Technical Note:
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Internal Standard Method for Size Calibration of Sub-Micrometer Spherical Particles by Electron Microscope*

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Key Words:

- Polystyrene
- Transmission Electron Microscope
- Standard Reference Material
- Uncertainty
- National Bureau of Standards (NBS)
- National Institute of Standards and Technology (NIST)
- NIST Traceable

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1. INTRODUCTION

Transmission Electron Microscopy (TEM) is an accepted method for measuring the mean diameter and size distribution of polymer latex microspheres[1,2]. However, errors in the size data of uniform latex particles from commercial suppliers[3], and the need for traceability of size data to the National Bureau of Standards (NBS)[4], raise doubts about the suitability of TEM size data for calibrating spherical size standards. In addition to needing accurate data, the authors' laboratory needed to calibrate smaller than usual latex microspheres. By omitting several sources of error in typical TEM calibration procedures[5,6] and by modifying the sample preparation procedure, we were able to make improvements in the method. This enabled us to calibrate a new series of polymer microspheres for use as certified particle size standards from 50 nanometers (nm) to 1 micrometer (µm).

TEM CALIBRATION PROBLEMS

Most of the methods of calibrating TEM's have problems which contribute to the uncertainty of the measurements. The most common method is to use the magnification directly to determine the size of the particles. A slight variation of this method is to photograph a grating replica to calibrate the magnification of the photomicrograph, which is then used to measure the diameter of the particles. The problem with these two procedures is that, in the authors' experience, the differences between stated and measured magnifications can be as much as 5%. The actual magnification can also vary as much as 2% between consecutive photos at the same magnification on the same instrument. In addition, the apparent distortion of polymer microspheres under the electron beam [2,3,6] contributes to measurement errors, along with the possibility of photographic prints stretching or shrinking during the drying process.

Another method is to mount the spheres on a diffraction grating replica and use the grating to provide the scale. The main problem with this method is the lack of certainty regarding the actual line spacing of the replica. Commercially available replicas are not certified for line spacing accuracy or traceability to NBS. While the 463 nm line spacing scale is adequate for larger diameter spheres, it is not suitable for spheres smaller than 200 nm. At such magnification the edge of the grating line is almost as wide as the particle being measured, and if the surface of the replica is rough, the coarseness bleeds through the image, making the sphere edges difficult to locate (Figure 1).

INTERNAL STANDARD METHOD

To reduce the error involved with grating replicas or other calibration methods, the spheres being analyzed are mixed with either NBS Standard Reference Materials 1691 (0.269 µm), 1690 (0.895 µm)[7,8], or Thermo Scientific Nanosphere Size Standards to provide an internal calibrant. Thus, the exact magnification can be determined for each photograph from the known diameter of the standard microspheres. For the measurement of monodisperse spheres, the calibration spheres need only be about 10% to 20% larger or smaller than the particles to be measured. For samples with a size distribution, the diameter of the calibration spheres should be outside the size range of the sample to avoid overlapping diameters.

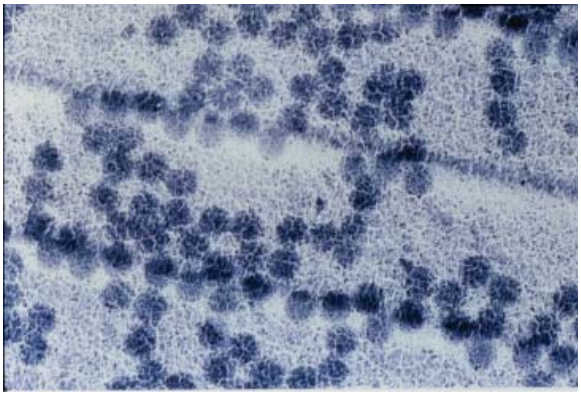

Figure 1. 100 nm latex microspheres on a 2160 line/mm grating

When used to calibrate polystyrene spheres, this method eliminates errors due to distortion of the spheres by the electron beam. Because the calibration microspheres are made of the same material as the sample, and are subjected to the same conditions, they are affected in the same way as the microspheres being measured. Since they are in the same picture, in the same field, they are at the same magnification. A variation of this method was used when the stage of the NBS metrology electron microscope was calibrated with SRM 1690 (0.895 µm) for the preparation of SRM 1691 (0.269 µm)[7].

