application note

Resolving Particle Size Mixtures of Concentrated Samples Using Dynamic Light Scattering

(This application note is an excerpt taken from M. Kaszuba, M.T. Connah, F. McNeil-Watson and U. Nobbmann (2007) Particle and Particle Systems Characterization 24, 159-162.

Introduction

Dynamic light scattering (DLS) is a non-invasive technique used for characterizing macromolecules in solution and particles in suspension. The technique measures the time-dependent fluctuations in the intensity of scattered light that occur because the particles are undergoing Brownian motion. The velocity of this Brownian motion is measured and is called the translational diffusion coefficient D. This diffusion coefficient can be converted into a hydrodynamic diameter (D_H) using the Stokes-Einstein equation.

DLS is inherently a low-resolution technique with limited capability of resolving different sized particles in a distribution. However, distribution algorithms, such as Non-Negative Least Squares (NNLS) and CONTIN, do have the ability to distinguish between different size populations in a sample. Whether this is possible in a particular sample is determined by a number of factors including:

- Relative sizes of the different populations present in the sample
- Relative intensity of scattering from the different populations present in the sample
- The polydispersities of the separate size distributions
- Quality of the sample preparation
- Quality of the data

As a test of the resolving ability of an algorithm, measurements can be done using mixtures of monosized latex standards prepared at different relative concentrations. This application note details measurements performed on a series of samples where the relative concentrations of different size latex standards were varied.

Measurements were made on mixtures of latex standards at neat concentration on a Zetasizer Nano S (173^{o}).

Experimental

All latex standards used for the measurements discussed in this application note were obtained from Duke Scientific, Palo Alto, California and were traceable to NIST, Gaithersburg, Maryland. Nanosphere^{TM} size standards from the 3000 series were used. Specifically, these were the 60nm (catalogue number 3060A), the 220nm (3220A) and 900nm (3900A) standards respectively. All of these standards are supplied at 1% w/v concentration.

The analysis model used in the Zetasizer Nano software was Multiple Narrow Modes.

Results

The Zetasizer Nano S incorporates non-invasive backscatter optics (NIBS) that allows for the measurement of concentrated samples. Therefore, measurements of mixtures of 60 and 220nm latex standards at different ratios were made at neat concentrations of 1% w/v. Table 1 summarises the results obtained for these measurements and contains the ratios of the 60 and 220nm latex mixtures, the z-average diameters, the polydispersity index values, the peak analysis of the intensity and volume size distributions obtained (peak modes and percentages). Conversions of the measured intensity size distributions into volume were done using Mie theory with particle refractive index values of 1.59 and particle absorption values of 0.01. The corresponding intensity and volume size distributions obtained for the various mixtures are shown in figures 1 and 2 respectively.

The z-average diameters obtained from the latex mixtures decreases as the concentration of 60nm latex in each mixture is increased. Conversely, the polydispersity index values increase as the percentage of 60nm latex contained in each sample increases.

The measured volume ratios are in good agreement with the actual volume ratios of the latex mixtures.

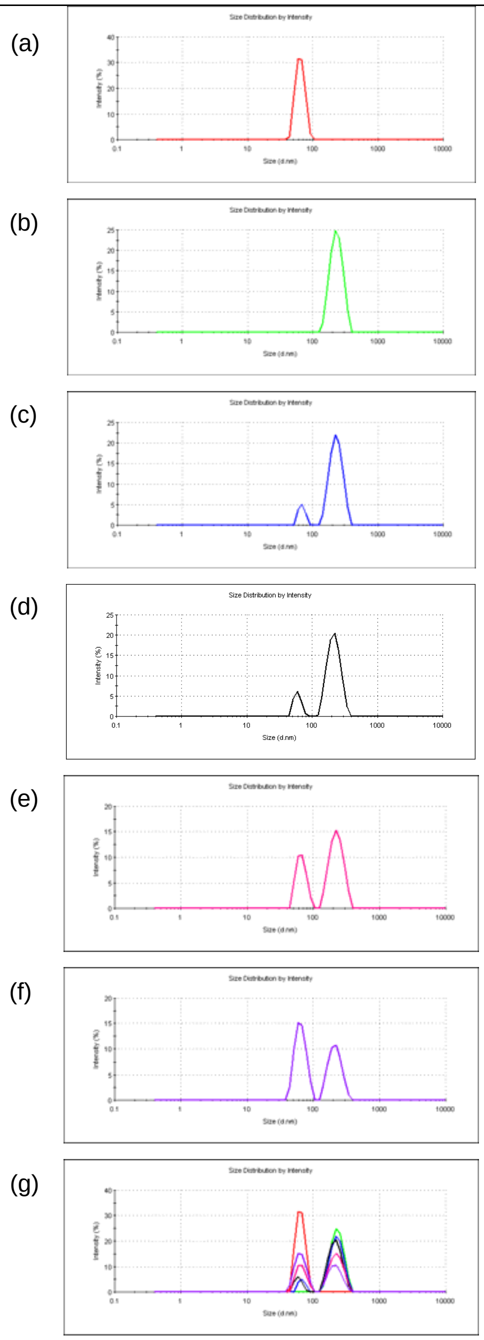

Figure 1: Intensity size distributions obtained for (a) 60nm latex standard (b) 220nm latex standard (c) 1:1 v/v mixture of 60:220nm latex (d) 2:1 v/v mixture of 60:220nm latex (e) 4:1 v/v mixture of 60:220nm latex (f) 12:1 v/v mixture of 60:220nm latex and (g) overplot of all the intensity size distributions

