\underset{|}{CH}}-CO_2^-$); polyfunctional compounds (functional groups are hydrophilic), i.e., carbohydrates (sugars), polyhydroxy compounds, polybasic acids, etc.
2	Monofunctional carboxylic acids with five carbons or fewer; arenesulfonic acids.
3	Monofunctional amines with six carbons or fewer.
4	Monofunctional alcohols, aldehydes, ketones, esters, nitriles, and amides with five carbons or fewer.
5	Strong organic acids: carboxylic acids with more than six carbons; phenols with electron-withdrawing groups in the ortho and para positions, β-diketones.
6	Weak organic acids: phenols, enols, oximes, imides, sulfonamides, thiophenols, all with more than five carbons. β-diketones, nitro compounds with α-hydrogens, sulfonamides.
7	Aliphatic amines with eight or more carbons, anilines (only one phenyl group attached to nitrogen), some oxy ethers.
8	Miscellaneous neutral compounds containing nitrogen or sulfur and having more than five carbon atoms.
9	Alcohols, aldehydes, methyl ketones, cyclic ketones, and esters with one functional group and more than five but fewer than nine carbons; ethers with fewer than eight carbon atoms, epoxides.
10	Alkenes, alkynes, ethers, some aromatic compounds (especially those with activating groups), ketones (other than those cited in class 9).
11	Saturated hydrocarbons, haloalkanes, aryl halides, diaryl ethers, deactivated aromatic compounds.

Carboxylic acid halides and anhydrides have not been classified because of their high reactivity.

[a] Taken from Ref. 212. A flowchart, which uses several of the solvents listed in Table XV, is contained in Ref. 212, and this scheme can be used to classify the material into the 11 solubility classes described above.

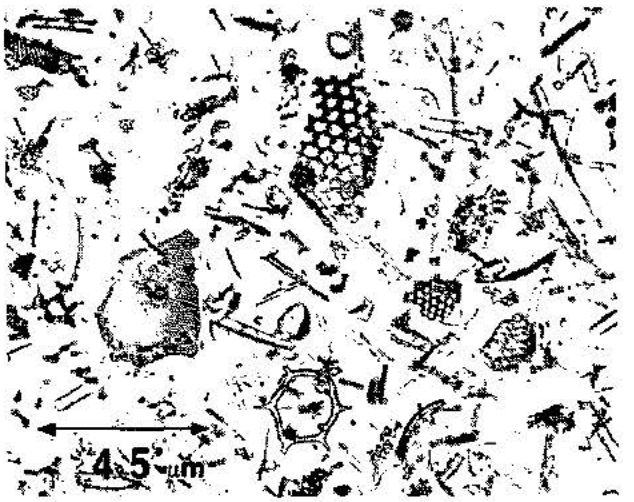

Figure 1—This is a photomicrograph of diatoms and radiolarians. The diatom is the main constituent of diatomaceous earth, which is a filter aid.

Hundreds of common substances from synthetic fibers to minerals such as hematite (Fe_2O_3) and calcite ($CaCO_3$) can be identified by their optical properties (Figs. 3 and 4). In addition, fragments of packaging components such as rubber and glass particles can also be characterized. In fact, one of the most characteristic tests for elastomer particles is the squeeze test, which is performed under the microscope (71). Finally, there is a large number of other particles, particularly those of crystalline inorganic compounds, that can be identified during a light microscopy analysis. For a detailed review of particles and characterization techniques, the reader should consult the Light Microscopy Atlases written by McCrone and coworkers (142, 145) and other references (219, 220).

Besides the measurements described above, there are other specialized microscopic techniques including dispersion staining, fluorescence, and interference microscopy which can be used to provide additional information (141, 145). Compared to the typical light microscopy measurements, these latter techniques have certain advantages.

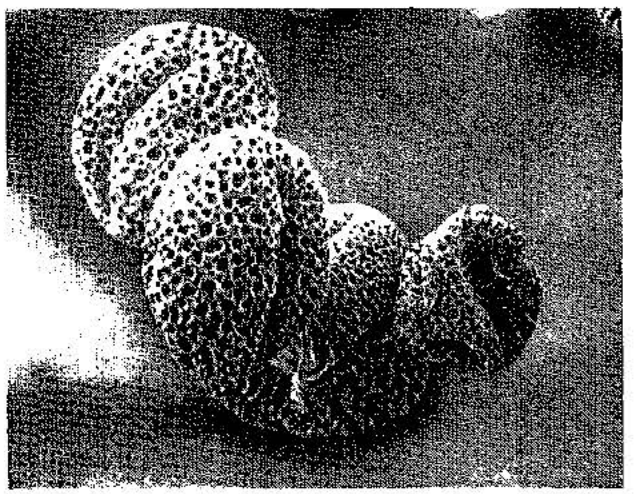

Figure 2—This is a micrograph of pollen grains, which are common air-borne particulate matter.

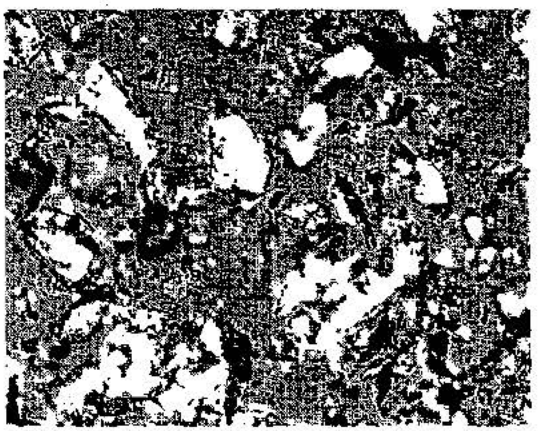

Figure 3—This is a photomicrograph of quartz (SiO_2) crystals, which are common in soil samples.

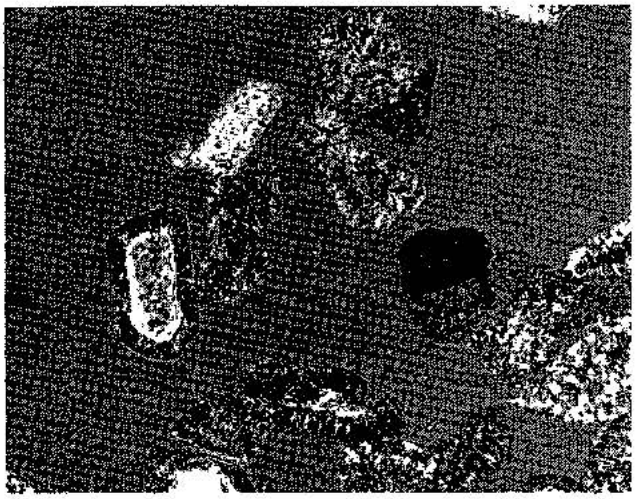

Figure 4—This is a photomicrograph of gypsum ($CaSO_4 \cdot 2H_2O$) crystals, which are common constituents of building materials.

For instance, dispersion staining can detect smaller particles than those normally observable in a light microscopy analysis, and it is very useful for the identification of trace components in the particulate population, the so-called needle-in-the-haystack analysis. Dispersion staining and other specialized light microscopy methods can provide useful information for the identification of particulate matter, but they are not as widely applicable as the other light microscopy measurements.

In general, even if an identification of the particles cannot be made using the light microscopy results, the data are normally useful. In many cases, the results of light microscopy analyses can be combined with the data from other techniques to identify the particulate matter. At the very least, the results of light microscopy experiments can be used to eliminate several possibilities. This can be especially helpful during the initial phases of particulate analyses. Finally, the information from the light microscopy measurements can be useful in directing further experiments that are to be performed on the particles.

(C) Atomic Spectroscopic Techniques: If microscopic measurements do not completely characterize the particles, elemental techniques are next used to gather atomic information concerning the particulate matter. This happens frequently, since many particles cannot be identified by microscopy alone. Even in some cases where the microscopic measurements are sufficient to characterize the particles, it is worthwhile to confirm the identification by performing elemental analyses.

