

Calibration of Spherical Particles by Light Scattering*

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ABSTRACT

Photon Correlation Spectroscopy, also known as Quasi-Elastic or Dynamic Light-Scattering, can be a convenient method of size measurement for suspensions of monodisperse spherical particles in the 20-500 nanometer (nm) size range [0.02 to 0.5 micrometer (μm)]. The results obtained vary widely, depending on the concentration and condition of the sample, as well as environmental factors. Techniques have been developed that improve the accuracy and precision of the measurements. These techniques include controlling the concentration, uniformity and dispersion of the sample. The improved method was verified by measuring particle size standards.

1. INTRODUCTION

Photon Correlation Spectroscopy (PCS) and Quasi-Elastic Light Scattering (QELS) are promising methods for the measurement of suspended particles from 20-500nm. In principle, these instruments do not require calibration, since they calculate the mean diameter of the particles by observing fluctuations of scattered light related to the diffusion coefficient of the sample. In a medium of known viscosity, temperature, and refractive index, the diffusion coefficient is directly related to the Brownian motion of the particles. In practice, the commercially available instruments, although relatively easy to operate, do not provide the kind of accuracy and precision required by our laboratory. In order to meet our objectives, we developed a series of procedures for preparing samples and optimizing their analysis to overcome the limitations of the instruments.

This report describes our methods for the benefit of those who want to optimize the performance of their PCS/QELS instruments for the measurement of polystyrene latex microspheres.

There are four principal considerations that have

proven to be of crucial importance for obtaining accurate size results from the PCS instruments: i) electrolytic activity in the analysis diluent; ii) particle concentration of the sample; iii) temperature conformity between the sample and the set temperature of the instrument; and iv) sample dispersion and uniformity.

2. ELECTROLYTIC ACTIVITY OF DILUENT

Suppliers of PCS instruments sometimes recommend using ultrapure water for the diluent. Most “ultrapure” water systems deliver water filtered to 0.2 μm or smaller and 18 M Ω resistivity. The problem with such water is that it does nothing to contribute to the dispersion stability of typical polystyrene latex microspheres, whose stabilization depends on the effect of negatively charged sulfate groups on the particle surface.

When there is a lack of effective conductance of the surface charges, a temporary hydrophobic interaction between the particles results. The associated particles will exhibit reduced Brownian motion and a larger diameter for the duration of the interaction. Thus, the PCS instrument will measure a larger particle diameter and usually, a higher polydispersity factor. For the sulfate groups to exhibit a sufficient repulsive force, a certain measure of electrolyte is needed to conduct the surface charges.

The electrolyte selected by our laboratory for all PCS measurements of polystyrene latex is a 1000 micromho conductivity solution of tetrasodium pyrophosphate (TSPP), approximately 0.16 wt%. This amount does not appreciably alter solution density or viscosity from that of pure water. The procedure is used exclusively for conventional polystyrene latex and does not apply to carboxylated and other surface-modified particles.

The optimum electrolyte should be somewhat basic in order to completely ionize the sulfate groups on the surface of the particles. It is also desirable for the electrolyte to function as an ionic dispersant, supplementing the polystyrene surface charges. In addition, the electrolyte must be used

Key Words:

- Polystyrene
- Dynamic Light Scattering
- Photon Correlation Spectroscopy
- Tetrasodium Pyrophosphate
- Concentration
- NIST Traceable

at a concentration that offers the best possible zeta potential for inhibiting the interaction of the particles. The TSPP diluent is basic, approximately pH 10 at the 1000 micromho conductivity recommended for the electrolyte. It is also only slightly water soluble, meaning that the TSPP molecules will have a certain affinity for the surface of the particles. Unlike a surfactant where a hydrophobic end is attracted to a particle surface, such dispersion stabilization occurs through simple solubility considerations. The ionic species are simply concentrated near the particle surfaces and add additional surface charges, meaning both pyrophosphate and sulfate groups aid in preventing association of particles in solution.

As with any PCS measurement, the diluent must be free of foreign particulates. It is thus best to filter this electrolyte solution using at least 0.2µm filtration, while minimizing contaminants from the filter media.

