Commercial Reference Shape Standards Use in the Study of Particle Shape Effect on Laser Diffraction Particle Size Analysis

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ABSTRACT

The purpose of this paper is to describe the use of LGC Promochem AEA 1001 to AEA 1003 monosized fiber-analog shape standards in the study of the effect of particle shape on laser diffraction (LD) particle size analysis (psa). The psa of the AEA standards was conducted using LD psa systems from Beckman Coulter, Horiba, and Malvern Instruments. Flow speed settings, sample refractive index values, and sample cell types were varied to examine the extent to which the shape effect on LD psa results is modified by these variables. The volume and number probability plots resulting from these measurements were each characterized by a spread in the particle size distribution that roughly extended from the breadth to the longest dimension of the particles. For most of the selected sample refractive index values, the volume probability plots were characterized by apparent bimodal distributions. The results, therefore, provide experimental verification of the conclusions from theoretical studies of LD psa system response to monosized elliptical particles in which this apparent bimodality was the predicted result in the case of flow-oriented particles. The data support the findings from previous studies conducted over the past 10 years that have called into question the verity of the tenets of, and therefore the value of the application of, the equivalent spherical volume diameter theory and the random particle orientation model to the interpretation of LD psa results from measurements made on nonspherical particles.

KEYWORDS: commercial reference shape standards, nonspherical particles, laser diffraction, equivalent spherical volume diameter, flow orientation, random orientation, mass equivalency.

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INTRODUCTION

Laser diffraction (LD) particle size analysis (psa) is one of the most commonly employed techniques within the pharmaceutical industry for the measurement of particle size distributions, with its use being strongly favored in quality control laboratories. The strengths of the technique responsible for this popularity include[1,2] (1) ease of use; (2) rapid data collection (measurements are typically completed within 60 seconds); (3) high reproducibility; (4) broad dynamic range (systems are available that cover the range from 0.02 μm to several millimeters); (5) volume distribution measurements (in those cases where the Mie theory is applicable, ie, for spherical particles less than 10 μm that are not opaque, volume-based results that can be equated to mass-based results can be obtained); and (6) flexibility (a variety of sample delivery devices have been manufactured that permit analysis of samples presented in different physical states).

Nonetheless, LD, like any analytical technique, has its limitations. The weaknesses of the present and past manifestations of LD psa systems include (1) intermanufacturer and system-generational result dependence[3-5] (differences in both the proprietary analysis algorithms and the detector configurations employed in different systems have resulted in significant differences in test results from measurements made on the same evaluation samples); (2) limit of detection[6] (subpopulations of large particles constituting up to 3% by volume of a sample can go undetected); (3) concentration dependence of results (measurements are restricted to relatively dilute samples); (4) shape dependence of results[7-21] (current algorithms assume spherical particle symmetry and provide inaccurate results when particle systems characterized by average aspect ratios even marginally greater than 1 are measured).

For a discussion of the general principles of LD, including instrumentation, sample requirements, the Mie theory, the Fraunhofer approximation, and data reporting, the reader is directed to the chapter "Size Characterization by Laser Light Diffraction Techniques" in Jillavenkatesa et al.[1]

The characterization of the limitations of LD psa has been complicated by the general absence of appropriate certified reference materials other than optically homogeneous spherical certified reference samples. This is especially true for the study of the influence of shape on psa results. Although the shape dependence of LD psa systems has received periodic attention over the past 10 years, definitive conclusions from these studies regarding the extent to which the various possible sources of variance have contributed to the differences observed between the measurement results and the expected results have been difficult to make, partly because the particle size analysts who have conducted these studies have had to try to either produce suitable standards or identify suitable samples from nature (see Table 1), and these samples have not been ideal for the intended purpose.

Since 1995, however, 3 varieties of commercial reference shape standards have been available for general purchase from either the Office of Reference Materials, Laboratory of the Government Chemist (Teddington, UK) or the supplier for this study, LGC Promochem (Teddington, UK).

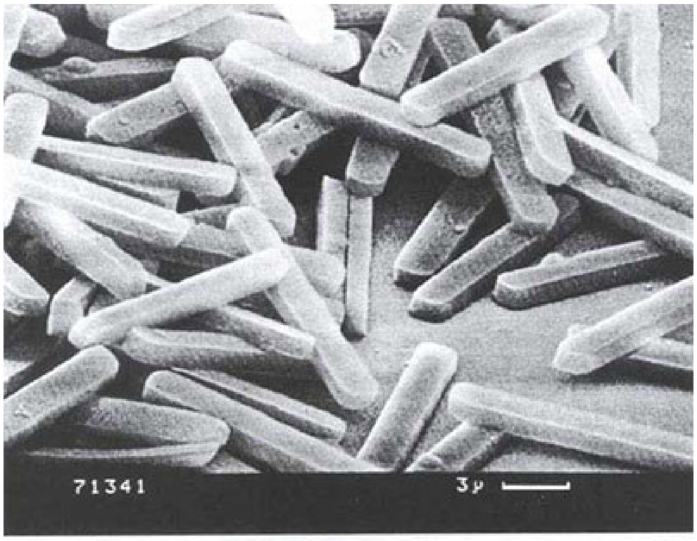
The reference standards, sold under the names AEA 1001, AEA 1002, and AEA 1003, are silicon dioxide micromachined monosized fiber-analog particles developed by Professor Paul Kaye (University of Hertfordshire, UK) and released as standards by AEA Technology (Dorchester, UK) as part of the Valid Analytical Measurement (VAM) program of the National Measurement System Policy Unit of the UK Department of Trade and Industry. The breadth and depth of the particles of each standard are essentially equal: 1.70 and 1.00 μm. The lengths are 3, 7.5, and 12 μm, respectively. Images of the 12-μm particles are presented in Figure 1.