Although many techniques can yield information on elemental content, there is a wide variation in their capabilities. Characteristics important to the analysis of particles include spatial resolution and imaging, multielement analysis, sensitivity, and sample preparation. Classical atomic absorption and emission techniques are poorly suited to the analysis of solid samples. Furthermore, dissolution of particulate matter to yield solution samples would negate the concentrating effect achieved during particle isolation. Bulk x-ray fluorescence analysis lacks sufficient sensitivity for most particle analysis problems. A natural extension of light microscopy which is well-suited to solids analysis is the scanning electron microscope equipped with energy dispersive x-ray fluorescence (SEM/EDXRA). This instrument, as well as the electron microprobe (EM), electron spectroscopy for chemical analysis (ESCA), and Auger electron spectroscopy (AES), will be described in terms of its applicability to particle analysis.

In the Scanning Electron Microscope (SEM), a beam of high energy electrons is rastered over a sample and an image of the sample is produced by means of low energy secondary electrons and backscattered electrons. The SEM gives a topographical picture of the material, including information concerning particle size, shape, and texture. An SEM/EDXRA system can provide elemental information by measuring the energy of the x-rays emitted from the sample when the material is exposed to an electron beam.

Only minimal sample preparation is necessary for an SEM/EDXRA analysis. Particles can be transferred directly to a specimen holder or if they were trapped on a filter, a section of the filter can be affixed to the holder. The sample is usually coated with a thin film of gold, carbon, or other conductive material prior to analysis to prevent charging by the electron beam. Modern SEM instruments can operate with high-efficiency sources at low electron energies to reduce this effect and make analysis of uncoated samples possible.

The SEM/EDXRA system is capable of excellent lateral spatial resolution, typically <0.1 μm and, consequently, the technique is ideally suited for detecting inhomogeneity among and within particles. However, the method has significantly lower capabilities for depth resolution since x-rays can be emitted from depths up to several microns (141, 159). The technique detects all x-ray energies simultaneously for all elements with an atomic number greater than or equal to 9 (*F*), although data have been generated for elements of lower atomic number (≥ 4) using thin window and windowless detectors. The method has adequate sensitivity for most of the aforementioned elements. With the exception of the lighter elements, an element that has a concentration >1–2% by weight in a particle can usually be detected.

The literature contains many reports of using SEM/EDXRA results to obtain qualitative elemental information about particulate matter (51, 52, 160–165, 221). For example, Boddapati and coworkers identified $BaSO_4$ particles in parenteral solutions based on the data obtained from light microscopy, SEM/EDXRA, and other techniques (160). In our studies with parenteral particulate matter, we have found that strong spectra of inorganic particles such as $BaSO_4$ can be obtained with very short integration times (Fig. 5). Even when a particle is an organic material, useable elemental information can be obtained using 60-sec signal integration (Fig. 6).

Although qualitative SEM/EDXRA measurements are often sufficient for some identification work, quantitative elemental analyses can also be very useful. Oles has described a semi-quantitative approach which uses the

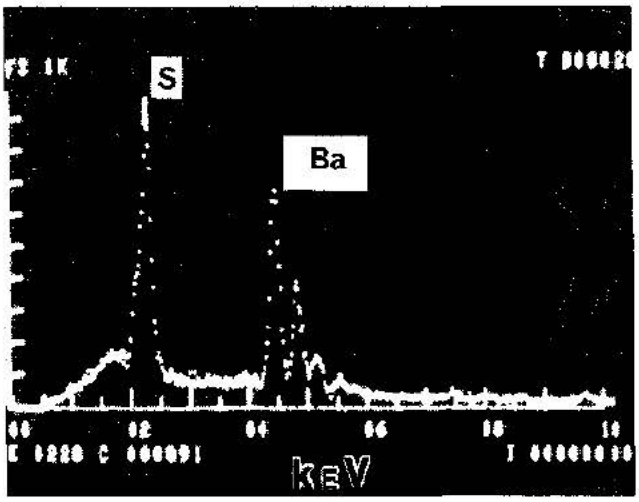

Figure 5—This is an SEM/EDXRA spectrum of a $BaSO_4$ particle. S has a peak at 2.31 KeV and Ba has a series of peaks at 4.47, 4.83, 5.13, and 5.45 KeV.

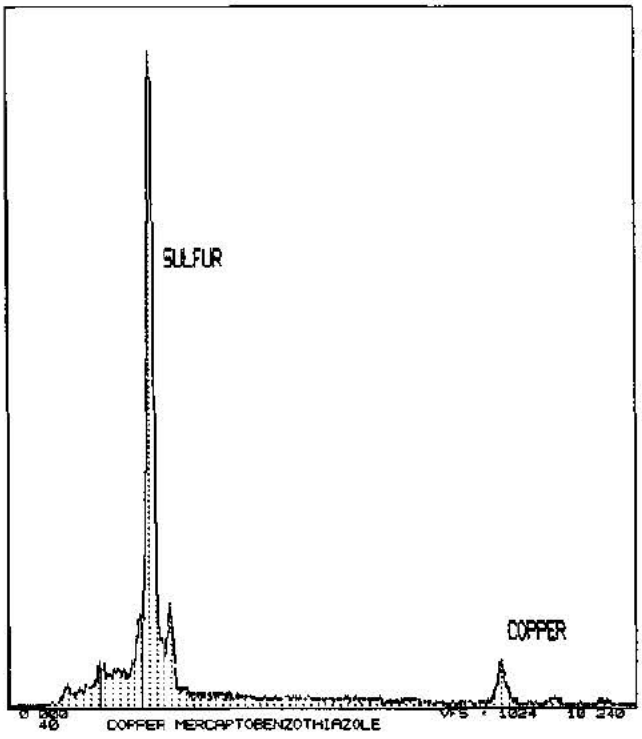

Figure 6—This is an SEM/EDXRA spectrum of a particle that was later identified as copper mercaptobenzothiazole. S has a peak at 2.31 KeV and Cu has peaks at 8.40 and 8.90 KeV.

TABLE XVII. Semi-Quantitative SEM/EDXRA Analyses for Known Particulate Compounds

Compound	Element Analyzed	Theoretical Percent	Measured Percent
$Na_2Cr_2O_7$	Cr	34.9	28
$CuSO_4$	Cu	39.8	36
SiO_2	Si	47.0	33
ZnO	Zn	80.0	72
Type 316 Stainless Steel	Cr	18.4	20
	Fe	64.4	63
	Ni	12.2	13

sample stub as an internal standard and normalizes the sample x-ray intensities with respect to the intensity observed for the Al K_α line generated from the aluminum specimen holder (166). Results of using this approach for some known particles are shown in Table XVII. Even though the approach is only semi-quantitative and has a relative accuracy of 10–25%, it can provide some very helpful information. This is especially true when one is trying to determine if the composition of the particulate matter is primarily organic or inorganic in nature.

Except for the analyses performed with windowless detectors, one disadvantage of the SEM/EDXRA measurements is the inability to detect elements lighter than fluorine. This can be a serious limitation, especially if most of the particles are organic and contain primarily C, O, N, and H. A technique that is capable of analyzing most of the lighter elements is the electron microprobe. As is true of the SEM/EDXRA system, EM uses a scanning electron beam to obtain images of the particles and measures the energy of the emitted x-rays to obtain elemental information. The electron microprobe has adequate spatial resolution capabilities (approximately 1 μm) and can detect all elements heavier than lithium (141, 167). The latter capability is achieved by using a wavelength dispersive detection system besides the energy dispersive x-ray analyzer. As expected, an EM analysis of a particle requires a significantly longer time (several minutes) than a SEM/EDXRA measurement of the same particle. Another difference between EM and SEM/EDXRA measurements is in the area of sample preparation. Prior to an EM analysis, particles are usually transferred to a Be plate. Also, it should be pointed out that EM has an excellent detection limit (<0.1%) for most elements and this combined with its ability to provide quantitative information makes it well-suited for particle identification work.

