MĀPAN-Journal of Metrology Society of India (September 2022) 37(3):469–474
https://doi.org/10.1007/s12647-022-00552-7

ORIGINAL PAPER

Development of Monodispersed Polystyrene Particles as Thailand Reference Materials (TRM)

J. Pongkasem[1], D. Crespy[1], S. Thaiboonrod[1], A. Klamchuen[1], R. Saenmuangchin[1], K. Mongkolsuttirat[2], N. Chanthawong[2], J. Buajarern[2] and J. Sumranjit[1]*

[1]National Nanotechnology Center, National Science and Technology Development Agency, 111 Phahonyothin Rd., Klongluang, Pathumthani 12120, Thailand

[2]National Institute of Metrology Thailand, 3/4-5 Moo 3, Klong 5, Klongluang, Pathumthani 12120, Thailand

Received: 23 January 2022 / Accepted: 22 March 2022 / Published online: 23 April 2022

Abstract: The purpose of this study was to develop monodispersed polystyrene particles as certified reference materials in accordance with the ISO 17034: 2016 and the ISO Guide 35. It can play a significant role especially during the COVID-19 pandemic since several covering items such as filtering facepiece respirators or medical masks must be investigated for the quality of operation by various sizes of polystyrene. The polystyrene particles were prepared in-house by National Nanotechnology Center (NANOTEC) using polymerization of styrene. Each batch was preliminary check for distribution, aggregation and averaged size by using dynamic light scattering. Then polystyrene particles were prepared to 1% solid suspension in deionized water for homogeneity testing, stability assessment and characterization using transmission electron microscope with ImageJ software. The 100 nm polystyrene as an example has been successfully synthesized fulfilled the criteria of size deviation from nominal value less than ± 10 nm and polydispersity index less than 0.05. Then, the particle size of polystyrene was statistically analyzed for screening test with the results of the coefficient of variation less than 10%. Stability assessment consisting of short-term stability testing with three different temperatures and long-term stability testing within 6 months observed was carried out. The results of short-term and long-term stability were presented within the maximum acceptable. The homogeneity tests for within bottle standard deviation and between bottle standard deviation were performed with randomly sampling. The results of homogeneity tests satisfied the criteria and therefore assigned the value as the certify value. Consequently, the certify value of 105.5 ± 4.6 nm of monodispersed polystyrene particles has been successfully developed as Thailand reference materials which were similar level of quality and accuracy to the standard commercial products.

Keywords: Reference material; Polystyrene; Traceability

1. Introduction

Certified reference material (CRM) is a reference material accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization to the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [1]. CRM is generally prepared in batches for which the property values are determined within the stated uncertainty limits by measurements on sample representative for the whole batch.

CRMs are widely used for calibration of an apparatus, method validation, assessment of method and instrument performance, establishing traceability or the measurement results, and determining the uncertainty of these results [2–6]. They are the most appropriate choice for quality control activities. Information obtained from their use would be the most extensive and reliable. Particularly important are the instructions for the use of the CRM as stated in the certificate. The certified values do only apply if the material is strictly used according to these instructions. The user needs to follow closely to the recommendations given for storage of the material, eventual drying

*Corresponding author, E-mail: jitapa@nanotec.or.th

procedures, and observe the indicated shelf life of an RM. It is not justified to assume the validity of the reference values beyond the expiry date of a given material.

Due to COVID-19 pandemic, filtering facepiece respirators, medical masks, and other face coverings are recommended as a simple barrier to help prevent respiratory droplets from traveling into the air and onto other people [7]. The respiratory droplets play role in the spread of the virus that causes COVID-19. Evidence from clinical and laboratory studies shows that masks reduce the spray of droplets when worn over the nose and mouth. COVID-19 spreads mainly among people who are in close contact with one another (within about 6 feet), so the use of masks is particularly important in settings where people are close to each other or where social distancing is difficult to maintain. Prior to the COVID-19 pandemic, similar medical masks were also widely used in public for the same purpose against PM2.5.

