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Assessing the Accuracy of Routine Photon Correlation Spectroscopy Analysis of Heterogeneous Size Distributions

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ABSTRACT

The aim of the current study was to investigate the ability of a fixed-angle routine photon correlation spectrometer (PCS) to resolve bimodal size distributions. The focus was on dispersions consisting of a majority of smaller and a minority of bigger particles. Monodisperse latex beads of sizes from 21 to 269 nm were measured first as single-size dispersions and then with various binary blends. For single-size dispersions, the mean diameters obtained were as indicated by the manufacturer, except for 21- and 34-nm particles, which were somewhat smaller. PCS analysis of blends of 21 + 102-nm and 34 + 102-nm particles resulted in bimodal distributions with particle diameters of the 2 peaks in the expected magnitude down to critical blending ratios of 0.002% and 0.08% of bigger particles, respectively. At these ratios, PCS results became inconsistent, and an increased number of monomodal results and/or high residuals were seen. For 21 + 102-nm blends, at even smaller ratios (0.001%), more consistent results were obtained again with predominantly monomodal distributions in the size range of the smaller particles (ie, the bigger particles were neglected). PCS analysis of blends of 21 + 269-nm particles yielded bimodal distributions with diameters within the expected magnitude as long as the content of bigger particles did not exceed 0.005%. Above this ratio, predominantly monomodal results with mean diameters in the magnitude of the bigger particles were obtained (ie, the smaller particles were neglected). In conclusion, a routine PCS instrument can resolve bimodal size distributions of colloidal dispersions only at certain ratios of the 2 subpopulations. Both low and high ratios lead to 1 of the 2 subpopulations being neglected.

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INTRODUCTION

In the field of drug delivery, preparations containing submicron particles are emerging as an important contribution for achieving either controlled (sustained-release) or targeted delivery of the active compound. Examples for such particulate drug carriers are polymeric and solid lipid nanoparticles as well as liposomes and submicron emulsions. For all these systems the particle size plays a key role[1-5] in their in vitro behavior (eg, loading-capacity, sedimentation/floating-behavior) as well as their fate in vivo, such as biodistribution and pharmacokinetics. Especially for preparations for intravenous applications, stringent control of size distribution is crucial, as the size of particles is a determinant for the site of action in the human body.[6] Photon correlation spectroscopy (PCS) is a widely used method for measuring submicron particles, and its range (from 5 nm up to 5 μm)[7] makes it especially convenient for measuring the size distribution of submicron particles. It is generally accepted that PCS analysis yields reliable results when monodisperse samples are measured. There are reports in the literature that micron-range contaminants in submicron particle dispersions are hardly detected by dynamic laser light scattering.[2, 4]

Advanced mathematical routines may allow the size distribution of samples consisting of 2 or more subpopulations of different particles in the submicron size range to be resolved, as demonstrated by various model blends.[5, 8] Little is known, however, about whether the size analysis of real colloidal dispersions is hampered by a minute amount of submicron particulate contaminant like flocs, aggregates, or dust particles.

The aim of this study was to check the ability of a routine PCS apparatus to detect the presence of a minor amount of significantly bigger particles in dispersions consisting of small (<<100 nm) colloidal particles. In order to do so, bimodal model systems consisting of Nanosphere (Duke Scientific Corp, Palo Alto, CA) size standards were analyzed. PCS instruments like the one used here (NICOMP 380, Particle Sizing Systems, Santa Barbara, CA) are in widespread use for routine particle size analysis of submicron particles. Most certainly, better results might have been achieved if more advanced equipment had been used (eg, multi-angle PCS) and if the manual adjustment of apparatus parameters had been performed. However, the aim here was to perform a study under routine conditions.

MATERIALS AND METHODS

Photon Correlation Spectroscopy

The PCS instrument used was a NICOMP 380 with a fixed 90° scattering and external fiber angle, and a 632.8-nm helium-neon laser. Each sample was measured in 10 parallels, where each cycle lasted for 5, 15, or 60 minutes, respectively. The autocorrelator was set to clear after each cycle (ie, every cycle represents an independent parallel). A new sample was prepared after every set of 10 parallels. The PCS instrument settings were as follows:

- Control Menu—channel width, autoset; liquid viscosity, 0.933cP; liquid index of refraction, 1.333; intensity set point, 300 kHz.
- NICOMP Input Menu—minimum diameter, 10 nm; plot size, 45; smoothing, 3; plot range, 100.