An electron microprobe is very expensive which is probably the major reason why it has not been utilized as extensively as the SEM/EDXRA technique for identifying parenteral particles. However, McCrone Associates and other microanalytical contract laboratories have used EM for this purpose. For example, Roseman and coworkers used an electron microprobe to identify some very small particles present in a product as borosilicate glass flakes (168).

Another method that can detect almost all elements of the periodic table is ESCA (141). ESCA is performed by bombarding a solid sample with soft x-rays. Photoelectrons emitted from the surface due to x-ray excitation are energy analyzed and all elements except for H and He that are present in a concentration >0.01–0.1 atom % can be identified from their binding energy. Compared to SEM/EDXRA and EM, the spatial resolution of ESCA is poor. Our spectrometer analyzes an area about 4 mm in diameter, although several commercial systems are now capable of analyzing a smaller area, approximately 250 μm in diameter. Even though the technique cannot be used to determine inhomogeneity among particles, it samples only the top 5 to 10 monolayers of a material and, hence, provides information concerning the surface of the

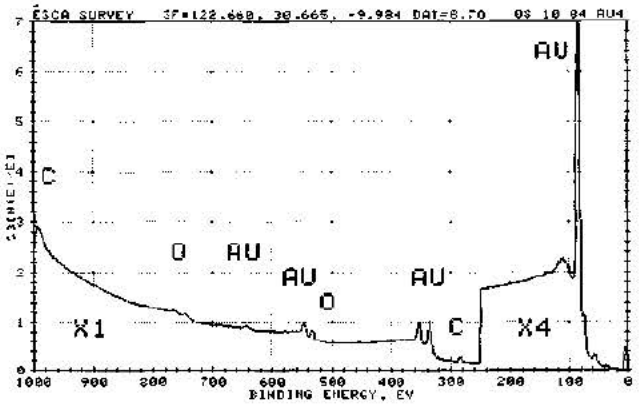

Figure 7—This is an ESCA spectrum of a Au-coated Nuclepore filter. Photoelectron counts vs. binding energy (eV). The gain for the 0–250 eV binding energy region is four times that of the rest of the spectrum.

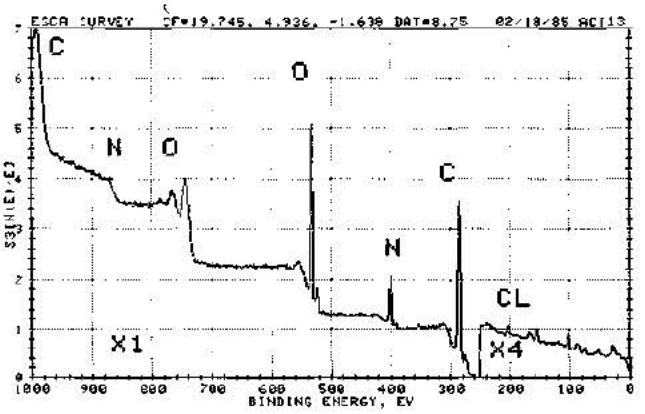

Figure 8—This is an ESCA spectrum of particles from an ampoule product trapped on a gold-coated Nuclepore filter. Quantitative studies gave the following results in atom % (H excluded): C = 65.2, O = 19.7, N = 12.0, and Cl = 0.4.

particles. Even if the surface composition is significantly different than the bulk composition of the particulate matter, this can be quite useful, since knowledge of the surface component may enable one to elucidate the mechanism of particulate formation. Also, besides qualitative elemental results, ESCA can provide quantitative information. A relative accuracy of 10–20% is typical without standards, but considerable improvement in relative accuracy (≃2–3%) can be attained when standards are utilized.

ESCA is a sensitive technique when the particulate matter can be concentrated on the surface of a substrate (141). We have found that the best sample preparation is to trap the particles on a Au-coated Nuclepore filter and place the filter directly on the sample stub. Typical ESCA analyses are nondestructive allowing the filter to be analyzed by other methods after the ESCA measurements.

Although there have been several studies that have used ESCA for the analysis of environmental particulate matter (169–174), the technique has not been used very often for the identification of parenteral particles. Our experience with organic particulate matter indicates that ESCA can be helpful when used in conjunction with other methods. For example, qualitative analyses of particulate matter found in ampoules of a parenteral solution showed that the major elements were C, O, and N, and the particles contained significantly less Cl than the active ingredient (Figs. 7–9). Quantitative analyses provided additional information and indicated that the insoluble material contained significantly more C and less O and N than the therapeutic agent.

Another surface analysis technique that can detect all elements except for H and He is Auger Electron Spectroscopy (AES). In AES, a sample is excited with an electron beam and the secondary electrons emitted from the surface are energy analyzed. With the exception of improved spatial resolution, the capabilities of AES are essentially the same as those of ESCA. AES has excellent spatial resolution, typically <1.0 μm in diameter, since it uses a focused electron beam for excitation. Although AES has been used for the identification of particulate matter in the microelectronics and metallurgy industries (175), it has not been widely used for the identification of parenteral particles. The primary reason is that in order to prevent charging problems the sample preparation of AES for insulating particles can be nontrivial.

Clearly, the information obtained from SEM/EDXRA and other atomic techniques is extremely helpful. In many cases, the elemental data and the results of light microsco-

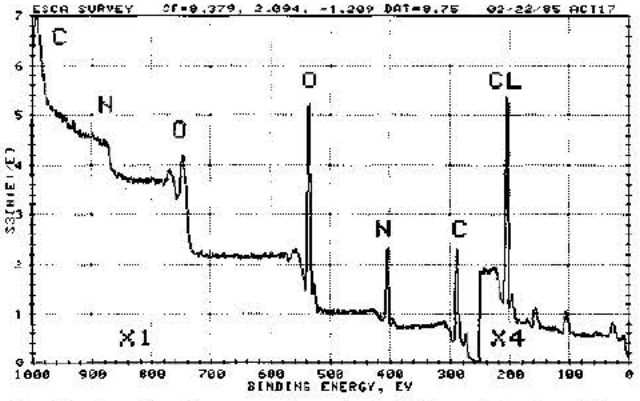

Figure 9—This is an ESCA spectrum of active ingredient for ampoule product which contained particles whose spectrum is shown in Figure 8. Quantitative studies gave the following results in atom % (H excluded): C = 47.2, O = 27.8, N = 17.6, and Cl = 7.4.

py analyses provide enough information to identify the particles. For some problems, the combination of atomic and microscopy data will not be sufficient to characterize the particulate matter, but the results may provide some useful clues concerning their composition. For example, the detection of Zn in particles that are primarily organic might suggest that the particles are related to a rubber closure ingredient. Finally, at the very least the results of light microscopy and atomic experiments can be used to eliminate several possibilities, and this is particularly helpful in guiding future identification work.

(*D*) Molecular Spectroscopic Methods: If the microscopic and atomic measurements do not completely characterize the particles, molecular techniques are next used to obtain structural and functional group information concerning the particulate matter. This can occur frequently, since some particles, especially those that are organic, cannot be identified solely on the basis of microscopy and elemental analyses. Even in those cases where the previous measurements were sufficient to characterize the particulate matter, it is often worthwhile to confirm the identification by performing molecular analyses.

Although several techniques can provide chemical structure and functional group information, there is a wide variation in their capabilities. In particular, not all the methods are applicable to most particles. For example, x-ray diffraction has been used for the identification of crystalline particulate matter (141, 153, 160, 165, 176, 177), but it cannot be used for amphorous materials. This is a definite disadvantage since the latter particles tend to occur frequently. Among the molecular techniques infrared, Raman and mass spectrometry are the most useful, and a discussion of each of these is given below.

A number of infrared techniques can be used for particle analysis. Three of the procedures have been used extensively. They involve a pin-hole aperture technique, diffuse reflectance Fourier Transform infrared spectroscopy (DRIFT), and transmission/reflection infrared microscopy.