As shown in Table 1, when clean 1000 micromho TSPP electrolyte was used as a diluent to analyze Thermo Scientific Nanosphere polystyrene size standards, the observed diameter was always smaller than for the same spheres in deionized water. As is shown later, the smaller diameter is usually the correct diameter. The instrument used was a Brookhaven BI-90 with a 633nm laser. The temperature was approximately 20-23°C and viscosity was set for the temperature.

Catalog PN Tested	PCS Diameter with TSPP (nm)	PCS Diameter with ultrapure water (nm)
3050	52.7	57
3060	62.8	69
3100	108	112
3150	155	166
3200	219	237
3269	270	285
3300	299	316

Table 1. Effect of Suspension Media on PCS/QELS Values

3. PARTICLE CONCENTRATION ADJUSTMENTS

The optical system of a PCS instrument can be constructed in various ways, and we have found a considerable difference in the optimum particle concentration required for instruments from different manufacturers. Some instruments, especially modern ones with more powerful lasers, require a more dilute sample concentration than does the Brookhaven BI-90.

Our laboratory achieved the greatest success in optimizing sample concentrations by measuring their turbidity on a spectrophotometer at the 633nm wavelength. This technique measures the turbidity of the sample and allowed us to adjust it at the PCS laser wavelength.

As shown in Table 2, when measuring the National Institute of Standards and Technology (NIST) 269nm standard (SRM 1691) [5], with TSPP 1000 micromho electrolyte, both the diameter and polydispersity values changed as a function of concentration on a Brookhaven BI-90 analyzer.

Absorbance @ 633 nm	Count Rate (Kcps)	Diameter (nm)	Polydispersity Index
0.020	142	272.5	0.040
0.036	221	271.8	0.036
0.076	458	270.1	0.043
0.101	537	268.4	0.062
0.145	648	267.2	0.066

Table 2. Effects of Sample Concentration on PCS/QELS Values

For analyses below about 300nm, the BI-90 instrument yielded the best data at or near 0.045 absorbance. For sizes above 300nm, it is necessary to increase the turbidity in successive amounts. Approximately 0.20-0.25 absorbance is optimal for the 0.5µm size.

It is desirable to establish a concentration curve which is generated using a range of appropriate standards in an electrolyte diluent. For each particle diameter there would then be an optimum absorbance value and particle concentration (Kcps) for obtaining the most accurate size.

We have since developed another method to determine the optimum concentration and use it for the preparation of all samples.

4. TEMPERATURE CONTROL

Since the diameter obtained by PCS is inversely proportional to sample viscosity, it is essential that the sample temperature matches the set point of the instrument sample holder. The procedure used at our laboratory was to measure the temperature before inserting it into the instrument and again immediately after the runs are concluded. Often adjustments are needed in the diameter value because the average temperature over the course of the runs does not equal the set point. These adjustments have ranged to as high as ±3% of the diameter value, and cannot be dismissed as insignificant when attempting to obtain the most accurate size data possible. Figure 1 shows the range of error that can be expected for discrepancies between true and set point temperatures.

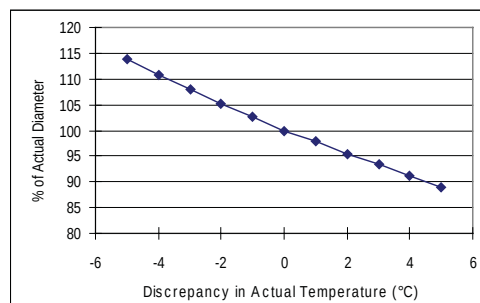


Figure 1. PCS/QELS Sensitivity to Temperature Variation

5. SELECTING AND PREPARING SAMPLES

Many materials used as PCS reference particles are not supplied in a dispersion of suitable quality to yield accurate size data. Frequently, polystyrene latex batches sold directly from the reactor contain a considerable amount of debris, and are at high solids concentrations. Some of these materials are relatively old and, in the course of aging, have formed hard aggregates which can not be dispersed by sonication or surfactants. Many of the difficulties encountered with the data from PCS instruments can be traced to poor quality in the initial particle dispersion.

