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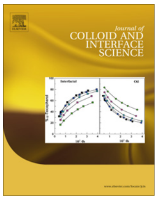

A comparative study of submicron particle sizing platforms: Accuracy, precision and resolution analysis of polydisperse particle size distributions

Will Anderson [a,*], Darby Kozak [a], Victoria A. Coleman [b,1], Åsa K. Jämting [b], Matt Trau [a,c,2]

[a] Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane 4072, Australia
[b] National Measurement Institute Australia, Nanometrology Section, Bradfield Road, West Lindfield 2070, Australia
[c] School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane 4072, Australia

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ABSTRACT

The particle size distribution (PSD) of a polydisperse or multimodal system can often be difficult to obtain due to the inherent limitations in established measurement techniques. For this reason, the resolution, accuracy and precision of three new and one established, commercially available and fundamentally different particle size analysis platforms were compared by measuring both individual and a mixed sample of monodisperse, sub-micron (220, 330, and 410 nm – nominal modal size) polystyrene particles. The platforms compared were the qNano Tunable Resistive Pulse Sensor, Nanosight LM10 Particle Tracking Analysis System, the CPS Instruments's UHR24000 Disc Centrifuge, and the routinely used Malvern Zetasizer Nano ZS Dynamic Light Scattering system. All measurements were subjected to a peak detection algorithm so that the detected particle populations could be compared to 'reference' Transmission Electron Microscope measurements of the individual particle samples. Only the Tunable Resistive Pulse Sensor and Disc Centrifuge platforms provided the resolution required to resolve all three particle populations present in the mixed 'multimodal' particle sample. In contrast, the light scattering based Particle Tracking Analysis and Dynamic Light Scattering platforms were only able to detect a single population of particles corresponding to either the largest (410 nm) or smallest (220 nm) particles in the multimodal sample, respectively. When the particle sets were measured separately (monomodal) each platform was able to resolve and accurately obtain a mean particle size within 10% of the Transmission Electron Microscope reference values. However, the broadness of the PSD measured in the monomodal samples deviated greatly, with coefficients of variation being ~2–6-fold larger than the TEM measurements across all four platforms. The large variation in the PSDs obtained from these four, fundamentally different platforms, indicates that great care must still be taken in the analysis of samples known to have complex PSDs. All of the platforms were found to have high precision, i.e. they gave rise to less than 5% variance in PSD shape descriptors over the replicate measurements.

1. Introduction

Many submicron particle suspensions have complex particle size distributions (PSDs) [1], in that the size distribution is broad (polydisperse), or they consist of several distinct particle populations of varying size (multimodal). Currently, most particle sizing techniques are only able to accurately measure an average particle size for simple monomodal systems [2–5]. For example, Bell et al. [6] recently demonstrated that six submicron particle sizing techniques, being, an Tunable Resistive Pulse Sensor (TRPS), Particle Tracking Analysis (PTA), Differential Centrifugal Sedimentation (DCS), Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Scanning Mobility Particle Sizing, generated similar mean values for the PSDs of several monomodal, submicron, Stöber silica particle suspensions. In contrast, obtaining the 'true' PSD of a multimodal or polydisperse sample is often more challenging due to fundamental limitations in the detection and analysis methodologies of sizing techniques. For example, it is well known that techniques such as light scattering, optical microscopy and electron microscopy are limited because

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Abbreviations: CV%, coefficients of variation; DCS, Differential Centrifugal Sedimentation; DLS, Dynamic Light Scattering; TRPS, Tunable Resistive Pulse Sensor; FWHM, full width at half maximum; PSD(s), particle size distribution(s); PTA, Particle Tracking Analysis; P10, P50, P90, percentile values 10%, 50%, and 90%; PS220, PS330, PS410, polystyrene particles with diameters of 220 nm, 330 nm, and 410 nm; SEB, Standard Electrolyte Buffer; SD, standard deviation; TEM, Transmission Electron Microscopy.

* Corresponding author. Fax: +61 7 3346 3973.

E-mail addresses: will.anderson@uqconnect.edu.au (W. Anderson), darby.kozak@izon.com (D. Kozak), Victoria.Coleman@measurement.gov.au (V.A. Coleman), Asa.Jamting@measurement.gov.au (Å.K. Jämting), m.trau@uq.edu.au (M. Trau).

[1] Fax: +61 2 8467 3752.

[2] Fax: +61 7 3346 3973.

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they either measure only an averaged sample size distribution, lack the resolution to observe such particles, or are too time consuming to be conveniently used to measure polydisperse or multimodal samples. These factors can result in skewed or misleading representations of the true PSD of a sample.

Herein we compare the resolution of three new particle measurement platforms, TRPS, PTA and DCS, for measuring the PSD of a highly polydisperse, multimodal mixture of 220 nm (PS220), 330 nm (PS330) and 410 nm (PS410) polystyrene particles, mixed in a weight ratio of 1:1:1. The size resolution of a technique is assessed by its ability to detect the three separate particle sets present in the multimodal sample, while technique accuracy is assessed from the deviation of PSD shape descriptors to those obtained from measurements of the monomodal particle sets by both TEM and each respective technique. Precision is assessed by repeatability of the measured PSD descriptors over several replicate measurements. For each technique, analysis of both the theory and the ways in which the PSD data was processed is presented for the purpose of explaining how comparisons between the dissimilar techniques are made.