In the pin-hole aperture method a finely pointed tungsten needle or a microspatula is used to transfer the particle directly to a thin (200–500 μm) and very small (1–2 × 3–4 mm) sodium chloride plate (178–181). In some cases, the particle has been embedded in a KBr disc by pressing the material onto a previously pressed micro pellet (180, 182). The latter is approximately 500 μm in diameter and 0.2 mm thick. After the sample has been prepared, it is centered in an aperture 50–200 μm in diameter and analyzed in a Fourier Transform infrared spectrometer (FTIR). Smaller aperture diameters have been used, but diffraction effects enforce a lower limit of approximately 10–20 μm (178, 181, 222, 223). Also, a beam condenser (4X–8X) is used to improve the sensitivity of this technique.

To prepare samples for the DRIFT method, particulate matter is trapped on a silver (Selas®) or a gold-coated Nuclepore filter. The analysis is then performed after placing the filter at the focal point of an on-axis ellipsoidal collecting mirror. The analysis is carried out in an analogous manner to that reported by Fuller and Griffiths for powdered materials which have been diluted with KCl (183). DRIFT is generally limited to cases where a large amount of particulate matter can be uniformly coated onto the filter substrate. It is not a technique for single-particle analysis in that it requires sample areas as large as a few mm^2.

Infrared microscopy is perhaps the technique best-suited to infrared analysis of individual particles or particulate inclusions in other matrices (184–186, 224, 225). Microscopic infrared analysis requires an all-reflecting microscope attached to an infrared spectrometer. Interferometric, rather than dispersive, detection is generally used in order to realize the multiplex advantage and avoid overheating the sample with an intense beam of infrared radiation. Whereas diffraction effects still limit apertures to sizes in the range of 10–20 μm, the combined capabilities of direct sample viewing and variable masking allow the analyst to accurately measure spectra of small areas within a large matrix. Importantly, these advantages greatly simplify sample preparation procedures.

Infrared microscopes are usually used in a transmission mode, requiring the transfer of a sample to an appropriate substrate such as BaF_2, KCl, or NaCl. Reflectance optics, available on many commercial instruments, allow a kind of double-pass transmission measurement, provided the sample is in close contact with a reflective surface and is not too thick. In fact, sample thickness and shape can have significant deleterious effects on infrared spectra. Fibers and irregularly shaped particles can create lensing and scattering effects, which can lead to baseline distortions and very inaccurate absorbance measurements. These problems can be of such a magnitude that spectral subtraction, a valuable method of interpreting spectra of mixtures, becomes impossible. Flattening the sample, either manually or in an KBr or diamond cell, might be necessary in some cases.

In general, infrared spectroscopy is very sensitive and is capable of recording useable spectra for subnanogram sample quantities (187). The infrared spectrum is useful because it can provide functional group information and it has a fingerprint region that is specific for each molecule. The latter is important since comprehensive spectral libraries are available with most infrared data systems.

The above techniques have been used for characterizing particulate matter in a variety of applications in the microelectronics, pharmaceutical and other industries. For example, in one of our investigations we identified particles in a product as a mixture of the active ingredient and silicone oil based on infrared spectra using the pin-hole aperture technique (Fig. 10). Spectral subtraction of the spectrum for silicone oil from the spectrum of the particulate matter gave the difference spectrum in Figure 11 and indicated that the particle contained very little besides the polysaccharide drug and silicone oil.

Raman spectroscopy has also been applied to the analysis of microscopic samples (145, 164, 188–191, 235, 236). In principle, the shorter wavelengths, usually visible, employed for Raman measurements allow higher spatial resolution than infrared spectroscopy. Raman spectra can be recorded with a spatial resolution of 1 μm, using conven-

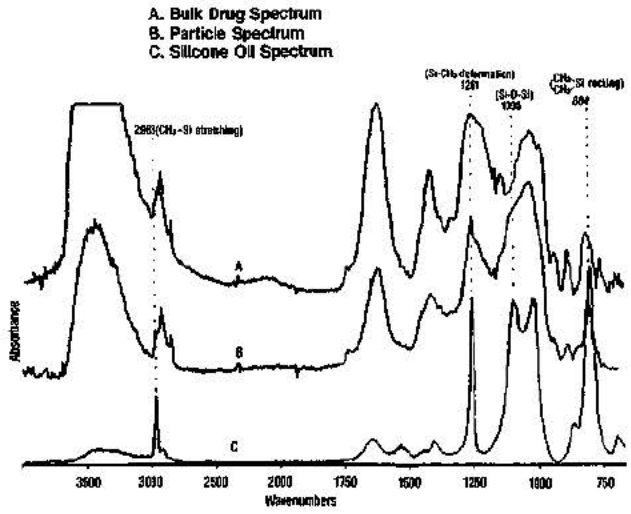

Figure 10—These are the infrared spectra in absorbance format of a polysaccharide drug (A), a particle (B), and silicone oil (C).

tional transmissive microscope optics. Such instruments can analyze sample quantities in the range of picograms to nanograms (145, 187).

A frequent problem with micro-Raman experiments is excessive sample heating. Low incident laser power and thermally conductive sample mounting can minimize this problem. Sample mounting procedures appropriate for micro-Raman work have been described in the literature (145, 192, 150). Polycarbonate and Teflon® membrane filters, commonly used for particle isolation, are unsuitable for Raman analyses because they exhibit intense and complex spectra themselves (191). Strong fluorescence can often dominate weak Raman scattering making some analyses virtually impossible. A decided advantage of Raman spectroscopy is the fact that spectra can be obtained of samples in aqueous solution or suspension. In addition to providing a good thermal sink, water yields only a weak Raman spectrum and, therefore, few spectral interferences.

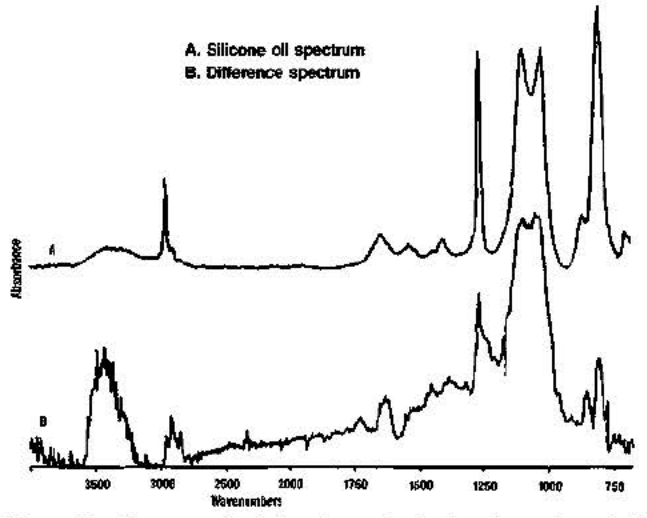

Figure 11—These are the infrared spectra in absorbance format of silicone oil and a difference spectrum. The latter was obtained by subtracting the spectrum for silicone oil from the spectrum for the particle [Fig. 10(B)].

Like infrared spectroscopy, Raman spectroscopy probes the vibrational motions of molecules. Both techniques yield functional group information as well as molecule-specific "fingerprint" spectra. In addition, certain Raman experiments detect polarization effects, which can provide further specificity for particle characterization. Unfortunately, only limited systematic collections of Raman spectra are available.

Numerous reports of particulate identification have used Raman spectroscopy (160, 162, 164, 182, 188–192). For example, Andersen used a combination of SEM/EDXRA and micro Raman analyses to identify calcium stearate particles in a parenteral product (164).

Mass spectrometry provides complementary structural information. Although there are a number of ways for obtaining mass spectra of particles including a Laser Microprobe Mass Analyzer (LAMMA), direct insertion on a wire probe, followed by thermal desorption, chemical ionization, or fast atom bombardment methods are useful for many types of sample. In the case of these direct approaches, the method of sample preparation limits the technique to particles greater than 5–10 μm in size. In contrast, the size range accessible to LAMMA analysis starts at approximately 0.2 μm (193, 194). Mass spectrometry is very sensitive and is capable of analyzing subnanogram quantities.