Processing of Results from PCS

Using single-size latex bead dispersions, a Gaussian distribution could be assumed. The following values were recorded: mean particle diameter, distribution width (SD), chi-squared (χ^2), baseline adjustment, average intensity, and amount of data in channel 1.

Chi-square: Any value close to (or below) 1.0 indicates an exceptionally good fit of the quadratic function to the reduced data. Assuming that sufficient statistics have been collected in the autocorrelation function to make the value of χ^2, a low value means that the Gaussian representation of the particle size distribution is a good assumption (ie, that no other distribution shape can offer a better fit to the data). A value of χ^2 exceeding 3, suggests that the Gaussian analysis result is inappropriate.

Baseline adjustment: The autocorrelation function C(t') decays toward the long-t' limiting value (baseline B); the value of B is adjusted by the software to achieve the lowest possible value of χ^2. A value of baseline adjustment in the range < 0.03 indicates that almost no adjustment in the value of B was needed to obtain the lowest value of χ^2. If values out of this range are achieved, NICOMP distribution should be applied.

Data in channel 1: a relatively large value, exceeding 1 million (= 1000 K), usually indicates a high degree of statistical accuracy. At this point, the results of the Gaussian analysis should have become stable, with relatively little change with additional run time.[7]

In order to compare the parallels, average values and SDs for all the above parameters were calculated for every set of 10 parallels. When measuring binary blends of latex bead dispersions, the so-called "NICOMP distribution model" was used. The following values were recorded: mean diameter peak 1 and 2, percentage peak 1 and 2, fit error, residual, average intensity, and amount of data in channel 1.

Fit error: This parameter provides an indication of how relatively stable, or settled, the distribution analysis results are. For more complex distributions, it is usually advisable to achieve a fit error below 1.5, or even approaching 1.0, to obtain the most accurate and reproducible results.

Residual: This value indicates whether neglible concentrations of aggregates are detected; hence the ideal value should be close to nil, where no aggregates are detected. If the value is close to or above 10, then, according to the increased amount of data collected, some observations can be made: the residual parameter will either remain high with no changes in the plot, or it will drop to a value near nil and the plot will be shifted toward higher diameters. Occasionally a new peak will appear in the high end of the scale.[7]

Dilution Medium

For dilution of Nanosphere-size standard stock dispersions (see **Table 1**), a tetrasodium pyrophosphate decahydrate (TSPP) (Fluka Chemie GmbH, Deisenhofen, Germany) solution, 0.117% wt/vol, corresponding to a conductivity of 1000 µS, was used.[10] TSPP was dissolved in freshly distilled water, and the conductivity was checked using a WTW LF 340 conductivity meter (Wissenschaftlich-Technische Werkstätten GmBH, Weilheim, Germany). Finally, the TSPP solution was filtered through a 0.2-µm cellulose acetate membrane filter in a pressure filter holder for inline filtration (Sartorius AG, Göttingen, Germany).

Table 1. Summary of Characteristics of Nanosphere Particles

Mean Diameter (nm)	Lot Number	Hydrodynamic Diameter Range (nm)
21 ± 1.5	22396	Not specified
34 ± 1.4	22557	Not specified
50 ± 2.0	23366	51 - 57
102 ± 3	22625	102 - 109
269 ± 7	21205	267 - 275

Sample Preparation

The sample preparation was performed according to guidelines for particle size analysis given by the International Organization for Standardization[9] and recommendations from Duke Scientific[10] with the following additional precautions. The preparation was done in a laminar airflow bench using powder-free vinyl gloves. Test tubes (borosilicate glass) for the PCS instrument and Eppendorf tubes were sonicated for 5 minutes in an ultrasonic bath containing TSPP solution and then flushed with freshly filtered TSPP solution (syringe filter unit, Millex-GS 0.22 µm, Millipore Corp, Billerica, MA) prior to contact with the sample. To reduce dust attraction to droplets, a needle with a large diameter (1.6 mm) was used.