The efficacy of such face coverings depends mostly on the filtration media and the seal with the wearer's face. While leakage must be assessed on an individual-by-individual basis, filtration efficiency can be measured using well-defined, universal test methods. Particulate filtration efficiency (PFE) of such covering item was measured using various size of polystyrene latex (PSL) particles as per many guidance document [8–10].

During the COVID-19 pandemic, barrier face coverings are in high demand and hence resulting in growth of face coverings manufacture worldwide including Thailand. Mask manufacturers in Thailand have increased from ten to hundreds within a year. As a result, the consumption level of PSL particles by the testing and calibration laboratories is increased dramatically. Not only that testing and calibration laboratories are continually requested to provide evidence in order to ensure quality of their operations. This is mandatory in cases where legislative limits are involved, e.g., in international trade, food and environmental analysis, clinical chemistry, etc. The best and easiest way for laboratories to formally demonstrate their quality is to adhere to an appropriate international quality standard and obtain formal accreditation/certification. However, basic quality requirements do not differ significantly. Due to a wide range of activities to which it can be applied and due to the well-established quality assessment structure, the ISO/IEC 17025 is commonly selected as a standard of choice whenever quality assurance in laboratory is to be demonstrated. The objectives are not only to reduce the operation cost but also promote the country's in-house facility.

PSL particles are typically synthesized by dispersion or emulsion polymerizations [11–13]. The synthesis method had been optimized in order to comply with specification as the TRM. The preparation of CRM is in accordance with the ISO 17034: 2016 [14] and the ISO Guide 35 [15].

For the characterization and certification, National Institute of Metrology Thailand (NIMT) has defined a protocol for developing PS TRM following ISO17034:2016 General requirements for the competence of reference material producers [14].

2. Experimental

Monodispersed PSL particles were prepared in-house by NANOTEC using polymerization technique based on the literatures [16, 17]. The suspension was diluted to concentration of 1% solid by deionized water (resistivity of 18.2 MΩ). The pH of the suspension was measured to be 9. The recipe is trade secret which is an intellectual property right on confidential information.

The PSL particles were statistically analyzed following the protocol based on ISO 17034: 2016 and the ISO Guide 35. General steps include:

- Synthesis of material
- Sample preparation (including homogenization, stabilization, bottling etc.)
- Homogeneity testing
- Stability assessment
- Value assignment (characterization)

Each batch was preliminary check for distribution, aggregation and averaged size by using dynamic light scattering (DLS), Zetasizer Nanoseries, NANO-ZS S4700 (Malvern Panalytical, UK). PSL particle suspension will further diluted at 1:100 and 1:500 by volume using deionized water. Batch that has size deviation from nominal value less than ± 10 nm, aggregation nor agglomeration free and polydispersity index (PDI) less than 0.05 will be further investigated. Otherwise, formula will be adjusted till properties meet criteria. Particle size analysis using DLS is conducted based on ISO 22412:2017 [18]. Measurement traceability to SI unit is established through the use of NIST CRM gold nanoparticle (RM 8012). Expanded

Table 1 Screening test results of PSL by using DLS

No.	Dilution 1:100 by volume		Dilution 1:500 by volume	
	Diameter (nm)	PDI	Diameter (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	109.10	0.021
#4	108.30	0.006	108.20	0.010
#5	108.30	0.010	109.00	0.014
Average	108.14	–	108.72	–