Mass spectrometry can be quite helpful in characterizing particulate matter. Many samples yield molecular weight information, and fragmentation patterns allow detailed structure elucidation. In one of our studies, a particle was identified as a zinc salt of mercaptobenzothiazole, a rubber stopper accelerator, based on its mass spectrum (Fig. 12). However, the technique does have some disadvantages. It can exhibit significantly greater sensitivity for volatile compounds which might comprise only a fraction of the particles. In an example mentioned earlier, dioctyl phthalate was detected by mass spectrometry in some of the isolated particles, even though the particulate matter was primarily a mixture of the active ingredient and silicone oil. For this reason, it is important to check the integrity of the sample before and after analysis to insure that the spectrum seen is truly representative of the bulk of the sample and not just a volatile component (Fig.

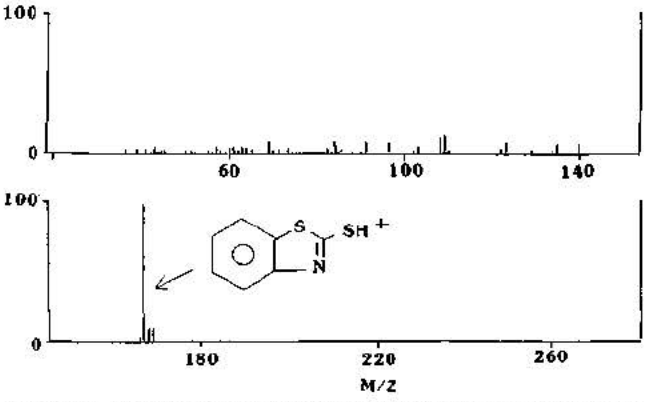

Figure 12—This is a mass spectrum of a particle that was identified as a zinc salt of mercaptobenzothiazole.

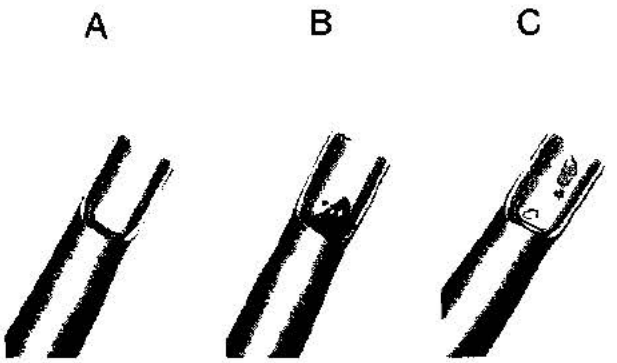

Figure 13—Particulate matter on a direct insertion probe should be examined both before and after a mass spectrometric analysis. Three possible outcomes, (A) Total Volatilization, (B) Pyrolysis, and (C) No Observable Change, are shown in this figure.

13). In addition, some particles are difficult to ionize which can prevent their analysis by this method.

In general, the information obtained from microscopic data, atomic measurements, and molecular analyses is sufficient to identify most particulate matter. For a small minority of particles, the results from these methods are not adequate to completely characterize the particulate matter, but the data usually provide fairly detailed structural clues concerning its composition.

(E) Chromatography: As noted above, the data from microscopic, atomic, and molecular techniques is sometimes not sufficient to identify the particles. For some of these situations it may not be practical to expend more effort characterizing the material. The available information could be utilized both to locate possible sources and to propose probable mechanisms of particle formation. However, for certain cases it is worthwhile to use chromatographic techniques such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) to aid the identification studies. For example, data from less sensitive techniques such as nuclear magnetic resonance (nmr) frequently require isolation of large quantities of material.

Chromatographic methods can be a help by separating the mixture into individual components which can be further identified using previous techniques. For instance, Pikal and Lang used a combination of GC and mass spectrometry to identify sulfur and paraffin wax components as the material responsible for haze in some reconstituted parenterals (195).

In a few special situations, chromatographic techniques should be used in the initial stages of identification studies and not always as a last resort. This is especially true when liquid droplets are responsible for majority of the particle counts. For example, we mentioned earlier that Horioka et al. found that when some PVC i.v. bags were shaken vigorously, di(2-ethylhexyl) phthalate (DEHP) was liberated and dispersed as microdroplets (55). The latter were recorded as particles by light blockage instrumentation, but they were not observed on a membrane filter under a microscope. These workers were able to identify the droplets as DEHP using GC analyses of the infusion fluids.

Although chromatographic and other separatory methods can be helpful, they have some disadvantages which limit their utility in particulate identification studies. One serious drawback is that during the use of these methods the particles are not in the field of view of the operator. Consequently, any subsequent identification must be verified. For instance, Pikal and Lang spiked solutions with sulfur and paraffin wax to confirm that these materials were indeed responsible for an observed haze formation (195). Another advantage is that isolation of trace levels of particulate matter using chromatographic procedures is nontrivial. In particular, it requires a significant effort and during the separation process the sample can be contaminated with compounds leached from the column. This column "bleed" can cause drastic interferences for many subsequent spectroscopic experiments.

(F) A Case History: This section has presented a general approach to identifying parenteral particulate matter. Of the variety of approaches which might be used, our experience suggests that the one outlined in this report is very effective for characterizing particles. The first steps, problem definition, sample selection, and particle isolation, are critical to the success of any identification project. Next, the particles are examined by light microscopy. If identification is not complete at this point, atomic and molecular spectroscopic techniques are employed to further characterize the particulate matter. Finally, in some cases chromtographic analysis must be used in conjunction with the various spectroscopic methods.

The following case history provides a good illustration of this approach. During the initial stages of the development of an ampoule formulation of a product, visible particulate matter was observed after a few weeks storage. The occurrence of particulate matter appeared to be related to the presence of a particular formulation excipient. In order to both test the existence of this relationship and obtain sufficient particulate matter for characterization, lab-scale lots of the product were manufactured under clean environmental conditions. Ampoules from these lots were inspected initially and at later times using both visual inspection and an Eisai automatic inspection system. Background defects present initially could be classified as environmental particulate matter (fibers, glass, etc.), and were present at a level of a few percent. Visual inspection results confirmed the occurrence of particulate matter over a period of a few weeks and established that, while the suspect excipient influenced the time required for the appearance of the particles, it was not essential for particle formation. (Importantly, later characterization established that the identities of particles from lots with and without the excipient were the same.) It is noteworthy that the fine/smoky/gossamer nature of the particulate matter partially masked its detection when using an ampoule spinner during manual inspection: the vigorous spinning tended to disperse the particles. In fact, the Eisai machine, which utilizes a vigorous spinning action, failed to reveal the presence of particulate matter long after it was visible to the naked eye.

A light microscopic examination of filtered particulate matter indicated a colorless, "greasy," amorphous solid with refractive indices in the range of 1.540–1.550. Slow

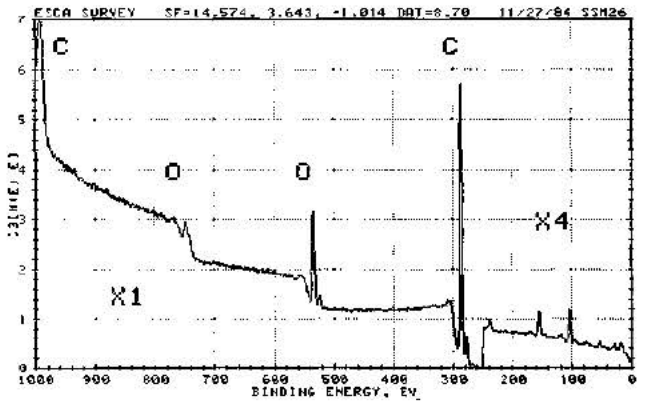

Figure 14—This is an ESCA spectrum of particles from an ampoule product trapped on a gold-coated Nucleopore filter.