To reduce the risk of particle aggregates, the bottles containing the stock dispersions of size-standard were bath-sonicated for 30 seconds. The first 3 drops from the flask were always discarded. In this study, the stock dispersion was diluted empirically until a count rate of 250 to 350 kHz was reached. Measurements out of this range can negatively affect the correlation function.[7] Blends of single-sized latex bead dispersions were prepared separately in Eppendorf tubes and vortexed for 60 seconds. The samples were then diluted with TSPP buffer solution in the test tube until the desired count rate was reached. The calculation of blending ratios was performed on the basis of the number of particles.

RESULTS AND DISCUSSION

Single-Size Latex Bead Dispersions

Single-size latex bead dispersions (Ø; 21, 34, 50, 102 and 269 nm) were measured in order to investigate whether the sizes specified in the analysis certificates of the manufacturer could be reproduced. Ten parallels were performed at 3 different run times (time periods of data collection: 5, 15, and 60 minutes) to find out whether prolonged run times improved precision and reduced variability. For 50-, 102-, and 269-nm beads, the mean diameters obtained were compared with the range of the hydrodynamic diameters as indicated in the manufacturer's certificates of analysis (results of PCS analysis) (see **Figure 1** and **Table 2**). For the smaller beads (21 and 34 nm), a hydrodynamic diameter range is not given by the manufacturer, only a mean hydrodynamic diameter. For quality assurance purposes, distribution widths (SDs), χ^2 values, and baseline adjustment values were recorded for each of the 10 parallels and are given as means ± SD (**Table 2**).

The measurements of the bigger particles (50, 102, and 269 nm), yielded mean diameters that were mostly within the hydrodynamic diameter range given by the manufacturer (see **Figure 1**), a finding that is in congruence with literature reports.[5, 8] Most χ^2 values were below 1.0, baseline adjustment values were below 0.02%, and the SDs, which represent a measure for the width of the distribution, were below 15% (see **Table 2**). These parameters thus fulfilled the requirements specified in the NICOMP manual,[7] and the results are therefore to be regarded as fully valid. In contrast, none of the series with 21- and 34-nm particles yielded an SD of 15% or lower. These particles also showed a significant deviation from the certified mean diameters. A repetition of the analysis series using different batches of 21- and 34-nm latex beads along with another PCS instrument confirmed our results (data not shown). The reason for the deviation from the manufacturer's certificates remains unclear. Since the mean diameters could be reproduced nicely, they were used as a basis for our further investigations.

Blends of Latex Bead Dispersions

In order to investigate how the system processed more complex systems of disperse particles, as is often the case with real submicron drug carrier systems such as liposomes, defined binary blends of latex beads were

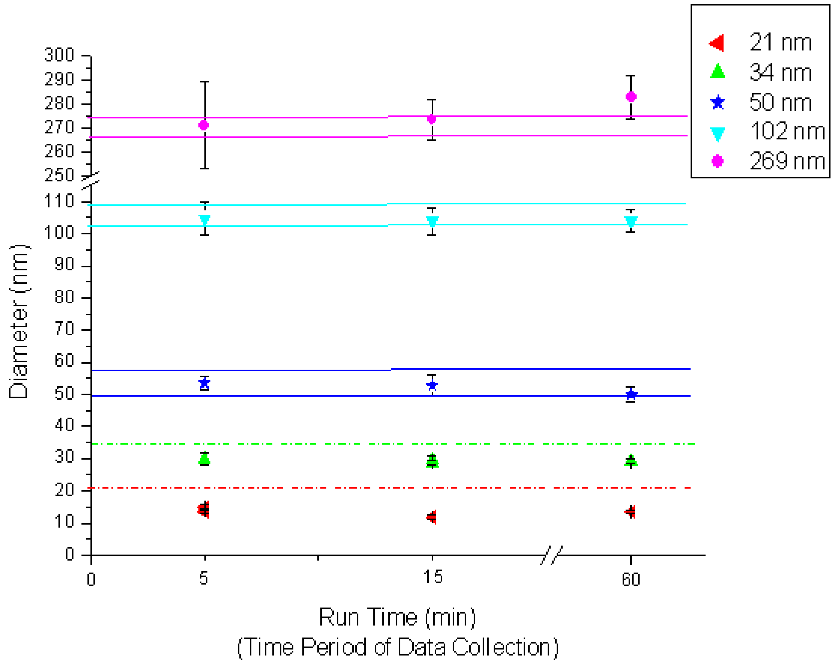

Figure 1. Mean diameters and SD of the mean (error bars) of sets of 10 parallels measuring single-size latex bead dispersions. Horizontal lines denote specified mean diameter (21 and 34 nm [dashed lines]) or specified range of size distributions for 50-, 104-, and 269-nm particles. Cycle time is 5, 15, and 60 minutes.