The AEA standards were developed for the purpose of characterizing the effect of particle shape on aerodynamic psa results. Because of this originally intended application, it appears that until the time of the present study the standards had not been used in the characterization of particle shape effects on the results from LD psa. Lack of knowledge of the existence of these standards outside the United Kingdom may have been a factor, although J. P. Mitchell[24]

Table 1. Examples of Materials Previously Employed in Studies on the Effect of Particle Shape on Laser Diffraction Particle Size Analysis Results*

Material	Shape	Size (μm)			Aspect Ratio	Reference
Stainless steel	Cubes	500 × 500 × 500			1	7
	Plates	700 × 700 × 150			1	
	Rods	1000 × 200			5	
Iron	Cubes	500 × 500 × 500			1	10
Copper fibers	Rods	250 × 40			6.25	
Aluminum oxide	Blocky	(0.71, 2.10, 4.69)	d_v10, d_v50, d_v90	LD	Not calculated	14
Barium titanate	Blocky	(0.72, 1.66, 2.87)	d_v10, d_v50, d_v90	LD		
Boron nitride	Flaky	(3.43, 8.79, 17.22)	d_v10, d_v50, d_v90	LD		
Silicon nitride	Whiskers	(0.76, 3.11, 10.91)	d_v10, d_v50, d_v90	LD		
Red sugar	Cubes	1018	d_v50	LD	Not calculated	21
Orange flavoring	Rectangles	793.5	d_v50	LD		
5-aminolevulinic HCl	Rods	300.4	d_v50	LD		
Magnesium stearate	Spheres	(5.6, 11.1, 20.1)	d_v10, d_v50, d_v90	LD	~1	8
Procarbazine HCl	Plates	(11.2, 88.8, 220.5)	d_v10, d_v50, d_v90	LD	~1	
Fleroxacin	Needles	(1.5, 3.5, 7.3)	d_v10, d_v50, d_v90	LD	>1	
Copper-zinc ferrite	Spheres	55.76	d_v50	LD	~1	11,12
Iron powder 1	Irregular	165.7	d_v50	LD		
Iron powder 2	Irregular	107.73	d_v50	LD		
Iron powder 3	Irregular	76.97	d_v50	LD		
Kaolin (Sedlec Ia)	Plates	5.91	d_v50	LD	~1	20
Kaolin (KDG)	Plates	5.01	d_v50	LD		
Kaolin (KD50)	Plates	11.83	d_v50	LD		
Wollastonite	Rods	d_v50: >100; breadth: ~20		photo	>10	22
Glass fibers	Rods	Diameter: 10; length: 25-500		IA	~2.5-50	15
Alumina	Plates	Thickness: 0.6; range: >0-20		LD	1	
Mica	Plates	Range: 10-1000		IA	~1	16
Copper oxalate	Rods	Range: 0.5-2.5		IA	>2	
Nitrofurantoin	Needle	Range: <18 to >290		LD	Not calculated	23
HMX	Rectangle	Mean length/breadth 35/23		IA	1.57	18
TATB	Rectangle	Mean length/breadth 23.1/14.8		IA	1.51	
PETN	Rod	Mean length/breadth 124/17		IA	7.5	

*LD indicates laser diffraction; IA, image analysis. HMX, TATB, and PETN are the acronyms for the highly energetic materials studies at Los Alamos National Laboratory—see Mang et al[18] for details.



12µm long particles after being freed from substrate

Figure 1. Images of the AEA 1003 shape standards as provided by LGC Promochem.

had described them in 1998. Cost may also have been a factor, as they are sold as 10-mL vials of aqueous dispersions, each containing approximately 1-2 $\times$ 10^7 particles at a cost of £150.

In 2002 we purchased samples of the AEA 1001 to AEA 1003 commercial reference shape standards and performed tests to determine their suitability for this new application. At the 2003 American Association of Pharmaceutical Scientists workshop on psa, we shared some of the details of the application of these shape standards to LD psa system suitability testing.[25] The findings of these studies are presented here in greater detail.

MATERIALS AND METHODS

Samples

AEA 1001, AEA 1002, and AEA 1003 were purchased from LGC Promochem (Teddington, UK); 3-, 5-, and 12-µm polymer microspheres were purchased from Duke Scientific Corp (Fremont, CA). The AEA samples were examined without prior sonication, except where noted.

Run Conditions

The default instrument conditions recommended by instrument manufacturers were initially employed. Conditions were thereafter varied to study the effect of flow rate, index of refraction, and analysis mode (optical model) selection. Refractive index values from 1.456 to 1.60 were initially used. The Fraunhofer model was also examined using the Beckman Coulter LS 13 320 (Beckman Coulter, Inc., Fullerton, CA). The sampling times were as follows: Horiba LA-920 (Horiba Instruments, Inc., Irvine, CA): 30 seconds, Beckman Coulter LS 13 320: 60 seconds, Malvern Mastersizer 2000 (Malvern Instruments, Inc., Worcestershire, UK): 20 to 60 seconds, and Malvern Mastersizer S (Malvern Instruments, Inc., Worcestershire, UK): 20 seconds. When the Malvern and Beckman Coulter LD systems were used, the background measurements were performed using deionized water as the carrier fluid. The water was then drained and the sample cells were filled with AEA suspension. Measurements were then executed.

In the case of the Horiba LA-920 measurements in which the fraction cell was employed, the cell was filled with 10 mL of a 50/50 vol/vol% mixture of glycerin and deionized water, and the background measurement was performed. Thereafter, 1 to 5 mL of AEA suspension was added and the measurement was executed. The data were evaluated with the form of the distribution identified as "sharp."

The general purpose analysis mode of the Malvern Mastersizer 2000 was first recommended. After the first run it was recommended to change the analysis mode to the single narrow mode (spherical). The multiple narrow modes analysis mode was also applied in one instance.

The lens range and beam length employed with the Malvern Mastersizer S were 300 mm and 14.30 mm, respectively.

Pump Speed Settings

LD measurements performed using the Beckman Coulter LS 13 320 initially were conducted at a pump speed setting of 50%. Pump speed settings from 0% to 90% were subsequently employed.

All LD measurements performed using the Horiba LA-920 were conducted with a pump speed setting of 10.

LD measurements performed using the Malvern Mastersizer 2000 initially were conducted at a pump speed setting of 2400 rpm. Pump speed settings from 0 to 3500 rpm were subsequently employed.