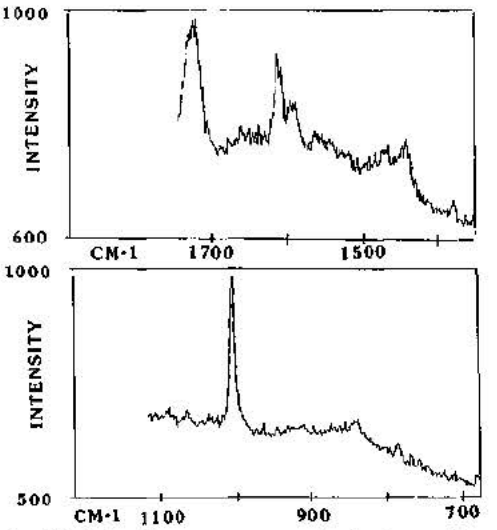

Figure 16—This is the Raman spectrum of a single particle.

melting was observed between 99 and 105 °C. The particles were partially soluble in dimethyl formamide, methanol, and acetonitrile, and insoluble in water, 5% NaOH, iso-octane, and acetone. An SEM/EDXRA analysis, which was limited to detection of elements with atomic number ≥9, showed no detectable elements in the primary, amorphous isolate. The presence of low levels of Si, Al, K, and Cr, was confirmed in a small number of particles. Microscopic analyses of this nature are important for assessing potential sample heterogeneity.

An ESCA spectrum detected primarily C and O (Fig. 14); other elements including Al, Si, and N were detected in trace amounts. High-resolution ESCA suggested the presence of an ester functional group and either aromatic or aliphatic carbon. Pin-hole aperture infrared spectra for both the particles and the active ingredient showed large differences, clearly indicting that the particulate matter was not the therapeutic agent or any closely-related analog (Fig. 15). The infrared spectrum of the particles contained a strong band at 1723 cm^{-1} and a doublet at 1304/1238 cm^{-1}, which were attributed to C=O and C—O—C stretching modes of an aromatic ester. Spectral searches comparing the particulate spectrum with those contained in the Hummel Polymer and Aldrich Neat libraries indicated that an isophthalate polymer, most likely poly(diethyleneglycol)isophthalate, PDEGI, was the best match for the particulate matter. Further infrared studies with an authentic sample of PDEGI showed some differences between the spectra of the particles and PDEGI in the 2850–2920 cm^{-1} region, suggesting the presence of another particle component, an aliphatic material. Raman spectra of PDEGI and the particles contained intense aromatic ring-breathing bands at 1000 cm^{-1} and confirmed the presence of an ester functional group (Fig. 16). Differences in the low-frequency spectrum were consistent with the presence of an aliphatic component. Finally, a careful study of the particles, PDEGI, and other model compounds by mass spectrometry confirmed the structure of PDEGI as a major particle component, along with an aliphatic material (Fig. 17).

Following this spectroscopic identification, chromatography experiments were undertaken in order to isolate the material leading to particle formation at its source. Importantly, the chromatographic isolates were examined by infrared and mass spectrometry to confirm the identity of

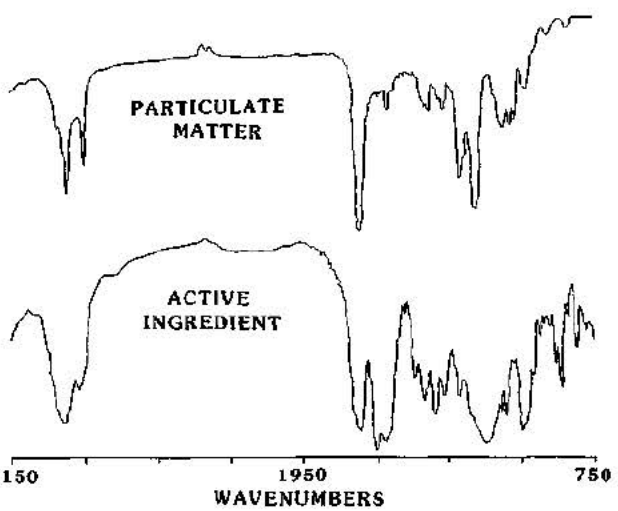

Figure 15—These are the infrared spectra of particulate matter and the active ingredient.

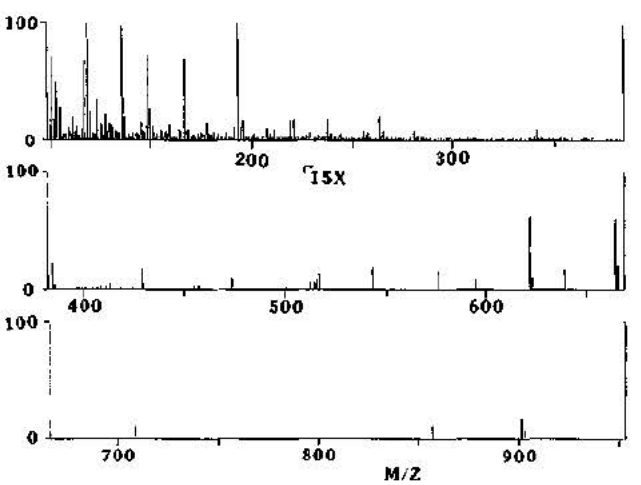

Figure 17—This is the mass spectrum of the particulate matter.

materials having similar retention behavior. This case history illustrates the use of inspection data to characterize a problem and the importance of complete particle identification using several independent and complementary analytical techniques.

3. Sources of Particles, Mechanisms of Their Formation, and Particulate Reduction Steps

The underlying objective for any particulate project is the reduction or elimination of the offending particulate matter. While the chemical composition or the identity of the particles frequently suggests their source, more often, a detailed understanding of how the particles arise is required for effective elimination. An added benefit of understanding the mechanism of particulate formation in specific cases is to highlight general areas where controls can be implemented to prevent reoccurrence of similar problems. For convenience, this section will be separated into three parts: (*A*) Sources of Particles, (*B*) Mechanisms of Their Formation, and (*C*) Particulate Reduction Steps.

(*A*) Sources of Particles: Previous review articles by other workers have thoroughly discussed the sources of particulate matter which reach the patient (4, 5). Even though any classification is arbitrary, it is useful to categorize the sources. In general, there are four broad categories including packaging materials, manufacturing factors, formulation components, and a miscellaneous group describing use factors not under the direct control of the manufacturer.

In the first category, packaging materials, particulate matter can originate from rubber closures, glass containers, or plastic components. In particular, it is important to note that the composition of a rubber stopper can be quite complex, and besides the elastomer includes some or all of the following ingredients: a vulcanizing agent, a filler, an accelerator, an activator, an antioxidant, a pigment, a plasticizer, and a lubricant (196). In addition to fragments of the elastomeric formulation, a variety of closure extractables can result in haze or particulate formation (1, 2, 4, 5, 47, 71, 164, 195, 197, 198, 226-230). For example, some of the extractables are not soluble in the parenteral solution and others can react with another component to form an insoluble material. Also, any surface debris that is not removed when the closures are cleaned can be sloughed off into the product (199) and the actual use of the product can generate particles via coring. Finally, even the volatiles from stoppers have sometimes caused haze formation for freeze-dried parenterals (195).

Glass vials, ampoules, and bottles have also been a source of particulate matter. Flakes have appeared in some parenterals which were due to materials leached from the container or caused by delamination of the glass surface by the product (4, 168, 200, 201). In addition, soluble extractables have occasionally reacted with a formulation component to form an insoluble material (160). Finally, surface debris can be a problem if the containers are not adequately cleaned, and the opening of ampoules as well as the handling of all glass containers can lead to the occurrence of glass fragments (4, 46, 65, 76, 117, 118).

Particulate matter has also come from plastic bottles and i.v. bags (4, 5). As was true of stoppers and glass containers, surface debris can slough off into the product. Finally, extractables from plastic containers have been responsible for elevated particle counts in some parenterals (4, 55, 147).