Table 2. Summary of Single-Size Latex Bead Dispersions

Specified Particle Size (nm)	Cycle Time (min)	Intensity (kHz) (mean ± SD)	χ^2 Mean ± SD	Baseline Adjustment (%) (mean ± SD)	Average Width of Distribution (SD%)
21 ± 1.5	5	261.6 ± 4.8	1.7 ± 0.3	0.02 ± 0.04	27
21 ± 1.5	15	270.3 ± 8.0	1.3 ± 0.2	0.03 ± 0.04	33
21 ± 1.5	60	256.3 ± 4.2	1.7 ± 0.5	0.00 ± 0.00	28
34 ± 1.4	5	257.6 ± 6.6	0.5 ± 0.1	0.06 ± 0.07	18
34 ± 1.4	15	336.8 ± 19.9	1.2 ± 0.2	0.03 ± 0.03	20
34 ± 1.4	60	216.2 ± 1.9	1.3 ± 0.2	0.01 ± 0.02	20
50 ± 2.0	5	293.9 ± 4.9	0.8 ± 0.4	0.02 ± 0.05	10
50 ± 2.0	15	333.2 ± 14.9	0.7 ± 0.3	0.02 ± 0.03	11
50 ± 2.0	60	252.0 ± 22.5	0.8 ± 0.2	0.05 ± 0.05	14
102 ± 3.0	5	346.1 ± 2.9	0.4 ± 0.1	0.09 ± 0.07	9
102 ± 3.0	15	257.4 ± 3.3	0.5 ± 0.2	0.07 ± 0.06	10
102 ± 3.0	60	289.7 ± 11.6	0.4 ± 0.1	0.08 ± 0.05	12
269 ± 7.0	5	309.4 ± 14.9	1.3 ± 1.2	0.13 ± 0.17	16
269 ± 7.0	15	324.7 ± 34.8	0.3 ± 0.1	0.19 ± 0.13	14
269 ± 7.0	60	236 ± 39.2	0.4 ± 0.2	0.14 ± 0.05	11

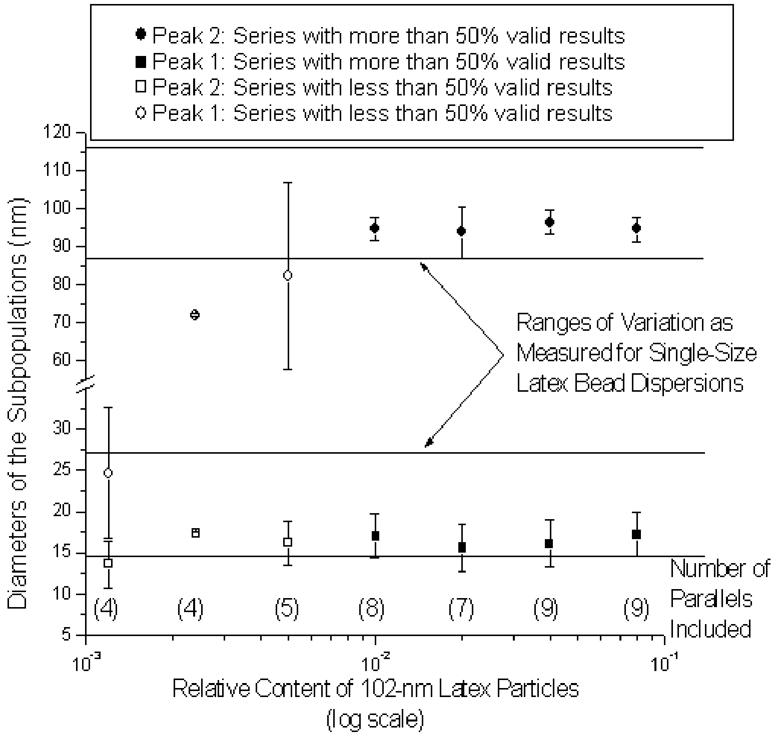

Figure 2. Mean diameters of the subpopulations detected in blends of 21- and 102-nm latex beads, 60-minute cycle. The results represent mean ± SD of up to 9 valid parallels. The range 0.0012% to 0.08% denotes the percentage of largest particles by number. Horizontal lines denote the range of specification as measured with single-size latex bead dispersions (see legend for Figure 1).

prepared. Three different sets of binary blends were investigated, 21 + 102 nm, 34 + 102 nm, and 21 + 269 nm. Blends with varying ratios from smaller and bigger particles were analyzed. Furthermore different run times were tried (5, 15, and 60 minutes). Ten parallels each were performed.

