Science and Innovation of Advanced Materials

The Materials Research Society of Thailand

Research Article

Statistical Analysis for Quality Control of Monodispersed Polystyrene Particles Certified Reference Materials

Benjarat Ngansalung*, Angkoonna Pringkasemchai, Jariya Buajarern

National Institute of Metrology (Thailand), Klong Luang, Pathumthani 12120, Thailand

* Corresponding author e-mail: benjaratn@nimt.or.th

Received: *July 28th, 2024* | ***Revised:*** *September 26th, 2024* | ***Accepted:*** *October 10th, 2024*

Abstract: Monodispersed polystyrene latex (PSL) particles serve as certified reference materials (CRMs) for ensuring quality, method validation, and calibration of particle size measuring instruments. They were produced in compliance with ISO 17034:2016 standards. However, limitations arise in the quality control of CRM characteristics due to challenges in synthesizing particles of consistent size across multiple batches. It is widely recognized that variations in particle size homogeneity between batches can occur in the particle manufacturing process even when conducted under identical conditions and ingredients. This paper aims to explain and demonstrate statistical analyses conforming to ISO Guide 35:2017 used for quality control and certifying reference values for monodispersed polystyrene particles. The procedure can be divided into three steps: 1) preliminary check by using dynamic light scattering (DLS), 2) preliminary check by using transmission electron microscopy (TEM), and 3) final homogeneity testing, along with short-term and long-term stability assessment and characterization. The described protocol can control the quality of particles from multiple batch productions by establishing criteria at each step to ensure particle size consistency. The certified value of a 100 nm diameter monodispersed polystyrene particle produced by the National Institute of Metrology, Thailand (NIMT) is 105.5 ± 4.6 nm, acceptable for measurement traceability for testing and calibration services.

Keywords: Quality control, polystyrene particles, certified reference materials

1. Introduction

Nanotechnology is being extensively studied and has a wide range of applications. One of the critical properties of nanomaterials is particle size and particle size distribution, as the size significantly impacts various aspects of their properties. Therefore, particle size analysis is essential for studying phenomena in the nanoscale. Techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) [1] are commonly used for particle size analysis [2].

Accurate and reliable measurements are essential to ensure the quality and consistency of nanomaterials. In accordance with ISO 17025:2017, laboratories must ensure that measurement results are traceable to the national system of units (SI units) and maintain metrological traceability [3]. One method to achieve traceability to SI units is by using CRMs calibration of particle sizing instruments, ensuring that measurements are accurate and reliable. CRMs provided by competent producers, with stated metrological traceability to the SI. Reference material producers that fulfil the requirements of ISO 17034:2016 [4] are considered reliable.

* The work was presented at The 7th Asian Symposium on Emulsion Polymerization and Functional Polymeric Microspheres (ASEPFPM 2024), 20-22 June 2024

Monodispersed polystyrene particles are used as CRMs for quality control and quality assurance of particle sizing instruments [5,6]. CRMs provide a benchmark for instrument calibration, ensuring accurate and reliable measurements. They also play a critical role in method validation, enabling laboratories to verify that their analytical procedures produce consistent and accurate results.

Various polymerization techniques, such as suspension, emulsion, dispersion, and precipitation, have been developed for synthetic polymeric colloidal particles [7-9]. In monodispersed particle synthesis, it is well-known that variations in particle size homogeneity can occur during particle production, both within and between batches. As shown in Table 1, Samples 1-4 are synthesized using emulsion polymerization under the same conditions. However, the results demonstrated that size distribution varied between batches, even with the same synthesized protocol.

Additionally, particle sizes measured using DLS and TEM can vary significantly, especially for non-homogeneous particles. This highlights the importance of maintaining particle homogeneity in size measurement and quality control.