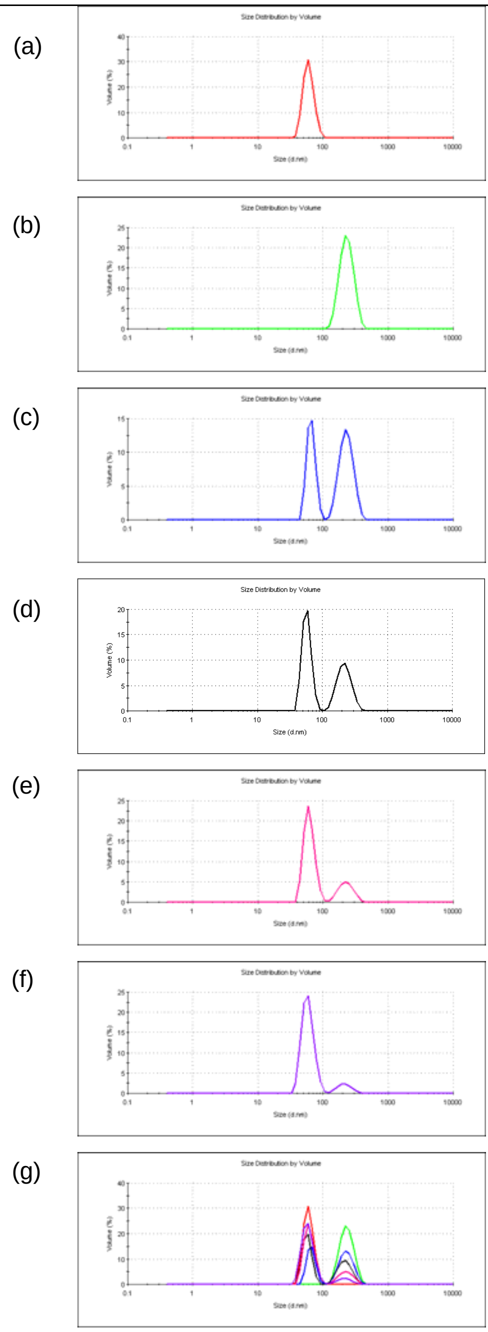

Figure 2: Volume size distributions obtained for (a) 60nm latex standard (b) 220nm latex standard (c) 1:1 v/v mixture of 60:220nm latex (d) 2:1 v/v mixture of 60:220nm latex (e) 4:1 v/v mixture of 60:220nm latex (f) 12:1 v/v mixture of 60:220nm latex and (g) overplot of all the intensity size distributions

Table 1: The results obtained for measurements of mixtures of 60 and 220nm latex standards at different ratios made at neat concentrations of 1% w/v. The table contains the ratios of the 60 and 220nm latex mixtures, the z-average diameters, the polydispersity index values, the peak analysis of the intensity and volume size distributions obtained (peak modes and percentages).

Volume Ratios of Prepared Latex Mixtures		z-Average Diameter (nm)	Poly-Dispersity Index	Intensity Size Distribution				Volume Size Distribution				Measured Volume Ratios of Latex Mixtures	
				Mode (nm)		Percentage		Mode (nm)		Percentage			
60nm	220nm			Peak 1 (nm)	Peak 2 (nm)	Peak 1	Peak 2	Peak 1 (nm)	Peak 2 (nm)	Peak 1	Peak 2	60nm	220nm
1	0	62.8	0.017	58.8	-	100	-	58.8	-	100	-	1	0
0	1	215	0.079	220	-	100	-	220	-	100	-	0	1
1	1	179	0.178	68.1	220	11	89	68.1	220	42	58	0.72	1
2	1	157	0.220	58.8	220	15	85	58.8	220	58	42	1.4	1
4	1	123	0.266	68.1	220	35	65	58.8	220	78	22	3.5	1
12	1	92.1	0.242	58.8	220	55	45	58.8	220	90	10	9	1

Conclusions

Even though dynamic light scattering is a low-resolution technique, the results presented in this application note have shown that not only can different size particles be resolved, but changes in the relative concentrations of each size population can be monitored.

The non-invasive back scatter (NIBS) optics incorporated in the Zetasizer Nano S allows for measurements to be made on concentrated, turbid samples.

Additional Reading

[1] Dynamic Light Scattering: An Introduction in 30 Minutes, Technical Note available from www.malvern.co.uk

[2] International Standard ISO13321 Methods for Determination of Particle Size Distribution Part 8: Photon Correlation Spectroscopy, International Organisation for Standardisation (ISO) 1996.

[3] Dahneke, B.E. (ed) Measurement of Suspended Particles by Quasi-elastic Light Scattering, Wiley, 1983.

[4] Pecora, R. Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy, Plenum Press, 1985.

[5] M. Kaszuba, M.T. Connah, F. McNeil-Watson and U. Nobbmann (2007) Particle and Particle Systems Characterization 24, 159-162.

Zetasizer Nano

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined static, dynamic, and electrophoretic light scattering measurements. The wide range of sample properties available for measurement with the Nano system include, particle size, molecular weight, and zeta potential.

The Zetasizer Nano system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications, along with the high concentration requirements for colloidal applications. Satisfying this unique mix of requirements was accomplished using a backscatter optical system and a novel cell chamber design. As a consequence of these features, the Zetasizer Nano specifications for sample size and concentration exceed those for any other commercially available dynamic light scattering instrument, with a size range of 0.6nm to 6µm, and a concentration range of 0.1ppm to 40% w/v.

These high sensitivity capabilities can also be applied to real time flow measurements, facilitating Absolute SEC and other HPLC measurements.

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