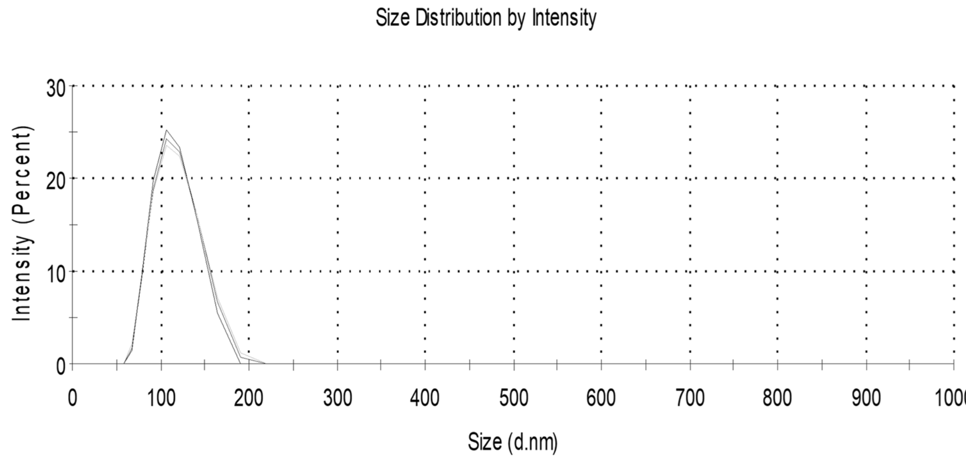

Fig. 1 Polydispersity of the PSL particles from DLS technique

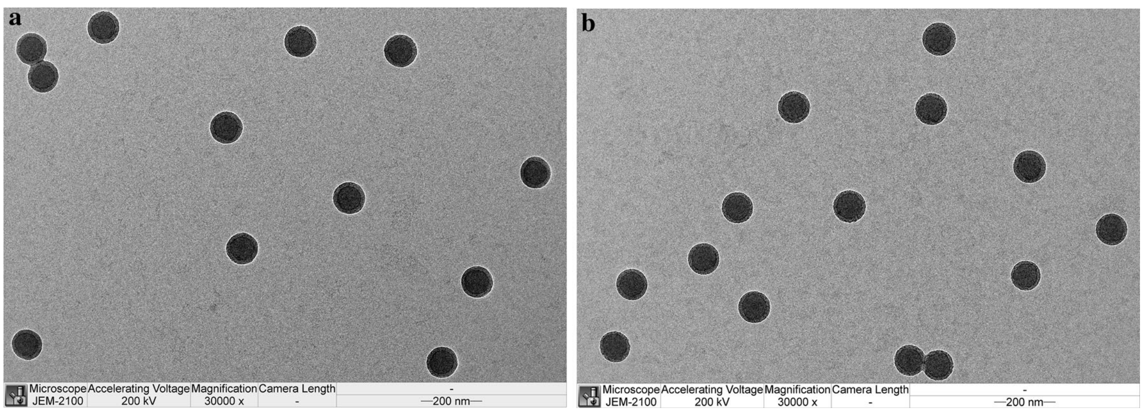

Fig. 2 TEM micrographs of the PSL particles

Table 2 Screening test result of PSL by using TEM

Sample vial	Mean diameter (nm)	σ_{n-1} (nm)	CV (%)
1	107.40	0.31	0.29
2	106.84	1.03	0.97
3	106.97	0.21	0.20
Average	107.07	0.29	0.27

Table 3 Short-term stability test results of PSL by using TEM

Temperature (°C)	Mean diameter (nm)	σ_{n-1} (nm)	CV (%)
4	104.41	0.14	0.14
25	103.85	0.36	0.34
40	104.45	0.20	0.20
Average	104.24	0.34	0.32

uncertainty for measurement using DLS is evaluated to be at ± 3 nm.

For further investigation, morphology and diameter of the polystyrene particles will be observed by JEOL (JEM-2100 Electron Microscope, USA) transmission electron microscope (TEM). The PSL particle suspension was dropped on to the copper grid and dried at room temperature. The electron micrographs were taken at the same magnification throughout the analysis. The particles sizes were measured from the electron micrographs using ImageJ software (National Institutes of Health, USA). Measurements of particle size and shape distributions by TEM is based on ISO 21363: 2020 [19]. Calibration and measurement uncertainty estimation of all measuring instrument used were conducted in accordance with the final report of APMP.L-S5 [20]. Magnification accuracy of TEM is ensured by using silicon crystal, {d220} lattice plane. Expanded uncertainty for measurement using TEM is evaluated to be at ± 1 nm.