Table 1 Data comparison between particle size with and without homogeneity

Samples	DLS			TEM	
	Z-average (nm)	PDI	Average size (nm)	Min - Max (nm)	SD
Sample 1	108.00	0.010	105.77	99.28 - 108.96	2.05
Sample 2	104.8	0.017	91.96	74.18 - 107.55	7.42
Sample 3	100.3	0.009	93.71	74.13 - 104.51	6.47
Sample 4	97.59	0.011	87.87	71.17 - 100.88	5.76

Therefore, in the production of particle reference materials, it is critical to implement stringent quality control measures, utilizing statistical tools to monitor every stage of the production process. This ensures that subsequent batches of particles synthesized using the same protocol consistently meet acceptable quality standards. Furthermore, controlling particle size uniformity is essential for ensuring the accuracy and precision of particle size measurements across various techniques.

2. Methodology

Monodispersed PSL particles were synthesized using surfactant-free emulsion polymerization based on [10,11]. Polystyrene in aqueous suspension was diluted to a concentration of 1% solids and suspended in deionized water before characterization. In the particle size analysis process, it is well known that TEM is an excellent tool for characterizing nanoparticles [12] due to its high resolution, but sample preparation can be more time-consuming and complex compared to the DLS technique. DLS is used for preliminary particle size analysis before performing size analysis by the TEM technique. When the criteria from both steps are passed, it will be bottled. After that, homogeneity and stability will be studied in the next step. The whole process is shown in Figure 1.

2.1 Preliminary check using dynamic light scattering (DLS)

Particle size analysis was performed using DLS, Zetasizer Nanoseries, NANO-ZS S4700 (Malvern Panalytical, UK) according to ISO 22412:2017 [13]. Due to concentration limitation, samples were diluted to 1:100 and 1:500 by volume. The average particle size should deviate from the nominal value by less than ±10 nm, and the polydispersity index (PDI) should be less than 0.05.

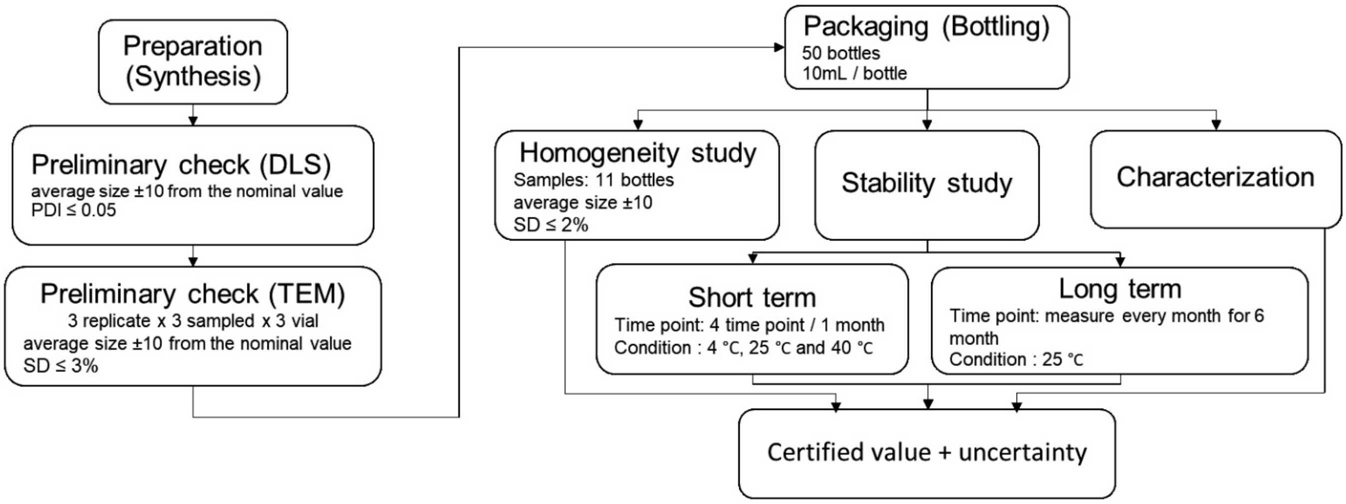

Figure 1 Schematic outline of monodispersed polystyrene particle.