21 + 102-nm and 34 + 102-nm Blends

The study of blends of 21 + 102-nm particles was performed with blending ratios from 0.0012% to 0.08% (of the largest particle by number), and either a Gaussian or a NICOMP distribution model, mostly bimodal, was automatically adopted for samples. The particle size range obtained for the samples was 13.7 to 21.5 nm for the smaller particles, and 26.8 to 109.2 nm for the larger particles, depending on blending ratios (see **Figure 2**).

Blends of 34- and 102-nm particles were studied with blending ratios in the range of 0.02% to 3.74%. The particle sizes obtained were in the range 15.9 to 35.3 nm for the smaller particles, and 46.1 to 112.1 nm for the larger particles, depending on the blending ratios (see **Figure 3**).

Both 21- and 34-nm latex bead dispersions, containing a minor fraction (<<5%) of 102-nm particles, showed similar behavior during PCS analysis: when the bigger particles were present in relatively high amounts, the automatic fit function of the PCS software mostly chose bimodal distribution models. The mean diameters of the 2 peaks obtained were well within the above established hydrodynamic size ranges (of the single-size dispersions). As the content of the 102-nm particles decreased, PCS analysis of the blends yielded inconsistent results (**Tables 3** and **4**). For an increasing number of parallels, the software chose other than bimodal distribution models (ie, monomodal or trimodal distributions). At the same time, an increasing number of parallels were hampered by unacceptably high residuals (>10), which indicated that the software was unable to adapt a distribution model that implemented all of the raw data. The mean diameters of the 2 subpopulations for the bimodal parallels deviated significantly from the hydrodynamic size ranges established

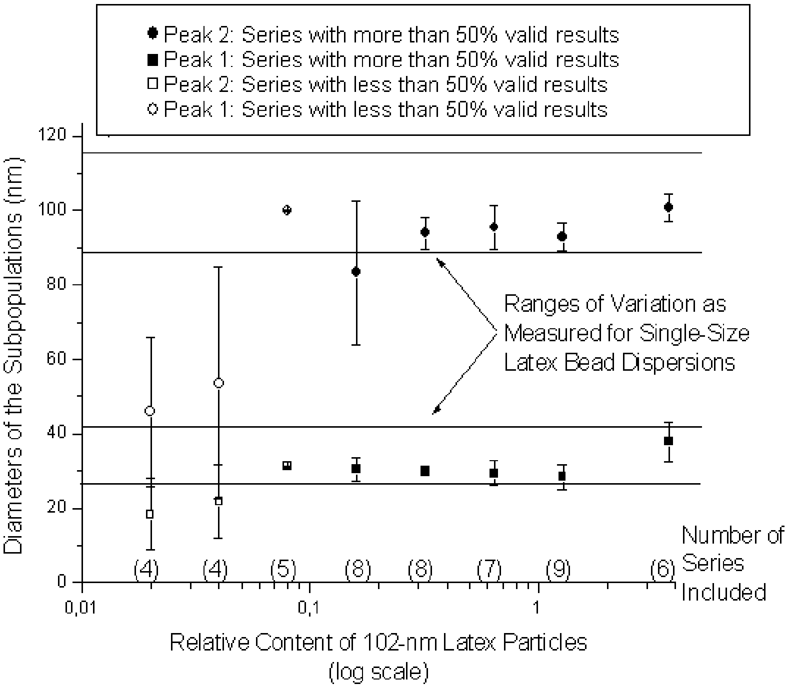

Figure 3. Mean diameters of the subpopulations detected in blends of 21- and 102-nm latex beads, 60-minute cycle. The results represent mean ± SD of up to 9 valid parallels. The range 0.0012% to 0.08% denotes the percentage of largest particles by number. Horizontal lines denote the range of specification as measured with single-size latex bead dispersions (see legend for Figure 1).