2. Measurement techniques

2.1. Transmission electron microscopy

TEM is an established technique that is capable of imaging samples based on the absorption of an electron beam as it passes through ultrathin ($\sim < 100$ nm) samples. The transmitted beam is typically projected onto a phosphorescent screen or detector, enabling micro- and nano-meter sized objects to be visualized. It has been a cornerstone of particle analysis for the last 50 years [7] and is widely applied for particle sizing. When applied to PSD analysis, TEM is a single particle technique that provides the added benefit of information on particle shape and composition. Particle size is measured in TEM from the diameter determined from a 2D projected area of a three dimensional particle. There are many possible diameters that can be reported, however the equivalent spherical diameter or the Feret diameter are the most common. In the case of spherical particles, all of the possible diameter measurements should be equivelent, however, for irregularly shaped particles, the choice of equivalent diameter can produce values that do not represent the sample geometry.

Despite the obvious benefits of being able to visually image a sample, the technique is often considered to be labor intensive as many hundreds or thousands (depending on the broadness of the PSD and the desired error) [8] of particles should be measured to obtain a statistically significant PSD measurement. In order to increase the throughput of PSD measurements, automated image analysis software is often used [9,10], however the requirement for user defined measurement parameters in these programs can often result in bias in PSD measurements. A further limitation of TEM is that it requires a high vacuum environment, preventing in situ sample analysis. The high energy electron beam used in TEM can also cause damage to a sample and must be taken into consideration, particularly for biological samples (which require chemical fixing to prevent sample destruction [11]), or polymer based samples which can shrink or charge under the electron beam.

Together with the low sample throughput, poor statistics, sample preparation requirements, harsh measurement conditions and inability to measure particles in situ, the requirement for rapid, bench-top PSD determination has lead to the development of many alternative sizing techniques, which are compared in this paper.

2.2. Dynamic light scattering

DLS, or photon correlation spectroscopy, is an established and popular technique for determining PSD and has been used as a particle characterization work horse since its development in the 1960s due to both its applicability to a wide spectrum of particles and dispersion media and its ease of use. DLS is an ensemble measurement that determines the average hydrodynamic diameter of a particle suspension by measuring the changes in the speckle pattern produced by particles scattering light as they undergo Brownian motion. Monodisperse suspensions produce an intensity autocorrelation function, $G_2(\tau)$, that can be described by [12]

$$G_2(\tau) = A\left[1 + B \cdot e^{(-2Dq^2\tau)}\right] \tag{1}$$

where τ is the time delay between intensity measurements, A and B are the baseline and intercept of the correlation function, D is the diffusion coefficient and $q = (4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index of the solvent, λ is the wavelength of the laser and θ is the scattering angle. For short time delays in τ the intensities will be highly correlated, while for long delays the correlation between intensity measurements will decay due to the movement of the particles undergoing Brownian motion. For a monodisperse system, the measured diffusion coefficient can then be related to the hydrodynamic diameter using the Stokes–Einstein equation.

$$d_{\mathrm{H}} = \frac{kT}{3D\pi\eta} \tag{2}$$

where d_{H} is the particle's hydrodynamic diameter, k is the Boltzmann constant, T is the temperature and η is the viscosity of the medium.

For a polydisperse sample, the correlation function is described by the sum of the exponential decays of the different populations of particles present in the sample. Unfortunately, inversion of this function to obtain the distribution of decay rates suffers from low resolution due to the ill-posed nature of the Laplace inversion [12]. To overcome this limitation a range of algorithms have been developed, such as Cumulants analysis, Non-negative least squares (NNLS) and CONTIN, that can be used to more reliably obtain information about the PSD of a polydisperse system. A more detailed analysis of DLS and the various algorithms can be found in the following articles [12,13]. To determine the volume or number-based PSD of a sample, these algorithms additionally require *a priori* knowledge of the optical properties of both the particle and its suspending medium.

A major limitation of DLS is that it is inherently sensitive to the presence of larger particles in a sample. This is because scattered light intensity is dependent on particle size. Using the Rayleigh approximation, the intensity, I, of scattered light is proportional to the sixth power of the particle diameter $d : I \propto d^6$ [12]. Thus even a few large particles in a sample will dominate the signal, resulting in an over-estimate in the mean diameter [4,14,15]. For this reason, DLS is not suitable for PSD analysis of highly polydisperse samples. However, the ease of use, high throughput nature, and the ability to analyze a large range of particulate sizes, materials and dispersion media has made DLS one of the most common and simplest measurement techniques for PSD determination.

2.3. Tunable resistive pulse sensor

TRPSs offer a new method for PSD analysis based on a size-tunable pore that measure objects via resistive pulse sensing. Resistive Pulse Sensors, or Coulter counters, were first pioneered in the 1950s by Coulter for high throughput, automated blood cell counting and sizing [16]. Historically, Resistive Pulse Sensors have been limited to characterizing micro-particles, however recent advances