2.2 Preliminary homogeneity testing using transmission electron microscope (TEM)

To further investigate particle size and identify any abnormalities in the polystyrene particles, morphology and diameter were examined using TEM, JEOL (JEM2100 Electron Microscope, USA) for preliminary homogeneity testing. Samples were dropped on copper grid and dried in clean environment at room temperature. One hundred particles were measured by TEM at magnification ≥ 20,000x One hundred particles were measured by TEM, and their sizes were analysed using ImageJ software [14]. The average size from nominal value less than ±10 nm and percent of Relative standard deviation (RSD) less than 3%. RSD can be calculated.

$$RSD(\%) = \frac{s}{\bar{x}} \times 100 \tag{1}$$

The standard deviation (s) and the mean ($\bar{x}$) can be determined.

$$S = \sqrt{\frac{\Sigma_{i=1}^{n}(x_i - \bar{x})^2}{n}} \tag{2}$$

$$\bar{x} = \frac{\sum_{i=1}^{n}(x - \bar{x})^2}{n} \tag{3}$$

For the statistical evaluation results to be reliable, samples were taken from the polystyrene suspension into three vials. Each vial prepared three samples, and 100 particles were measured per sample, with the relative standard deviation maintained at less than 3%. Particle size and shape distributions were measured according to ISO 21363:2020 [15] standards. The expanded uncertainty for measurements using TEM was evaluated to be ±1 nm.

2.3 Homogeneity testing, along with stability testing and characterization

After passing preliminary checks by DLS and TEM, PSL suspension was filled in 50 bottles. Homogeneity and stability study will be designed according to ISO 17034:2016. Statistical analysis processes will be used according to ISO Guide 35:2017 [16] to study and evaluate the usability of the certified reference material (CRM).

Homogeneity

The ISO Guide 35 requires RM producers to quantify variations between and within bottles. Between bottles demonstrates whether the processed batch is sufficiently homogeneous with respect to the certified property. Within the bottle, repeatability and particle size variation are combined in different sub-samples from one bottle [17].

Reference materials are produced in small batches. The minimum number of units for a homogeneity study should be assessed on 10% of batch size and simple random use as a sampling strategy for a homogeneity study. Eleven bottles were randomly selected for study within bottles and between bottles. Each bottle was analyzed by three sampling per bottle, three replicates per sampling, and each replicate analysed 100 particles. The criterion of homogeneity is RSD than 2%. Analysis of variance (ANOVA) was used as an analysis tool to determine within-bottle standard deviation and between-bottle standard deviation.

Stability

Two types of stability, short-term and long-term, are associated with the distribution behaviour of RM in different temperature conditions. Long long-term stability, study distribution during storage conditions for six months, the candidate RM was kept at a controlled temperature of 25 °C and measured using TEM every month. Trend analysis using the LINEST function in Microsoft Excel. The regression parameter from the LINEST function will be calculated automatically by the slope b_1 and standard deviation of slope s_{b_1} used for calculated t_{cal} and t_{crit} consider by t-table if $\mathrm{t}_{cal} < \mathrm{t}_{crit}$ Therefore, no trend was observed. It can be discussed that candidate RM has long-term stability.

$$\mathrm{t}_{cal} = \frac{|b_1|}{s_{b_1}} \tag{4}$$

Short-term stability, study under extreme conditions what might happen during transport conditions at 4 °C, 25 °C and 40 °C for a month where 25°C is the reference temperature and experiment design using isochronous stability testing. The maximum acceptable for short-term relative standard deviation is less than 2%. Regression analysis is used as an analysis tool for long-term stability testing.