3. Results and Discussion

PSL particles were synthesized by polymerization of styrene. Size of particles was controlled by temperature, concentration and time. Product from each recipe was preliminary checked for distribution, aggregation and average size using DLS technique because it is a simple and fast measurement technique. The criteria are size

Table 4 Long-term stability test results of PSL by using TEM

Month	Mean diameter (nm)		
	#1	#2	#3
1	104.26	104.54	104.43
2	104.84	104.51	104.82
3	103.68	104.66	104.13
4	104.82	103.98	104.14
5	103.90	104.71	103.98
6	103.79	104.67	103.25
Variation (nm)	1.16	0.73	1.57
Variation (%)	1.1	0.7	1.5

Table 5 Homogeneity test results of PSL by using TEM

No.	Mean diameter (nm)	σ_{n-1} (nm)
1	104.85	0.39
2	105.70	0.19
3	105.59	0.25
4	105.40	0.13
5	105.41	0.28
6	105.36	0.25
7	105.47	0.22
8	105.58	0.06
9	105.45	0.17
10	105.45	0.12
11	105.96	0.59
Average	105.47	0.27

deviation from nominal value less than ± 10 nm and PDI less than 0.05. Table 1 and Fig. 1 demonstrate DLS measurement result of 100 nm PSL suspension from 5 reproduced batches.

After passing preliminary checking, PSL particles were prepared to 1% solid suspension in deionized water. Homogeneity testing, stability assessment and characterization were conducted using TEM. Uncertainty of TEM measurement was evaluated according to JCGM 100:2008 [21]. The combined standard uncertainty of TEM measurement is 0.43 nm. This accuracy level is suitable for characterization and certification of reference material for nanoscale measurement.

Figure 2 illustrates TEM images of the 100 nm PSL particles at magnification 30 k. The measurement result was analyzed according to ISO 21363:2020 [19]. Since TEM measurement and data analysis require a lot of man-hour of machine and operator, screening test is additionally included in the statistically analysis.

A portion of sample was taken from the prepared PSL particle suspension and divided into 3 vials. Then each vial was sampled 3 times. TEM images were then taken where each image shall contain at least 10 PSL particles. Particle size of 100 particles from each sampling was measured using ImageJ software. The criterion is the coefficient of variation (CV) of the particle size and shall be less than 3%. Table 2 presents statistically analysis result from 100 particles × 3 sampling × 3 vials where CV is well below 3%.

When the sample has passed the screening test, homogeneity testing and stability assessment were proceeded. The PSL candidate was bottled to 50 portions, 10 mL in volume each. Eleven bottles and 9 bottles were randomly sampling for homogeneity testing and stability testing,