Error sources due to spherical edge uncertainty and grating line definition are eliminated by mounting smaller particles (<300 nm) on smooth substrates rather than rough grating replicas (Figure 2).

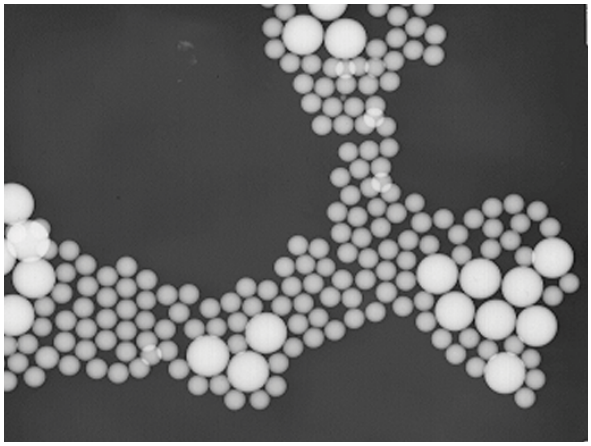

Figure 2. 100 nm microspheres on a smooth substrate with NBS SRM 1691 as an internal standard.

Several photomicrographs are taken of each sample. To eliminate errors due to the magnification and preparation of photographic prints, the spheres are measured directly from the negatives. At least 200 spheres and as many of the standard particles as possible are measured with a loupe fitted with a 200 division scale. The measured diameters of the calibration spheres are averaged; their reference diameter is used to determine the magnification and calculate the mean diameter for each negative. The standard deviation of the mean diameters of the negatives is the random error. Then the individual sphere diameters are used to calculate the mean and standard deviation of the sample.

RESULTS AND CONCLUSIONS

A series of monodisperse polystyrene spheres made in the authors' laboratories were calibrated using the internal standard method. Their diameters were compared with data from two other NBS traceable methods: photon correlation spectroscopy (PCS)[9] and optical array microscopy[10]; the results are given in Table 1. The average percent variation between the measured and reference values is 0.77%. Figure 3, a graph of the data, shows very high correlation (0.999991) between the observed and reference values.

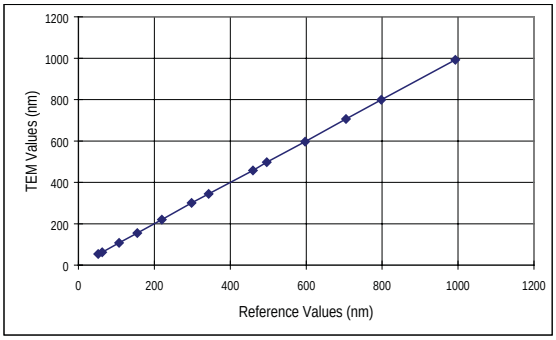

Figure 3. TEM Internal Standard Method Values vs. PCS and Optical Array Methods

In summary, the internal standard method of TEM particle size measurements is a convenient and effective way of obtaining accurate and precise NBS traceable calibration of polystyrene microspheres. This method, when used with certified particle size standards, can be applied to a broad range of particle size analysis problems using electron microscopy. It also has the potential for automated TEM image analysis.

Reference Material	Observed Diameter TEM (nm)	Mean Diameter IntensityWeighted** (nm)	Reference Values (nm)	Difference (nm)	Difference (%)
3050	47.8	52.1	53.6 (PCS)	1.5	2.9
3060	60.0	62.9	62.6	-0.3	-0.48
3100	107	107	108	1	0.93
3150	155	155	155	0	0
3200	220	220	220	0	0
3269	269	269	270	1	0.37
3300	298	298	301	3	1.01
3350	343	343	344	1	0.29
3450	460		458 (Array)	-2	-0.43
3500	496		497	1	0.20
3600	597		597	0	0
3700	705		706	1	0.14
3800	798		799	1	0.13
4009	993		992	-1	-0.10

*Intensity Weighted TEM Mean Diameter = $\Sigma nd^6/\Sigma nd^5$

Table 1: TEM Internal Calibration Method vs. PCS and Optical Array Methods.

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