Characterization

The value assignment x_{CRM} has been certified for the diameter of the polystyrene nanoparticle in an aqueous solution by using the TEM technique, and can be calculated.

$$x_{CRM} = y_{char} + \delta_{homo} + \delta_{lts} \tag{5}$$

x_{CRM} is the property value, y_{char} is the property value obtained from the characterization of the batch, δ_{homo} is an error due to heterogeneity and δ_{lts} is an error for stability effect under storage conditions, where y_{char} can be calculated.

$$y_{char} = \frac{1}{p}\sum_{i=1}^{p} y_i \tag{6}$$

The measurement uncertainty was evaluated according to JCGM 100:2008 [18] and can be calculated.

$$u_{CRM} = \sqrt{u_{char}^2 + u_{homo}^2 + u_{lts}^2} \tag{7}$$

where u_{CRM} is standard uncertainty of CRM and u_{Char} is standard uncertainty from characterization. u_{homo} is standard uncertainty from homogeneity and u_lts is standard uncertainty from stability.

3. Results and discussion

Preliminary check using DLS

The criteria are the average size ±10 from the nominal value and the PDI ≤ 0.05. Table 2 shows the DLS measurement result of PSL 100 nm from 10 replicates.

Table 2 Results testing of PSL by using DLS [19]

No. of replicate	Dilution 1:100 by volume		Dilution 1:500 by volume	
	Average size (nm)	PDI	Average size (nm)	PDI
#1	107.80	0.015	109.30	0.006
#2	108.10	0.011	108.00	0.008
#3	108.20	0.011	109.00	0.021
#4	108.30	0.006	108.20	0.010
#5	108.30	0.010	109.00	0.014
#6	106.80	0.011	109.10	0.015
#7	108.80	0.013	108.50	0.006
#8	108.40	0.018	107.80	0.010
#9	107.40	0.007	107.00	0.006
#10	107.40	0.015	108.40	0.021

Figure 2 shows the size distribution of PSL particle size 100nm, replicate number 1, and dilution 1:100 using the DLS technique. The figure shows the uniform distribution of particle sizes.

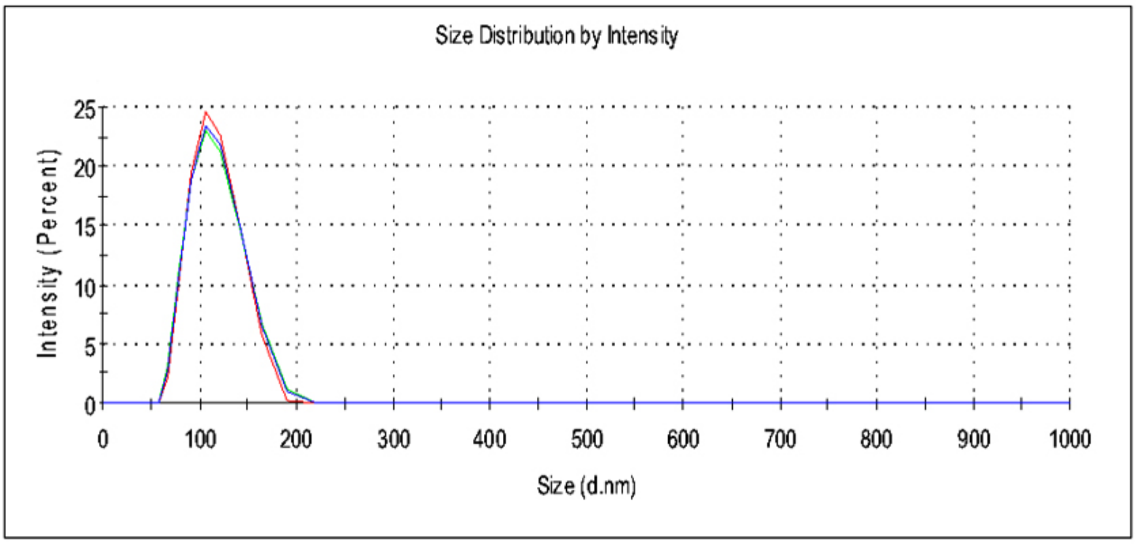

Figure 2 Size distribution of PSL particle from DLS technique.