All LD measurements performed using the Malvern Mastersizer S were conducted with a pump speed setting of 50%.

Stop-Flow Experiment

The particles were first circulated at the default pump speed setting. The flow was then stopped and data acquisition was initiated.

RESULTS AND DISCUSSION

Many aspects of the design of LD psa system hardware and software remain the same today as when the systems were first introduced to the market almost 20 years ago. The basic LD system hardware components include a laser light source, a sample cell, and some variation of the concentric ring detector design. A simplified version of the theory of operation of LD psa systems as presented by LD psa system manufacturer representatives is as follows: While flowing through a sample cell during LD psa, particles interact with the laser-emitted light, resulting in the production of diffraction patterns. The time-averaged diffraction patterns, as detected by a series of concentrically spaced detectors, are interpreted according to an analysis algorithm based on the assumption of spherical particle symmetry to produce the particle size distribution results. The analysis algorithm is appropriately applied to nonspherical particles because during the particles' transit through the flow cell the particles undergo x, y, z plane rotation as well as translation, with each possible random orientation of the particle being statistically equivalently presented to the laser, thereby allowing the equivalent spherical volume diameter of the particle, actually the size class of particles, to be calculated. The following assumptions are typically made in this operating model: (1) particles are optically homogeneous, (2) no multiple scattering exists, (3) particles are spherical, (4) particles are randomly oriented in the measurement zone (ie, the random particle orientation model is assumed), (5) data are reported in terms of equivalent spherical volume diameter (ie, the equivalent spherical volume diameter theory is assumed to be correctly and meaningfully applied), and (6) volume percentage data can be directly equated to mass distribution data.

Since 1994 it has been known that assumptions 3 to 6 are invalid for a large fraction of real-world samples as a result of their nonspherical shape. The Mie analytical expression appropriate for spheres when applied to the inversion of the diffraction patterns produced by nonspherical particles does not yield accurate equivalent spherical volume diameter data, with the degree of inaccuracy being proportional to the particle system's average aspect ratio. An appreciation of the source of this inaccuracy can be obtained from the visual comparison of the diffraction patterns produced from the illumination of pinhole and rectangular slit apertures (Figure 2).

As seen from the images in Figure 2, particle asymmetry leads to diffracted light intensity asymmetry. The greater the average aspect ratio of the particle system, the greater the diffracted light intensity asymmetry. Therefore, it is not to be expected that an analysis algorithm based on the assumption of particle spherical symmetry would provide accurate size results for nonspherical particles. The diffracted light from different dimensions of an irregularly shaped particle will register on different detectors. For an unknown sample in which neither particle size nor shape distributions are single valued, current hardware and software are insufficient to deconvolute the light intensity data. The systems cannot distinguish between smaller particles whose longest dimensional diffraction registers on a particular set of detectors and the signals from the same detectors resulting from diffraction from the smaller dimensions of larger particles.

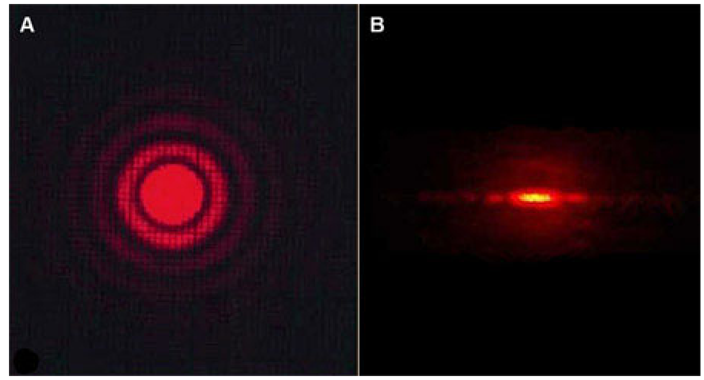


Figure 2. Diffraction patterns produced from illumination of a pinhole aperture (A) and a rectangular slit aperture (B).

The effect of shape on LD psa results as revealed from the measurements of the fiber-analog AEA commercial reference shape standards conforms to the expectations based on the above-considered points.

Shape Effect on LD Particle Size Distribution Variance

Number Probability

Figure 3 shows the number and volume distribution results for AEA 1001 to AEA 1003 using the Beckman Coulter LS 13 320 configured with the Micro Liquid Module. Figure 3A demonstrates that the primary effect of increasing deviation from spherical particle symmetry on the number probability LD particle size distribution is increasing apparent particle size distribution variance. The number probability frequency curves of the LD psa data are monomodal. The results are in agreement with the observations of Gabas et al.[7] As the custom-made stainless steel particles employed by Gabas et al were characterized by dimensions on the order of several hundred microns, result agreement indicates that the shape effect on LD psa results is not significantly modified by particle size.

Volume Probability

The primary effect of particle asymmetry on the LD volume probability data is the same as that on the number distribution (Figure 3B). However, the AEA 1002 and AEA 1003 frequency data are bimodal in appearance for almost all selected refractive index values when the Mie theory is applied (Tables 2 and 3 and Figure 4), and this behavior is not significantly influenced by the analysis mode or flow speed selected (Figure 5A-D). In the case of the AEA 1001 test results, the data do not appear bimodal, but it is