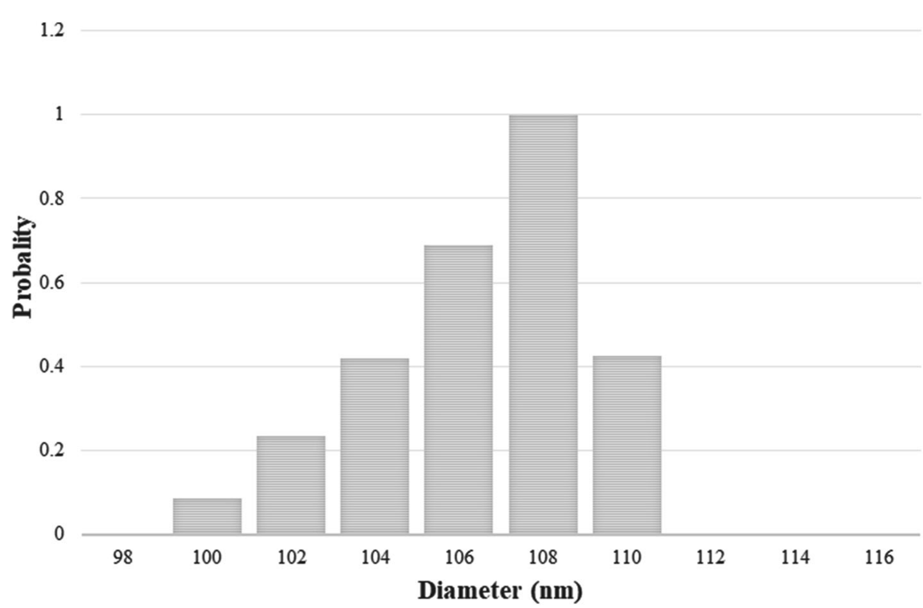

Fig. 3 Histogram of the PSL particle size from TEM technique

respectively. Stability assessment consists of short-term stability testing and long-term stability testing. For short-term stability testing, 9 bottles were stored at temperature controlled at 4 °C, 25 °C and 40 °C (3 bottles at each temperature) for a month. Then measurement was all carried out under repeatability condition. Number of replicates per unit is three. The maximum acceptable short-term stability is 0.5%. Table 3 illustrates the short-term stability test result of 100 nm PSL candidate.

Long-term stability was observed for 6 months. The candidate was kept at 25 °C, and measurements were performed using TEM every month. Number of replicates per unit is three. The maximum acceptable short-term stability is 1.5%. Table 4 illustrates the long-term stability test result of 100 nm PSL candidate.

Homogeneity test results are summarized in Table 5, and size distribution of the PSL candidate is demonstrated in Fig. 3. TEM images of 100 particles × 3 replicates from 11 randomly sampling bottles were taken at 200 kV with magnification of 30 k. Analysis of variance (ANOVA) was used as an analysis tool to determine within bottle standard deviation and between bottle standard deviation. Since homogeneity is one of the uncertainty components that plays a crucial role to the accuracy of the certified value of PSL reference material, criterion of homogeneity not exceeding 2% is set.

The certified value was calculated from measurement result in Table 5. Measurement uncertainty was evaluated according to JCGM 100:2008. The 100 nm monodispersed PSL TRM was successfully certified as 105.5 ± 4.6 nm. Follow up testing was done by using such TRM to calibrate Zetasizer Ultra, model Ultra, manufactured by Malvern. Another exercise was done by utilizing our PSL TRM for mask testing facility. Upon comparison with the commercially available PSL, our PSL TRM has similar quality and level of accuracy.

It should be noted that PSL particles of size range from 100 nm to 2.5 µm are under development and assessment to be TRM.

4. Conclusion

In this paper, the 100 nm monodispersed PSL particles were prepared by polymerization technique. The statistical analysis according to ISO 17034:2016 and ISO Guide 35 applied to PSL particles. TEM was used to determine size, homogeneity, stability and characterization, and the uncertainty of measurement was evaluated according to JCGM 100:2008. The results showed that the 100 nm PSL had an excellent result in all short-term stability, long-term stability, and homogeneity tests. The 100 nm monodispersed PSL TRM was certified as 105.5 ± 4.6 nm, which was equivalent quality to the standard commercial CRM products.

Acknowledgements This work is supported by the Platform technology of the National Nanotechnology Center Thailand (NANOTEC).

References

[1] ISO (2015) Reference materials—selected terms and definitions, ISO Guide, 30:2015.

[2] A. Kriukova, D. Lytkin, M. Marchenko and I. Vladymyrova, Development of green production technology and research of harpagophytum procumbens root dry extract, ScienceRise Pharm. Sci., **4** (2021) 43–49. https://doi.org/10.15587/2519-4852.2021.239332.

[3] D.T. Zhao, Y.J. Gao, W.J. Zhang, T.C. Bi, X. Wang, C.X. Ma and R. Rong, Development a multi-immunoaffinity column LC–MS–MS method for comprehensive investigation of mycotoxins contamination and co-occurrence in traditional Chinese medicinal materials, J. Chromatogr. B Anal. Technol. Biomed. Life Sci., **1178** (2021) 122730. https://doi.org/10.1016/j.jchromb.2021.122730.

[4] M.M. Phillips, K.E. Sharpless and S.A. Wise, Standard reference materials for food analysis, Anal. Bioanal. Chem., **405** (2013) 4325–4335. https://doi.org/10.1007/s00216-013-6890-5.