using single-size dispersions; the diameters found for the subpopulation of the bigger particles were too small. The monomodal distributions had mean diameters in the magnitude of the smallest latex bead. The critical blending ratio, below which inconsistent results are obtained, appears to be around 0.0024% (percentage of bigger particles) for blends of 21- and 102-nm particles. Blends of 34 + 102-nm particles appear to be analyzed with consistent results down to a content of 0.08% of bigger particles. Below this, the results are inconsistent. With even smaller contents of bigger particles (<0.002%) for 21 + 102-nm blends, the results appear to become more consistent again. A majority of monodisperse distributions is obtained here (**Table 3**) with mean diameters in the magnitude of the smaller particles. This result indicates that the bigger particles are disregarded.

All of the above holds true irrespective of the data collection time used. Longer run times do not seem to have any effect on the measured particle size nor on the ability to resolve bimodal distributions, as compared with 5-minute runs. In conclusion, blends of 21 + 102-nm as well as 34 + 102-nm particles can be analyzed properly down to a certain critical percentage of bigger particles.

21 + 269-nm Blends

Blends of 21- and 269-nm particles were tested in a similar manner, with blending ratios in the range from 0.0012% to 0.08%. The particle size range obtained for the samples was 17.9 to 90.5 nm for the smaller particles, and 199.4 to 311.2 nm for the larger particles, again, depending on the blending ratios (see **Figure 4**).

Again, mono-, bi-, and trimodal models were adopted by the PCS software (**Table 5**). A lower critical blending ratio was not observed here. In contrast, a tendency toward monomodal distributions was seen above the critical blending ratio of 0.005% (percentage of larger particles by number). The mean diameters of the monomodal results were mostly in the magnitude of 270 nm (ie, near the size of the bigger particles). For blending ratios up to 0.005% (percentage of larger particles by number), mostly bimodal distributions were obtained, with mean diameters of the 2 subpopulations well in agreement with the single-size dispersion re

Table 3. 21 + 102-nm Blends of Latex Beads

Blending Ratio (% of Largest particle)	**Run Time (min)**	**Average Intensity ± SD**	**Average Fit Error ± SD**	**1 Peak**	**3 Peaks**	**Residual >10**
0.0012	60	266.0 ± 3.5	2.4 ± 0.2	6/10	0/10	0/10
0.0024	60	267.5 ± 0.7	1.8 ± 0.2	3/10	0/10	3/10
0.005	60	279.2 ± 4.3	1.5 ± 0.2	1/10	1/10	3/10
0.01	60	253.5 ± 4.7	1.1 ± 0.1	0/10	2/10	0/10
0.02	60	329.4 ± 1.3	1.0 ± 0.1	2/10	0/10	1/10
0.04	60	280.9 ± 5.8	1.1 ± 0.1	0/10	1/10	0/10
0.08	60	336.5 ± 1.4	3.3 ± 0.4	0/10	1/10	0/10

Table 4. 34 + 102-nm Blends of Latex Beads

Blending Ratio (% of Largest Particle)	**Run Time (min)**	**Average Intensity ± SD**	**Average Fit Error ± SD**	**1 Peak**	**3 Peaks**	**Residual >10**
0.02	60	305.8 ± 2.9	1.6 ± 0.1	2/10	0/10	0/10
0.04	60	280.8 ± 3.3	1.5 ± 0.1	1/10	0/10	4/10
0.08	60	251.3 ± 4.4	1.5 ± 0.1	1/10	0/10	4/10
0.16	60	299.5 ± 6.3	1.2 ± 0.2	0/10	0/10	2/10
0.32	60	278.4 ± 8.9	1.1 ± 0.1	0/10	2/10	0/10
0.64	60	289.0 ± 4.5	1.3 ± 0.1	0/10	1/10	2/10
0.64	60	329.1 ± 2.5	0.9 ± 0.1	0/10	1/10	0/10
1.28	60	261.2 ± 2.5	1.2 ± 0.1	1/10	0/10	0/10
3.74	60	239.9 ± 48.6	1.2 ± 0.2	2/10	1/10	0/10

sults. Longer cycle times (15 and 60 minutes) did not improve the result as compared with 5-minute cycles. Blends of 21 + 269-nm particles can be analyzed properly up to 0.005% of bigger particles. As the blending ratio increases, the smallest latex particles (21 nm) cannot be detected at all. Obviously, as a result of the large difference in scattering power between the particles, the smaller particles are neglected.