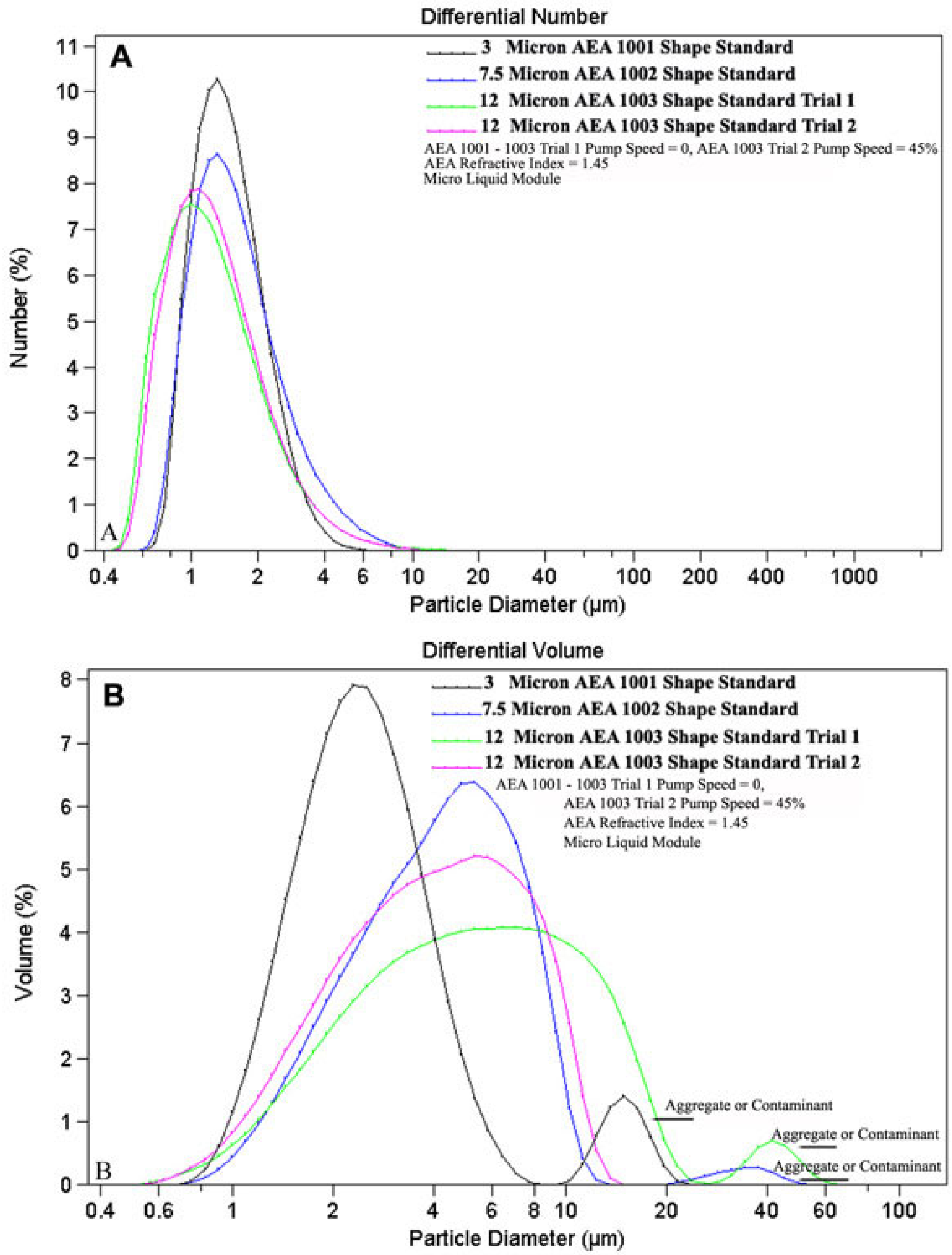


Figure 3. AEA 1001 to AEA 1003 number distribution (A) and volume distribution (B) results from the measurements using the Beckman Coulter LS 13 320 configured with the Micro Liquid Module.

believed that LD psa system resolution limitations are responsible for the apparent monomodal appearance. The results of the LD psa system resolution tests conducted by Schellhamer et al[26] provide support for this contention.

The data obtained using the Horiba LA-920 also appear monomodal (Figure 6). Horiba representatives, however, have indicated that this is the result of 2 factors: (1) the measurements were made at extremely low concentration (0.2% obscuration), and (2) the "sharp" analysis mode was chosen, based on prior knowledge of the monosized nature of the AEA standards. The Horiba representatives indicated that rod-shaped particles characterized by aspect ratios greater than 3 typically produce apparent bimodal distribution results when analyzed using Horiba's line of LD psa systems.

Matsuyama et al predicted the possibility of apparent bimodality in the measurement of asymmetric monosized materials when the particles were flow oriented within the LD psa system sample cells. They also predicted that for randomly oriented particles the apparent volume probability particle size distribution would appear monomodal.[19] It was predicted that in the case of the measurement of elliptical particles the modes of the apparent bimodal distribution would have values approximately equal to the breadth

Table 2. Intermanufacturer LD psa AEA 1001 to AEA 1003 Measurement Results Comparison*

Sample ID	AEA 1001				AEA 1002				AEA 1003			
Physical dimensions (µm)	3 × 1.7 × 1				7.5 × 1.7 × 1				12 × 1.7 × 1			
Equivalent spherical volume diameter (µm)	2.14				2.90				3.39			
Equivalent circular area diameter (µm)	2.55				4.03				5.10			
	Statistics: Volume Diameters (µm)											
LD psa system ID conditions	d10	d50	d90	d(4/3)	d10	d50	d90	d(4/3)	d10	d50	d90	d(4/3)
Beckman Coulter LS 13 320; RI = 1.54 for 3 µm	1.68	2.46	3.76	2.61	1.83	4.00	6.53	4.78	1.58	5.33	13.31	6.51
Horiba LA-920	2.29	2.55	3.26	NC	3.34	4.05	5.31	NC	NM	NM	NM	NM
Malvern Mastersizer2000 general purpose mode	1.78	2.94	5.59	3.36	NM	NM	NM	NM	2.23	4.60	10.75	5.66
Malvern Mastersizer 2000 single narrow mode (spherical)	NM	NM	NM	NM	2.02	5.23	6.76	4.71	2.07	5.34	12.71	6.48
Malvern Mastersizer 2000 single narrow mode (spherical) flow stop	NM	NM	NM	NM	NM	NM	NM	NM	2.25	5.26	9.93	5.69
Malvern Mastersizer 2000 multiple narrow mode	NM	NM	NM	NM	NM	NM	NM	NM	2.00	4.96	12.42	6.13
Malvern Mastersizer S polydisperse	NM	NM	NM	NM	1.49	4.07	9.43	5.21	1.53	3.90	11.48	5.40
	Duke Scientific Corp Polymer Microspheres											
Sample ID	**3-µm Spheres**				**5-µm Spheres**				**12-µm Spheres**			
	Statistics: Volume Diameters (µm)											
LD psa system ID conditions	d10	d50	d90	d(4/3)	d10	d50	d90	d(4/3)	d10	d50	d90	d(4/3)
Beckman Coulter LS 13 320 (University Liquid Module)	2.69	2.98	3.29	2.97	4.40	4.87	5.40	4.88	10.62	11.87	13.32	11.89
Malvern Mastersizer 2000 single narrow mode (spherical)	3.06	3.22	3.38	3.22	NM	NM	NM	NM	8.48	11.27	14.91	11.51

*LD indicates laser diffraction; psa, particle size analysis; ID, identification; RI, refractive index; NM, not measured; NC, not calculated. For a discussion of reporting of psa results, including definitions of (d) values, see the chapter "Reporting size data" in Jillavenkatesa et al.[1]

and length of the flow-oriented particles. In contrast, in the case of randomly oriented particles it was predicted that the mode value would be approximately equal to the breadth of the particles.