[5] ISO (2017) Particle size analysis—dynamic light scattering (DLS), ISO 22412:2017.

[6] ISO (2014) Particle size analysis—image analysis methods—part 1: static image analysis methods, ISO 13322:2014(en).

[7] V. Babaahmadi, H. Amid, M. Naeimirad and S. Ramakrishna, Biodegradable and multifunctional surgical face masks: a brief review on demands during COVID-19 pandemic, recent developments, and future perspectives, Sci. Total Environ., **798** (2021) 149233. https://doi.org/10.1016/j.scitotenv.2021.149233.

[8] R.M. Jones and D. Rempel, Standards for surgical respirators and masks: relevance for protecting healthcare workers and the public during pandemics, Ann. Work Exposures Health, **65** (2021) 495–504. https://doi.org/10.1093/annweh/wxab008.

[9] ASTM (2017) Standard test method for determining the initial efficiency of materials used in medical face masks to penetration by particulates using latex spheres ASTM F2299/F2299M-03.

[10] ASTM (2018) Standard specification for performance of materials used in medical face masks ASTM F2100-11.

[11] S.T. Ha, O.O. Park and S.H. Im, Size control of highly monodisperse polystyrene particles by modified dispersion polymerization, Macromol. Res., **18** (2010) 935–943. https://doi.org/10.1007/s13233-010-1008-9.

[12] M. Antonietti and K. Landfester, Polyreactions in miniemulsions, Prog. Polym. Sci., **27** (2002) 689–757. https://doi.org/10.1016/S0079-6700(01)00051-X.

[13] Q. Hongfei, H. Weichang, X. Huaizhe, Z. Junying and W. Tianmin, Synthesis of large-sized monodisperse polystyrene microspheres by dispersion polymerization with dropwise monomer feeding procedure, Colloid Polym. Sci., **287** (2009) 243–248. https://doi.org/10.1007/s00396-008-1979-7.

[14] ISO (2016) General requirements for the competence of reference material producers ISO 17034:2016.

[15] ISO (2017) Reference materials—guidance for characterization and assessment of homogeneity and stability, Guide 35:2017.

[16] F.P.T. Yohanala, R.M. Dewa, K. Quarta, W. Widiyastuti and S. Winardi, Preparation of polystyrene spheres using surfactant-

free emulsion polymerization, Mod. Appl. Sci., **9** (2015) 121–126. https://doi.org/10.5539/mas.v9n7p121.

[17] N.I. Prokopova, I.A. Gritskovaa, O.P. Kiryutinaa, M. Haddazha, K. Tauerb and S. Kozempel, The mechanism of surfactant-free emulsion polymerization of styrene, Polym. Sci. Ser. B, **52** (2010) 339–345. https://doi.org/10.1134/S1560090410050106.

[18] ISO (2017) Particle size analysis—dynamic light scattering (DLS) ISO 22412:2017.

[19] ISO (2020) Nanotechnologies—measurements of particle size and shape distributions by transmission electron microscopy ISO 21363:2020.

[20] H.L. Lin, W.E. Fu, H.F. Weng, I. Misumi, K. Sugawara, S. Gonda, K. Takahashi, K. Takahata, K. Ehara, T. Takatsuji, T. Fujimoto, J. Salas, K. Dirscherl, J. Garnãs, J. Damasceno, J.C.V. de Oliveira, E. Emanuele, G.B. Picotto, C.S. Kim, S.J. Cho, C. Motzkus, F. Meli, S. Gao, Y. Shi, J. Liu, K. Jämting, H.J. Catchpoole, M.A. Lawn, J. Herrmann, V.A. Coleman, L. Adlem, O.A. Kruger, J. Buajarern, E. Buhr, H.U. Danzebrink, M. Krumrey and H. Bosse, Nanoparticle characterization—supplementary comparison on nanoparticle size, Metrologia, **56** (2019) 04004. https://doi.org/10.1088/0026-1394/56/1A/04004.

[21] JCGM 100:2008—Evaluation of measurement data—guide to the expression of uncertainty in measurement.