For the handling of unknown samples the following approach appears promising:

- run 10 parallels of at least 5 minutes data collection time each;
- exclude parallels with residual >10;
- identify the distribution model (mono-, bi-, and trimodal) that appears most frequently;
- exclude parallels with other distribution models.

CONCLUSSION

The investigations of particle size distributions of homogeneous single-size latex bead dispersions by a routine, 90° fixed-angle PCS instrument yielded reproducible results for all particle sizes studied. Results for particles in the size range of 50 to 269 nm were in congruence with the hydrodynamic diameter range given in the certificate of analysis by the manufacturer of the standards. For smaller particles (ie, 21 and 34 nm, the measured sizes were somewhat smaller than stated by the manufacturer of the standards but could be reproduced nicely.

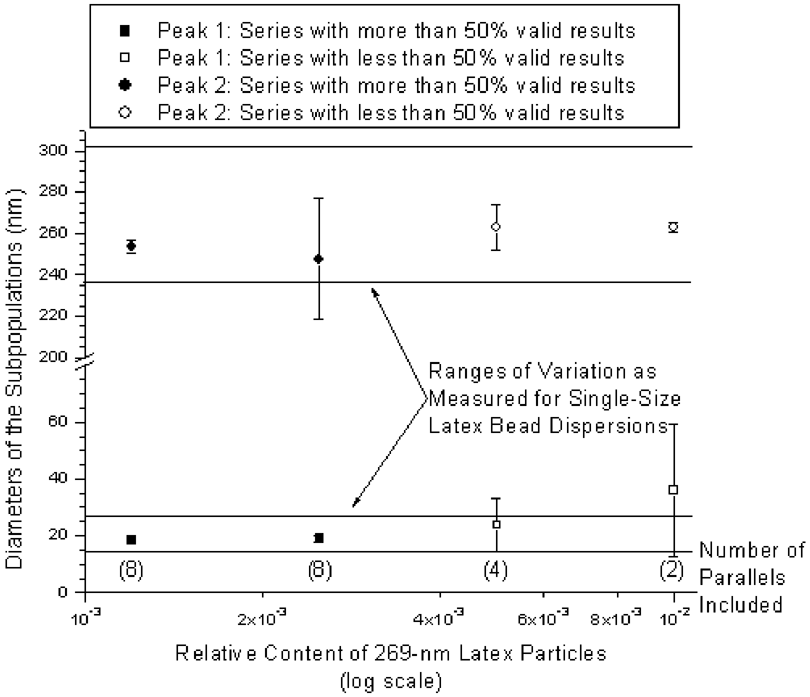

Figure 4. Mean diameters of the subpopulations detected in blends of 21- and 269-nm latex beads, cycle time 15 minutes. The results represent mean ± SD of up to 8 valid parallels. The range 0.0012% to 0.08% denotes the percentage of largest particle by number. Horizontal lines denote the range of specification as measured with single-size latex bead dispersions (see legend for Figure 1).

Table 5. 21 + 269-nm Blends of Latex Beads

Blending Ratio (% of Largest Particle)	Run Time (min)	Average Intensity ± SD	Average Fit Error ± SD	1 Peak	3 Peaks	Residual >10
0.0012	15	326.0 ± 7.1	1.8 ± 0.4	1/10	0/10	1/10
0.0025	15	315.2 ± 12.0	2.5 ± 1.2	2/10	0/10	1/10
0.005	15	349.9 ± 6.0	2.3 ± 0.7	6/10	0/10	0/10
0.01	15	305.6 ± 2.1	1.4 ± 0.0	8/10	0/10	0/10
0.02	15	261.6 ± 8.7	2.0 ± 0.5	9/10	0/10	0/10
0.08	15	253.6 ± 50.4	2.8 ± 0.8	9/10	1/10	0/10

For all 3 blends that were investigated, a limitation in terms of ability to resolve the bimodal size distribution was seen both below and/or above certain blending ratios. A specific range of detection for bimodal blends depending on particle size, difference in particle size, and blending ratio can be postulated. The size of this range decreased as the difference in particle size increased. It is obvious from these results that the PCS technique is restricted to specific ranges of blending ratio when measuring unknown bimodal samples. Outside this range, reliable results cannot be expected in terms of accurately detecting the sizes of both subpopulations.

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