Circulation Speed Effect on LD psa Results—Flow Orientation

It has been claimed that equivalent spherical volume data for high-aspect-ratio samples can be obtained from LD psa measurements if the experiments are conducted using the stop-flow method of data acquisition. As the name implies, stop-flow experiments are those in which the sample is introduced into the sample cell and circulated for a period of time, the circulation (flow) is stopped, and data acquisition is initiated. The supposed effect of the stop-flow step is the induction of random orientation in the case of particle systems that have been observed to be or are suspected of being flow aligned. Integration of the measurements of the particles in each of their statistically equivalent random orientations supposedly would provide the desired equivalent spherical volume diameter.

However, in the case of the stop-flow experiment conducted using the Malvern Mastersizer 2000, although the results obtained were significantly altered from those obtained using the default parameter settings, they were not rendered any more easily interpretable in terms of the equivalent spherical volume diameter theory (Figure 5D). When the Micro

Table 3. Effect of Refractive Index Choice Sample: AEA 1002 Beckman Coulter LS 13 320 Universal Liquid Module*

	Volume (µm)						Number (µm)				
Conditions	d10	d50	d90	Mean	Mode 1	Mode 2	d10	d50	d90	Mean	Mode
PS 10% RI 1.456 Obs 1%	1.643	3.906	7.376	4.234	5.878	NA	0.932	1.438	2.771	1.708	1.204
PS 10% RI 1.60 Obs 1%	1.885	4.683	7.901	4.569	2.11	6.45	1.682	2.003	2.554	2.195	1.919
PS 10% RI 4.2 Obs 1%	1.374	3.202	7.975	4.088	7.08	2.11	0.963	1.341	2.202	1.525	1.204

*PS indicates pump speed; RI, refractive index; obs %, percent obscuration; NA, not applicable.

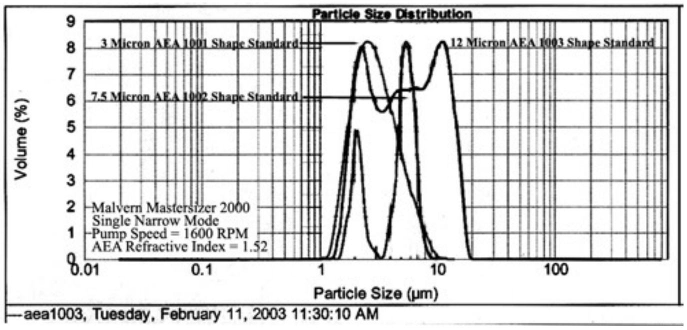


Figure 4. AEA 1001 to AEA 1003 volume distribution results from the measurements using the Malvern Mastersizer 2000 configured with the Hydro 2000µP module.

Liquid Module of the 13 320 is used, a difference does seem to exist between the results obtained from testing a freshly introduced sample without stirring and the results obtained from testing performed at all other stir speeds (Table 4). The particle size distribution variance of the noncirculated sample test results is larger than that of all other collected results, with the range extending to twice the longest dimension of the particles. The mode values appear to be increased and appear to correspond to the known longest dimension of the particle system. This differs from the results of the circulated samples, in which the mode occurs at significantly smaller values. The particles of a sample freshly introduced into a sample cell can reasonably be expected to exist in a state of highly random particle orientation. As discussed below, even at low circulation speeds this state is probably made to transition to a more ordered state in which particles are flow aligned. The difference between the ordered and disordered particle states in the noncirculated and circulated samples is probably responsible for the noted differences in the respective particle size distribution results. Some aggregation is noted in the images resulting from the Malvern FPIA 2100 analysis of a mixture of AEA 1002 and AEA 1003 samples (Figure 7). Therefore, aggregation does contribute to the greater variance of the noncirculated sample test results, but it is not believed to be the sole factor. The absence of this effect in the circulated samples indicates that the aggregates are weakly associated.

A significant influence of circulation speed between 10% and 90% on the results of the tests conducted in this study using the Coulter LS 13 320 configured with the Universal Liquid Module was not noted (Table 5). This circulation speed independence was expected based on the following points. In the 2003 edition of *Powder Sampling and Particle Size Determination,* Terence Allen[27] states as a matter of fact that within the flow cell of the Beckman Coulter RapidVUE automated image analysis system, particles are flow oriented. The orientation of nonspherical particles within the flow cell of the RapidVUE was also observed in our laboratory during a demonstration of the technology. It was also noted that the design of the RapidVUE flow cell is very similar to that of the Universal Liquid Module of the Beckman Coulter LS 13 320. Therefore, it was expected