Manufacturing variables including air flow, air quality, filters, equipment, personnel, and housekeeping procedures comprise the second category. Both the air flow and air quality in the preparation and filling areas can have a significant impact on the level of particulate matter in parenterals (4, 5). Consequently, to minimize the contamination from the air many manufacturers use laminar air-flow devices and class-100 filling environments. Particles can also originate from filters. This is a source which is often overlooked since it is assumed that filters remove and do not contribute particulate matter to solutions. Another source of particles is the equipment that the product contacts, such as formulation tanks, rubber and plastic tubing, filling devices, pipe lines, valves, stills, storage tanks, and other components. Finally, personnel and housekeeping procedures can also contribute to the level of particulate matter in parenterals. In particular, ultraclean techniques are essential to ensure clean manufacturing conditions. Since operators disseminate a large number of particles as a result of metabolic processes, personnel can be the largest source of air-borne particles in filling areas, and a meticulous approach is necessary to control particulate contamination from this source.

A third category is the parenteral formulation (4, 5). The media, the active ingredients, or any of the excipients can be a source of particulate matter even when the formulation is filtered prior to filling. Since particulate problems often involve trace quantities of material, any small change in the formulation such as product degradation or interaction with packaging extractables can lead to particulate formation.

The last category is a miscellaneous group. It includes the devices used to administer the solution to the patient and the manipulations involving both the medical team and the patient (4). For example, as noted in the section on subvisible particles, any addition of solvent or change in the nature of the medium can affect the level of particle counts (55, 119). In addition, agitation, storage time, and/or temperature can influence the results of particle counting (53, 140).

Even though we have not given a detailed listing of all the possible sources of particulate matter, the discussion in this section has summarized the key areas of concern. In general, among the four categories people have found that packaging materials, especially rubber closures, have been major sources of particles (47). In fact, as mentioned earlier, the differences in USP, LVP, and SVP regulations can be rationalized if one assumes that the containers and closures are the prime contributors to the particulate load. Although the latter assumption is valid for many parenterals, one should not ignore the other possibilities when determining the particulate source in a specific product.

(B) Mechanisms of Their Formation: Once the source(s) of the particulate matter are defined, it is often useful to determine how the particles were formed. There are at least three mechanisms that have been observed. They are direct contamination/addition, precipitation/crystallization, and agglomeration.

Probably the most common mechanism is direct contamination or addition. In this mechanism, particulate matter is introduced as insoluble material. Any surface debris such as rubber closure fragments that can be sloughed off into the product is an example of direct contamination. Insoluble compounds that are extracted into the product from filters, packaging components, and other materials also illustrate this mechanism. For example, glass delamination, which is the result of attack on the glass surface by the solution, is an example of direct contamination (168, 201).

Another mechanism that has been observed is precipitation or crystallization. Some particles may be the result of precipitation of one of the components of the parenteral formulation. Generally, products are not intentionally formulated near or above the solubility limits of the components, although this is a possibility which should not be overlooked. Careful delineation of solubility as a function of pH and excipient concentration in the preformulation process provides little protection against drug or excipient precipitation that is a result of temporary storage at cold temperatures. For example, sodium phosphate buffers yield the relatively insoluble $Na_2HPO_4{\cdot}12H_2O$ at 4 °C. A more likely situation involves the presence of a contaminating species which has been leached from a closure or inadvertently introduced prior to filling. This species can interact with solution components to form a compound at a concentration above its solubility limit. For example, subvisible particles of barium sulfate that were found in several parenteral solutions were formed by the reaction of barium ions extracted from the borosilicate glass container with sulfate ions that originated from the anion of the drug and/or the bisulfate ion of the antioxidant (160). Precipitation of $Ca_3(PO_4)_2$ and/or $AlPO_4$ in parenteral phosphate buffer solutions also illustrates this mechanism (202–206).

The surfactant nature of many parenteral products can cause several unique effects relating to particle formation. Surfactants, especially when present at concentrations above a critical level at which micelle formation occurs, can dramatically influence the solubility of some excipients. The hydrocarbon-like environment in the interior of normal micelles can act as a distinct solution phase in which hydrophobic molecules can dissolve. Occasionally, the solubility of the active ingredient itself in a parenteral formulation might depend on the existence of these micelles. Components dissolved in a SVP might precipitate following dilution into an infusion solution if the dilution brought the surfactant concentration below the critical micelle concentration. Alternatively, a seemingly insignificant increase in the level of an impurity might lead to the precipitation of another species dissolved in the micellar phase if the impurity alters the solubilizing ability of the micelles. The prediction of micelle behavior in the presence of various excipients is not straightforward. Of course micellar solubilization can also be influenced by temperature. Mazer et al. have observed the growth of cholesterol microcrystals in bile-salt solutions following a drop in temperature from 60 °C to 10 °C (231).

Non-micelle-forming excipients can also act as cosolvents and affect the solubility of formulation components (232). Dilution or variations in excipient concentration can change solubilities even in the absence of a separate micellar phase. In addition to an oversaturation condition, precipitation requires the existence of nucleation sites. Thus the chance introduction of potential nucleation centers, such as contact with a closure surface, a glass defect, or a syringe needle, might cause the sudden formation of large numbers of particles. This phenomenon or excessively slow rates of particle growth might lead to unpredictable behavior.

In addition to influencing solubility, surfactants can alter rates of particle growth. Polysorbate 80 and sodium dodecyl sulfate have been shown to influence the growth rate of methylprednisolone particles without substantially affecting their overall solubility (233).

Discussions of the particle aggregation process itself are available (237, 238). The proliferation of biotechnology research will result in many polypeptide products, which are especially vulnerable to denaturation and aggregation. These products will present new challenges in the areas of small particle detection and identification.

(C) Particulate Reduction Steps: Knowledge of the identity of the particles, their source(s) and the mechanism of their formation will usually suggest the type of action that can be taken to reduce the level of particulate matter. In many cases, more than one alternative may solve the problem, although not all possibilities may be practical. A few of the numerous particulate reduction steps are discussed below.

A revision of one or more of the manufacturing procedures could lead to a reduction in the level of particulate matter. If surface debris from the environment or the packaging materials is responsible for the particles, a change in one or more of the cleaning protocols may solve the problem. In particular, there are several literature articles that present different methods for cleaning rubber closures (199, 208–210). An example of a direct contamination mechanism related to filling equipment comes from one of our investigators. Elevated defect rates for visible particulate matter prompted the examination of several defective vials of a parenteral product. Each of these contained small metallic fragments, all of which were characterized as stainless steel 316, a metal typically used for pharmaceutical equipment. Close inspection of the vials revealed tiny scratches in the vial necks (Fig. 18) aligned along the long axis of the inner glass surfaces, which were encrusted with the same type of metal flakes that were found in the solutions (Fig. 19). Further examination of the filling line showed that, for the size vial and filling head used for that product, misalignment of the reciprocating stainless steel 316 filling needles (Fig. 20) would result in contact with the glass and subsequent deposition of small metal particles. The filling needles

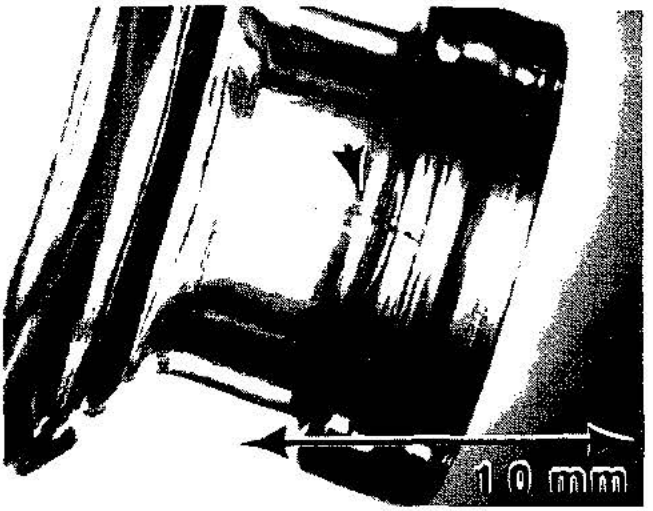

Figure 18—This is a photograph of a scratch on a vial neck, which was caused by the reciprocating filling needle.