In some instances, a polystyrene latex suspension may contain an abundance of off-size small and large particles which can skew a PCS analysis. More typically, the number of such undesirable particles is small and the impact upon analysis may be more subtle. The greatest problem is with near-oversize particles and with aggregates of the main population, since PCS systems are most sensitive to these particles. In the case of very small particles, it may be useful to use a 0.1, 0.2, 0.45, or 1.0µm filter to remove oversize particles and debris from the diluted sample.

Unless steps are taken to remove undesirable particulates and debris from bulk polystyrene latex samples, they can reduce the accuracy and reproducibility of PCS evaluations. A summary of these adverse effects is shown in Table 3.

Problem	Effect
• Debris from latex reactor	• Oversizes and broadens the distribution
• Undispersed clumps of particles	• Oversizes and broadens the distribution
• Near-sized large particles	• Oversizes and broadens the distribution
• Near-sized small particles	• Undersizes and broadens the distribution
• Ultrafine particles	• Negligible, depending on quantity

Table 3. Effects of Off-size Particulates and Dispersion Quality on PCS/QELS Size Measurements

6. RESULTS AND CONCLUSIONS

The procedures employed here will reduce the artifacts induced by factors such as dispersion, concentration, temperature, and sample anomalies in PCS measurements. Our laboratory has found very good agreement between PCS and accurate transmission electron microscopy (TEM) measurements, but only when these conditions are optimized (Table 4 and Figure 2). The TEM number-weighted values were adjusted to the intensity-weighted values typically measured by PCS instruments. Since the instruments vary in performance by make and model, these optimized measurements will permit more effective evaluation and use of the instruments. Using the optimized methods described, the Thermo Scientific Nanosphere line of size standards has been developed at our laboratory.

Catalog PN Tested	TEM* Diameter (nm)	TEM Intensity** Weighted (nm)	PCS/QELS Diameter (nm)
3050	50.5	52.1	52.7
3060	63.7	62.9	62.8
3070	73.1	74.3	74.0
3080	81.4	83.0	82.9
3090	96.0	97.9	97.0
3100	107	107	108
3125	126	126	126
3150	155	155	155
3220	220	220	219
3269 (NIST)	269	269	270
3300	298	298	299
3350	343	343	344

* Diameter calculated as $\Sigma nd/\Sigma n$

** Diameter calculated as $\Sigma nd^6/\Sigma nd^5$

Table 4. Optimized PCS/QELS Values vs. Reference Standards data updated 1/3/2000

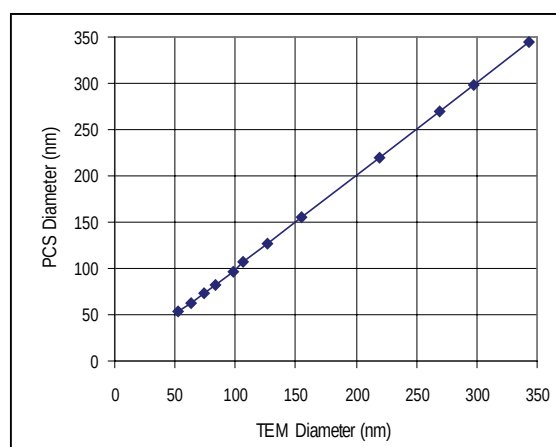


Figure 2. Optimized PCS/QELS Values vs. Reference Standards

7. INSTRUMENT COMPANIES

1. Brookhaven Instrument Company
Brookhaven Corporate Park
750 Blue Point Road
Holtsville, NY, 11742
2. Beckman Coulter
4300 N. Harbor Boulevard
P.O. Box 3100
Fullerton, CA 92834-3100.
3. Malvern Instruments Inc.
117 Flanders Road
Westborough, MA 01545.
4. Hach Company
P.O. Box 389
Loveland, CO 80539-0389.

8. STANDARDS ORGANIZATIONS

1. National Institute of Standards and Technology
Department of Commerce
Gaithersburg, Maryland, 20899.

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