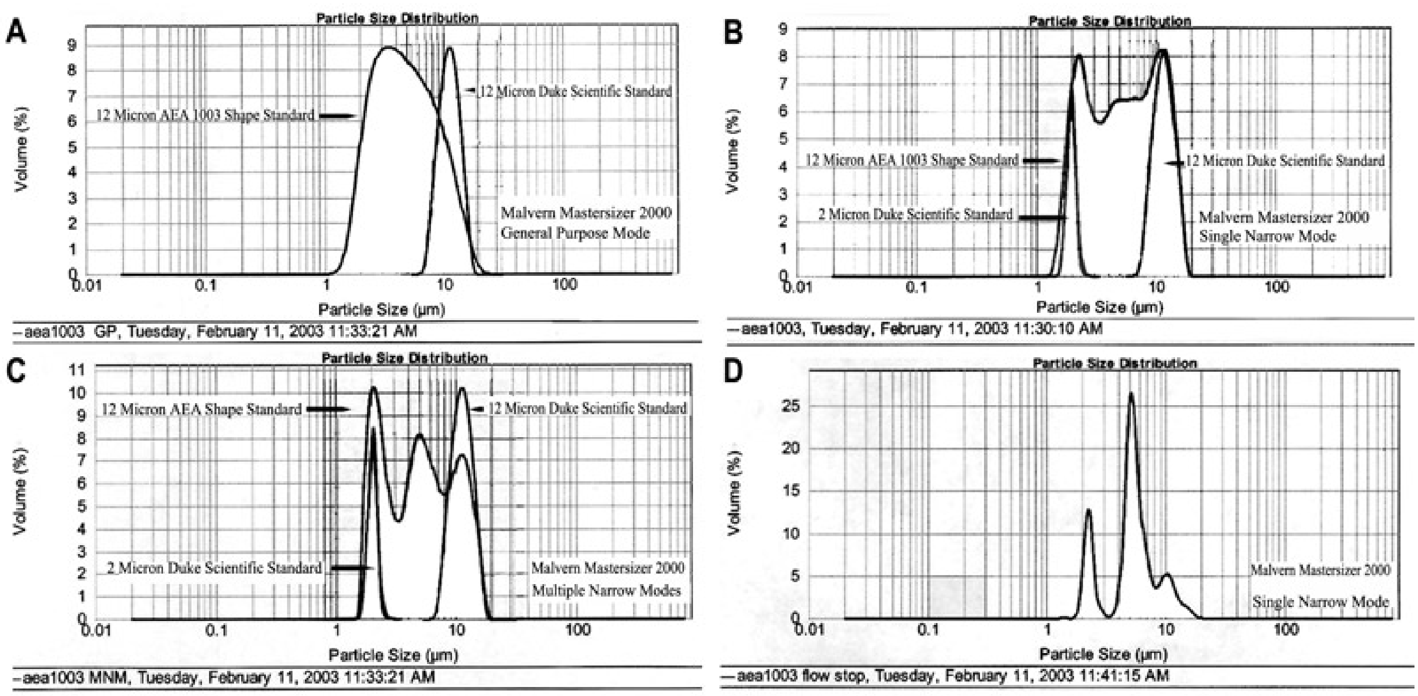


Figure 5. AEA 1003 volume distribution results from the measurements using the Malvern Mastersizer 2000 configured with the Hydro 2000µP module: (A) general purpose mode, (B) single narrow mode (spherical), (C) multiple narrow modes, (D) flow stop: single narrow mode (spherical).

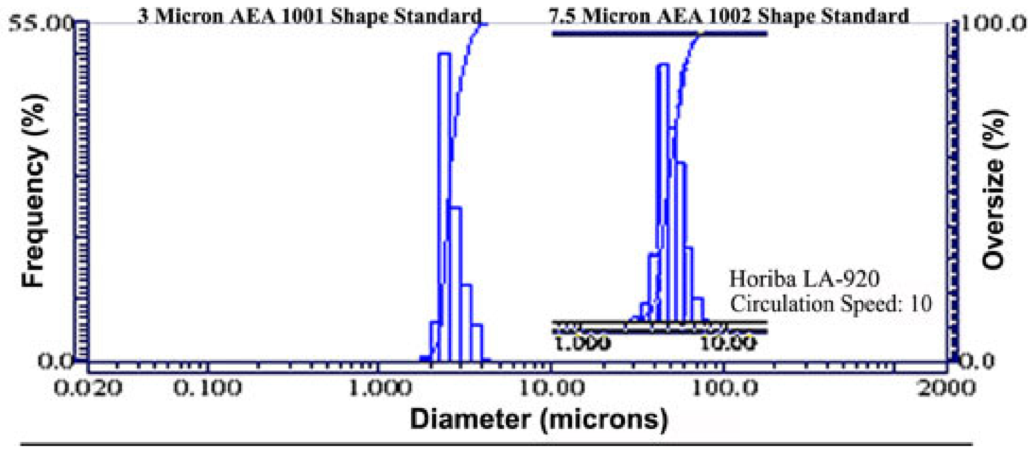


Figure 6. AEA 1001 to AEA 1002 volume distribution results from the measurements using the Horiba LA-920 configured with the fraction cell.

that at all nonzero flow speeds, nonspherical particles would also be to some extent flow oriented within the Universal Liquid Module.

Other factors that contribute to the expectation of flow speed independence as a result of flow orientation include (1) the demonstration by Berthold et al[22] of laminar flow within LD psa system flow cells and their further demonstration of the orientation of fiberlike particles in the direction of the laminar flow; (2) recently conducted chemical engineering experimental and theoretical studies[28,29] on the orientation of solids in laminar flow pipes showing that laminar flow conditions characterized by even modest Reynolds numbers (>100) result in a flow orientation of particles with little dependence on particle aspect ratio or density; and (3) the statement from Xu and Di Guida[21] in their 2003 publication that "for non-spherical particles larger than about 20 microns the angular scattering patterns for LD are mainly produced by the projection of particles perpendicular to the beam" (page 150).

The results of this study, therefore, indicate that flow orientation of nonspherical particles is a phenomenon that occurs in LD psa flow cells to particles that are as small as 3 μm in length and to particles that have aspect ratio values as small as 1.7.

Implications for the Correlation of LD psa Results to the Results From Other Techniques— Image Analysis Example

Equivalent spherical volume diameter is the measure of size that LD psa system manufacturers most often claim is provided by their systems. However, as seen from the data presented in Table 2, whereas there is no obvious correlation between any of the values of the statistical descriptors from the LD psa measurements of the AEA standards and the AEA equivalent spherical volume diameters, the d_v50 values do appear to correlate with the equivalent circular area diameter values of the AEA standards.