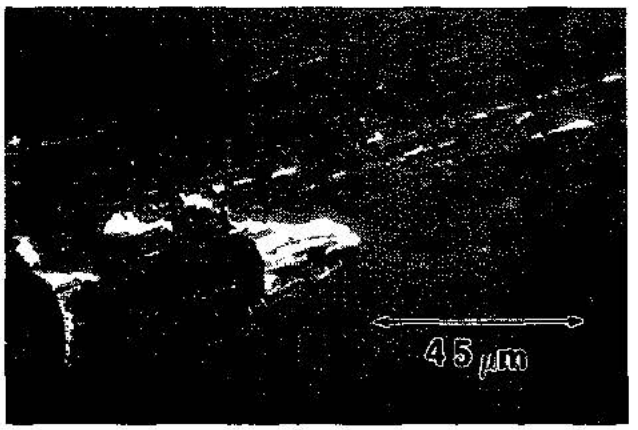

Figure 19—This is a micrograph of metal particles on glass surface in the region of the scratch. The particles were deposited by the reciprocating filling needles.

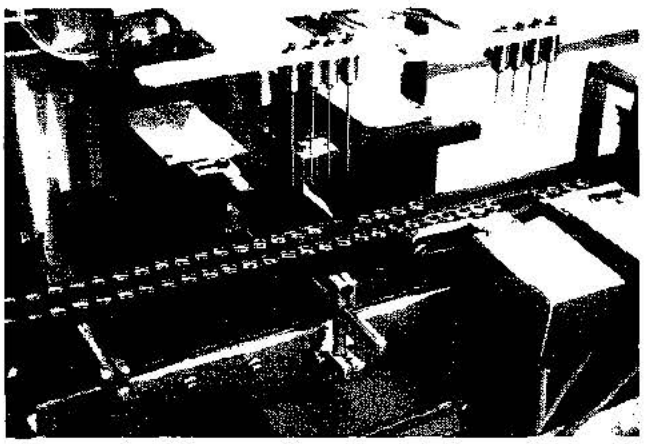

Figure 20—This is a photograph of filling line needles.

were realigned and the defect rates returned to normal levels.

In addition to steps that may be implemented at the pharmaceutical manufacturer's facility, some changes at a supplier's plant may be necessary to reduce the level of particles. For example, the source of a particulate problem in one product was traced to the quality of rubber closures sent by one of our suppliers, which had recently started a new manufacturing plant. All of the stoppers that we were receiving at that time were being made at the new facility, as compared to the closures used during the development of the product, which had been manufactured at an older plant. Initial lots of the product resulted in defect rates of 2–4%, which, even though low, was unacceptable. Identification of the particulate matter in the defective containers revealed that the particles were predominantly rubber closure fragments. Next, the closures from the defective containers were examined and found to contain ragged trim-edges and rough, pitted rims on the closure flange. It was thought that during our processing of the closures the bad trim-edges contributed rubber particles, some of which settled on the product-facing surfaces of the closures. After filling, these particles could become dislodged and add particulate matter to the product. When compared to the quality of the supplier's original samples, it was apparent that the closures being used for the new lots were of marginal quality. A visit to the supplier's new facility revealed that certain production problems were the likely cause of the marginal closures. After the supplier corrected the problems, newly manufactured closures were used for subsequent lots of product and the inspection results showed a return to acceptable defect rates (Table XVIII).

If the particulate matter is caused by an extractable from one of the packaging materials, the specific component could be replaced by a more suitable material. As noted earlier, the occurrence of barium sulfate particles in various parenterals were the result of an extraction of Ba ions from barium containing borosilicate glass containers into the solutions (160). One solution to this problem would be to use only non-barium borosilicate glasses. In fact, Kimble Glass recently developed such a glass composition because of the comments they received from the pharmaceutical industry (207). In another case, Pikal and Lang had found that the haze in a reconstituted freeze-dried parenteral was caused by volatile extractables from the rubber closures (195). Their solution to the problem was to select a different elastomeric formulation based on a comparison of the volatile extractables from several closures using a sublimation test that was specifically developed for this problem.

In some situations, one of the ingredients of the product

TABLE XVIII. Lot % Defect History Comparison

Lot	Closure Quality	Defect (%)
A	Bad Trim	2.1
B	Bad Trim	3.8
C	Bad Trim	4.2
----------	Problem Corrected	
D	Good Trim	1.1
E	Good Trim	0.9
F	Good Trim	0.9

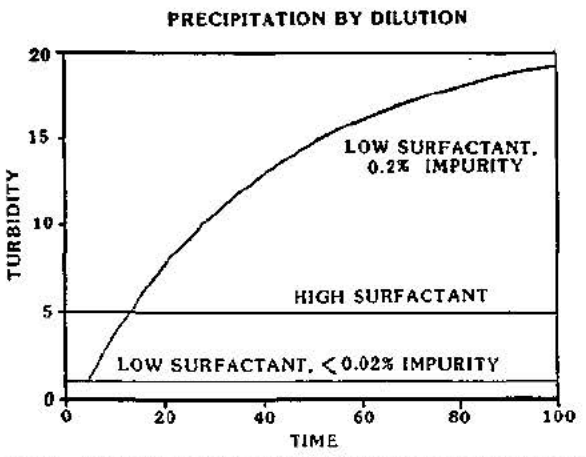

Figure 21—Turbidity data (in arbitrary units) is plotted as a function of time for various concentrations of surfactant and an insoluble impurity. One curve shows the precipitation behavior for a solution containing a high surfactant concentration. The other two curves show the precipitation behavior for a solution containing a low surfactant concentration with two different concentrations of an impurity, <0.02 and 0.2%.

is involved in the mechanism of particulate formation. In these cases, a revision in the parenteral formulation may reduce the level of particles. For instance, Hasegawa and coworkers found that the particulate matter in phosphate buffer solutions was primarily $Ca_3(PO_4)_2$ and $AlPO_4$, which they attributed to the reaction between Ca and Al ions from pH-adjusting agents and phosphate (202–206). They reported that one solution was to add a material such as EDTA to the formulation which would complex the Ca and Al cations.

A change in the specifications for the bulk drug or one of the excipients may also lead to a reduction in particles. For example, an impurity in one of the ingredients which has an extremely low solubility in the formulation may lead to particulate formation. This can easily occur for micellar solutions where the insoluble material is dissolved by the micelles at high surfactant concentration. When the solution is diluted below the critical micelle concentration, the solubilization capacity is reduced and the insoluble material can precipitate (Fig. 21). One of many possible solutions to this problem could be to significantly reduce the level of this impurity by changing the acceptance criteria for the affected material.

Finally, whatever steps are taken, it is essential to check that the level of particulate matter has actually been reduced using the appropriate visual and subvisual detection systems described in an earlier section. If the action was not successful, the approach presented in this article must be repeated. In particular, the identification of the particles in the parenterals, which were manufactured after action was taken, might indicate that there has been a reduction in the previously identified particles, but it might also reveal that there is another source of particulate matter.

Summary

The volume and depth of the literature on particulate matter in parenteral products attests to the commitment of the pharmaceutical industry to address this complex issue. Visible inspection and subvisible counting techniques have progressed to the point where an objective assessment of particulate quality is possible. Advances in analytical techniques have made particle identification more feasible and an understanding of particle formation an attainable goal. The development and maintenance of a high quality product depends upon the use of a comprehensive approach. This approach should be based on an accurate assessment of product quality and careful control of both the manufacturing environment and the formulation ingredients. When particulate levels exceed established limits, a scientific approach as outlined in this article can be an efficient means for solving the problem.

Acknowledgments

In addition to the ideas put forth in the general literature, the practices and methodologies which we have found to work best draw heavily from the experiences and contributions of our colleagues throughout the Upjohn Company. In particular, we want to acknowledge E. A. Hardwidge for his participation in the bulk of our particulate investigations.

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