Preliminary homogeneity testing using TEM

The criteria are the average size ±10 from the nominal value and the RSD (%) ≤ 3%. Table 3 shows the measurement results of PSL. The mean diameter represents the particle diameter from each vial, and the average size represents the average diameter of 3 vials. Results are specified criteria.

Table 3 Results of preliminary testing of PSL by using TEM

No. of vial	Mean diameter (nm)	Relative standard deviation (%)
#1	107.40	0.29
#2	106.84	0.96
#3	106.97	0.20
Average	107.07	0.27

Homogeneity testing, along with stability testing assessment and characterization

Homogeneity testing

The criteria are the average size ±10 from the nominal value and the RSD (%) ≤ 2%. Eleven bottles from 50 bottles were randomly selected for testing. The size distribution within the unit was considered the RSD of each bottle, and between units considered average of 11 bottles. From the results in Table 4, considering the RSD, it can be indicated PSL is homogenous because measurement results are within acceptable criteria.

Table 4 Results of homogeneity testing within and between bottles of PSL by using TEM

No. of bottles	Mean diameter (nm)	Relative standard deviation (%)	
#1	104.85	0.37	Pass
#2	105.70	0.19	Pass
#3	105.59	0.23	Pass
#4	106.66	0.21	Pass
#5	105.41	0.26	Pass
#6	105.36	0.23	Pass
#7	105.47	0.21	Pass
#8	105.58	0.61	Pass
#9	105.45	0.16	Pass
#10	105.45	0.11	Pass
#11	105.96	0.55	Pass
Average	105.59	0.42	Pass

ANOVA can determine that the standard uncertainty of homogeneity is 1.142 nm.

Stability testing

a) Short-term stability

The criteria are the average size ±10 from the nominal value and the RSD (%) ≤ 2%. The mean diameter and RSD under extreme conditions for a month are shown in Table 5. From the results, considering RSD, PSL is stable at room temperature 25 °C and extreme temperature 4 °C and 40 °C measurement results are within acceptable criteria. It can be indicated that PSL is stable during transport conditions.

Table 5 Results of short-term stability testing of PSL by using TEM

Temperature (°C)	Mean diameter (nm)	Relative standard deviation (%)	
40	107.41	0.14	Pass
25	103.85	0.34	Pass
4	104.45	0.20	Pass

From ANOVA, the standard uncertainty of homogeneity is 0.196 nm.

b) Long-term stability

In statistical analysis, the t-statistic was used to test the significance of the slope at a 95% confidence level. The calculated t-value (t_{cal}) of 0.085 was obtained using equation 4. The critical t-value (t_{crit}) for a two-tailed test at a 95% confidence level (with a p-value of 0.05) was found to be 2.7764, as shown in Table 6. Since $t_{cal} < t_{crit}$, the null hypothesis cannot be rejected, indicating that no significant trend was observed. Therefore, during the long-term storage at room temperature, no noticeable change in particle size, either increasing or decreasing, was detected.

Table 6 Results of long-term stability testing over 6 months and regression parameter from LINEST function

Time point (months)	Mean diameter (nm)	Regression parameter from LINEST function		
1	103.558	slope, b_1	0.009	104.112
2	104.723	the standard deviation of the slope s_{b_1}	0.105	0.408
3	104.159		0.002	0.438
4	104.316		0.007	4
5	104.199		0.001	0.768
6	103.904		0.009	104.112

Characterization

The value assignment x_{CRM} has been certified for a diameter of the polystyrene nanoparticle of 105.475 nm calculated following Equation 5 and combined standard uncertainty of homogeneity, stability, characterization, and source of uncertainty of measurement including from measurements of RM, sensor (CCD), image drift, and thermal effect, u_{CRM} is 4.6 nm.

4. Conclusion

From the defining statistical evaluation of each step, the quality of CRM production meets standards according to ISO 17034:2016, equivalent quality to the standard commercial CRM products. The 100 nm monodispersed polystyrene particles produced by NIMT have a specification of 105.5 ± 4.6 nm, comparable to other commercially available 100 nm particles. The adoption of CRMs thus ensures high standards of accuracy and reliability in particle size measurements, contributing to the overall integrity and credibility of analytical results.