This observation is in agreement with the findings of Brewer and Ramsland,[8] who in 1994 compared image analysis and LD psa results from the measurements of 3 samples characterized by spherical, platelike, and needle-like morphologies. In their study, the measured image analysis cumulative

Table 4. Circulation Speed Examination Results Sample: AEA 1003 Beckman Coulter LS 13 320 Micro Liquid Module*

Beckman Coulter LS 13 320 Micro Liquid Module											
	Volume (μm)						Number (μm)				
Conditions	d10	d50	d90	Mean	Mode 1	Mode 2	d10	d50	d90	Mean	Mode
PS 0% RI 1.456 Obs 8%	1.58	5.33	13.31	6.49	11.29	NA	0.48	0.87	1.97	1.11	0.689
PS 43% RI 1.456 Obs 3%	1.60	4.05	8.59	4.61	5.35	Shoulder ~4.60	0.73	1.21	2.50	1.48	1.10
Mean SD	1.59	4.69	10.95	5.55			0.60	1.04	2.23	1.30	
	0.01	0.91	3.34	1.33			0.17	0.24	0.38	0.26	
PS 0% RI 4.2 Obs 8%	1.39	5.08	13.45	6.37	11.29	2.11	0.74	1.11	2.00	1.30	1.00
PS 43% RI 4.2 Obs 3%	1.32	3.54	9.10	4.47	8.54	2.11	0.77	1.15	2.09	1.35	1.00
Mean SD	1.35	4.31	11.27	5.42			0.76	1.13	2.05	1.33	1.00
	0.04	1.09	3.08	1.34			0.02	0.03	0.06	0.03	

*PS indicates pump speed; RI, refractive index; obs %, percent obscuration; NA, not applicable.

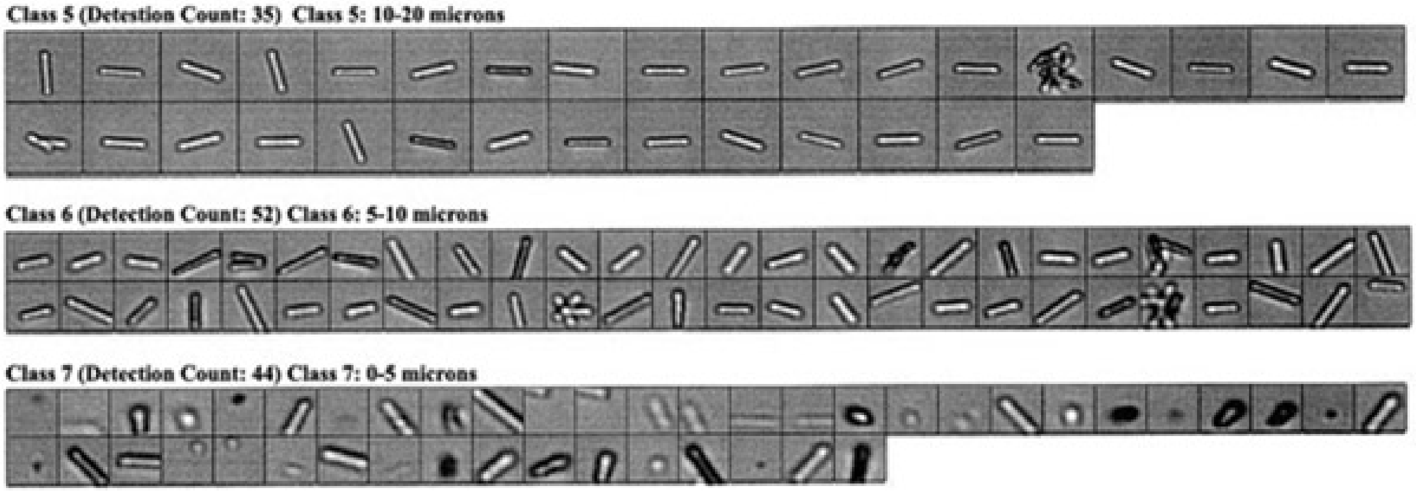


Figure 7. Images from the Malvern FPIA 2100 analysis of a mixture of AEA 1002 and AEA 1003 showing evidence of aggregation.

area percent data, which is not to be confused with its equivalent circular area diameter transformation, was compared with the so-called LD cumulative volume percent data. In the cases of the spherical and platelike particles, strong positive correlations were established.

Choice of the Appropriate Size Descriptor: Multiple Size Descriptor Nature of LD psa Results

In the past 10 years, investigators who have attempted to compare LD and image analysis results from the measurements of the same samples have for this purpose (in all cases we know of, at least) chosen a single size descriptor from the many available from image analysis.[8,11,12,14-18,20,21]

Most often, the size descriptor chosen has been some variation of the equivalent circular area diameter or the equivalent spherical volume diameter.

However, the bimodal nature of the volume probability frequency presentation of the LD psa data collected from the measurements of the fiber-analog AEA standards indicates that multiple size descriptors from image analysis would be required for a successful correlation with the complete LD psa data set. As the first mode of the AEA graphical

Table 5. Circulation Speed Examination Results Sample: AEA 1002 Beckman Coulter LS 13 320 Universal Liquid Module*