References

[1] B. J. Berne, R. Pecora, Dynamic light scattering: With applications to chemistry, biology, and physics, John Wiley, New York (1976).

[2] T. G. F. Souza, V. S. T. Ciminelli, N. D. S. Mohallem, A comparison of TEM and DLS methods to characterize size distribution of ceramic nanoparticles, *J. Phys.: Conf. Ser.* **733** (2016) 012039.

[3] International Standard, ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories, International Organization for Standardization (ISO) (2017).

[4] International Standard, ISO 17034:2016 General requirements for the competence of reference material producers, International Organization for Standardization (ISO) (2016).

[5] B. Ngansalung, N. Chanthawong, J. Buajarern, Enhancing particle size characterization performance through proficiency testing program: Result and insights from inter-laboratory comparison, *J. Phys.: Conf. Ser.* **2653** (2023) 012008.

[6] M. Effendi, F. S. Rahmah, L. Susanti, N. T. Rochman, Preparation of monodisperse polystyrene spheres by physical method, *IOP Conf. Ser.: Mater. Sci. Eng.* **509** (2019) 012088.

[7] V. Chaudhary, S. Sharma, Suspension polymerization technique: Parameters affecting polymer properties and application in oxidation reactions, *J. Polym. Res.* **26** (2019) 102.

[8] Y.-S. Cho, C. H. Shin, S. Han, Dispersion polymerization of polystyrene particles using alcohol as reaction medium, *Nanoscale Res. Lett.* **11** (2016) 46.

[9] S. T. Ha, O. O. Park, S. H. Im, Size control of highly monodisperse polystyrene particles by modified dispersion polymerization, *Macromol. Res.* **18** (2010) 935-943.

[10] N. I. Prokopov, I. A. Gritskova, O. P. Kiryutina, M. Khaddazh, K. Tauer, S. Kozempel, The mechanism of surfactant free emulsion polymerization of styrene, *Polym. Sci. Ser. B* **52** (2010) 1043-1049.

[11] F. Y. P. Tyassena, R. M. Dewa, K. Quarta, W. Widiyastuti, S. Winardi, Preparation of polystyrene spheres using surfactant-free emulsion polymerization, *Mod. Appl. Sci.* **9** (2015) 121-126.

[12] J. E. Bonevich, W. K. Haller, Measuring the size of nanoparticles using transmission electron microscopy (TEM), NIST-NCL Joint Assay Protocol PCC-7 (2010).

[13] International Standard, ISO 22412:2017 Particle size analysis – Dynamic light scattering (DLS), International Organization for Standardization (ISO) (2017).

[14] S. Zhang, C. Wang, Precise analysis of nanoparticle size distribution in TEM image, *Methods Protoc.* **6** (2023) 63.

[15] International Standard, ISO 21363:2020 Nanotechnologies – Measurements of particle size and shape distributions by transmission electron microscopy, International Organization for Standardization (ISO) (2020).

[16] International Standard, ISO/Guide 35:2017 Reference materials – Guidance for characterization and assessment of homogeneity and stability, International Organization for Standardization (ISO) (2017).

[17] A. Braun, V. Kestens, K. Franks, G. Roebben, A. Lamberty, T. P. J. Linsinger, A new certified reference material for size analysis of nanoparticles, *J. Nanopart. Res.* **14** (2012) 1021.

[18] JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement, Joint committee for Guides in Metrology (JCGM) (2008).

[19] J. Pongkasem, D. Crespy, S. Thaiboonrod, A. Klamchuen, R. Saenmuangchin, K. Mongkolsuttirat, N. Chanthawong, J. Buajarern, J. Sumranjit, Development of monodispersed polystyrene particles as Thailand Reference Materials (TRM), *MAPAN* **37** (2022) 469-474.