	Volume (μm)						Number (μm)				
Conditions	d10	d50	d90	Mean	Mode 1	Mode 2	d10	d50	d90	Mean	Mode
PS 10% RI 1.456 Obs 9%	2.03	4.08	6.46	4.16	6.452	NA	1.46	2.05	3.78	2.37	1.75
PS 20% RI 1.456 Obs 1%	1.91	4.32	6.54	4.21	5.878	NA	1.46	1.95	3.47	2.26	1.75
PS 51% RI 1.456 Obs 2%	1.83	4.00	6.53	4.06	5.878	NA	1.34	1.84	3.22	2.13	1.75
PS 90% RI 1.456 Obs 9%	2.10	4.34	7.13	4.48	7.083	NA	1.50	2.11	3.89	2.45	1.92
Mean	1.97	4.19	6.66	4.23			1.44	1.99	3.59	2.30	1.79
SD	0.12	0.17	0.31	0.18			0.07	0.12	0.30	0.14	0.09
PS 10% RI 1.60 Obs 9%	1.89	4.25	6.56	3.99	2.11	6.45	1.70	2.02	2.60	2.24	1.919
PS 20% RI 1.60 Obs 1%	1.90	4.79	7.29	4.44	6.45	2.11	1.69	2.01	2.58	2.22	1.92
PS 51% RI 1.60 Obs 2%	1.88	4.47	7.32	4.31	2.11	6.45	1.67	2.00	2.58	2.20	1.92
PS 90% RI 1.60 Obs 9%	1.93	4.67	7.30	4.47	7.08	2.11	1.72	2.04	2.63	2.29	1.92
Mean	1.90	4.55	7.12	4.30			1.70	2.02	2.60	2.24	1.92
SD	0.02	0.24	0.37	0.22			0.02	0.02	0.02	0.04	0.00
PS 10% Fraunhofer Obs 9%	1.21	2.68	6.06	3.23	5.88	2.11	0.75	1.13	2.01	1.31	1.00
PS 20% Fraunhofer Obs 1%	1.22	3.04	7.71	3.88	7.08	1.92	0.736	1.10	1.95	1.28	1.00
PS 51% Fraunhofer Obs 2%	1.21	2.93	7.54	3.75	1.92	6.45	0.71	1.07	1.94	1.25	1.00
PS 90% Fraunhofer Obs 9%	1.23	2.73	6.75	3.46	6.45	1.92	0.75	1.14	2.01	1.31	1.00
Mean	1.22	2.85	7.01	3.58			0.74	1.11	1.98	1.29	1.00
SD	0.01	0.17	0.76	0.29			0.02	0.03	0.04	0.03	

*PS indicates pump speed; RI, refractive index; obs %, percent obscuration; NA, not applicable.

results roughly corresponds to the breadth of the particle system and the second mode roughly corresponds to the particle system's longest dimension, it is speculated that for particles similar in shape to the AEA standards, at least 2 size descriptors might be required (eg, breadth and longest dimension). Alternatively, at least in the case of particle systems in which the breadth and the longest dimension distributions do not significantly overlap, perhaps a reintegration of the LD psa data would be sufficient to render the LD psa data comparable to a single size descriptor from the image analysis data. Reintegration would deconvolute the LD psa data by essentially subtracting the contributions from all but one significant dimensional source of diffraction. The problem is determining the correct integration limits.

Mass Distribution Equivalency of LD Equivalent Spherical Volume Diameter Particle Size Distribution Data

It is often claimed[30-34] that if the particles within a sample are characterized by constant density, then the volume percent data presentation from the LD psa of the sample is equivalent to the sample's mass-weighted particle size distribution. The premise of this claim is that LD psa systems provide equivalent spherical volume diameter data. Because it has been demonstrated that this is generally not true, especially in the case of nonspherical particles, it must be concluded that in general LD psa volume percent data cannot be equated to mass-weighted particle size distribution data.

Implications for the Quality Control Application of LD psa Systems

The quality control application of psa techniques is typically in the monitoring of lot-to-lot particle size distribution consistency of product constituents and/or products. In this application, accuracy is often irrelevant, which is a point that is often asserted by advocates of the quality assurance application of LD psa but then forgotten in subsequent discussions of the merits of Mie versus Fraunhofer analysis routines. As shown above, the LD psa system signal is both size and shape dependent. When the quality control suitability of any LD psa system is considered, regardless of whether the system interprets the diffraction pattern using a Mie- or Fraunhofer-based analysis algorithm, it must be determined whether the system is sufficiently sensitive to allow the degree of change control desired. This determination would ideally be made by means of simulation studies in which standards would be used to model the samples of interest. Unfortunately, such experiments are difficult to perform, as only 3 commercial reference shape standards exist (ie, the AEA shape standards), and these standards are not ideally suited for this purpose, because they are limited in size range and shape and are present as only relatively low concentration aqueous suspensions that render, because of their cost, their routine use financially prohibitive. This point having been made, attention should be directed to the possible limitation of LD psa systems in the detection of significant changes in the percent contribution of fines in particle systems consisting of high-aspect-ratio particles. At a 2001 US Food and Drug Administration Science Day event, Prasanna et al[23] presented a poster in which the results of a comparative study of the performance of an ensemble psa technique (ie, LD psa) and particle counting techniques (eg, image analysis) in the psa measurement of an acicular particle system—nitrofurantoin—were reported. One conclusion from this study was that "the large population of fines was only detected by the particle counting techniques and not by the ensemble technique." A possible explanation for the observed apparent insensitivity of LD psa to the fines within the nitrofurantoin samples is based on signal interference. Given the results from the LD psa testing of the AEA standards, it is to be expected that the apparent particle size distribution of the larger acicular nitrofurantoin particles overlapped the apparent particle size distribution of the fines. As LD intensity is a function of the square of particle cross sectional area, the signal from the breadth of the larger acicular particles may have rendered the intensity contribution from the fines indistinguishable from total intensity random fluctuation, thereby rendering LD psa insensitive to changes in the concentration, the size distribution, and the shape distribution of the fines.

The above discussion underscores the importance of discrimination testing during method development by means of the employment of orthogonal techniques and cross validation/verification of the results, especially in analysis of nonspherical particles, and especially if the monitoring of fines is critical. This testing may indicate the necessity of the use of multiple psa techniques in the psa monitoring of quality control samples.

CONCLUSIONS

The LGC Promochem AEA 1001 to 1003 study data show that these commercial reference shape standards can be successfully applied to the study of shape effects on LD psa results. The data support the findings from studies conducted over the past 10 years that have called into question the verity of the tenets of, and therefore the value of the application of, the equivalent spherical volume diameter theory and the random particle orientation model to the interpretation of LD psa results from measurements made on nonspherical particles. This is especially true if the goal is the correlation of LD psa measurement results with the results from other psa techniques. The study has also highlighted the need for additional shape standards of varying geometry that are affordable and available in a variety of monosize and polydisperse wet and dry preparations to allow the simulation studies required in the case-by-case determination of the appropriateness of the quality control application of LD psa systems.

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