Calculation of the Budget of Uncertainty on Measurements Size Nanoparticles Using Dynamic Light Scattering

Adriana N. Geraldes, [ORCID] **,a Wilmmer A. A. Rosero,a Maria Elisa C. M. Rostelatoa and Jorge E. S. Sarkis* [ORCID] a

aInstituto de Pesquisas Energéticas e Nucleares-Comissão Nacional de Energia Nuclear (IPEN-CNEN), Av. Prof. Lineu Prestes, 2242, 05508-900 São Paulo-SP, Brazil

Accurate nanoparticle characterization is essential since it can significantly affect its physicochemical and biological properties. Among physicochemical properties used to characterize nanomaterials, size and size distribution are essential and should be assessed before surveying poisonousness or biocompatibility. Several methods are suitable to evaluate these characteristics including the dynamic light scattering. The aims of the present paper were to propose a methodology to measure nanoparticle size and present the estimation of the particle size uncertainty using the dynamic light scattering technique. The reliability of measurements was ensured by a series of handling precautions and quality criteria for good measurements to be applied for methodology validation using reference material polyvinylpyrrolidone coated silver nanoparticles. The identification and quantification of input quantities to the measurement uncertainties were estimated. The uncertainty concerning the equipment was 1.2% while the repeatability obtained was 1.4%, within the range of values stipulated in the reference standard (less than 5%). The relative standard uncertainties of trueness and repeatability were below the thresholds defined by the International Organization for Standardization. The result of the expanded uncertainty was 3.9% with 95% coverage probability for the reference material.

Keywords: nanoparticles, reference material, dynamic light scattering, uncertainty

Introduction

Nanomaterials (NM) are increasingly used for different purposes[1-11] such as health and medicine, energy, cosmetics, environment and many others, requiring the use of robust and reliable procedures for their production and characterization.

The identification, quantification and characterization of NM, therefore, brings a series of scientific and technological challenges since these materials have chemical, physical, electrical and magnetic characteristics that are different from the materials found in micro and macro scales. These materials may undergo modifications depending on the environment or the matrix in which they are present, which could form aggregates on their surface with other compounds present in the sample, making their identification difficult. Thus, its quantification, characterization and evaluation of functionality necessarily require the use of different techniques with different analytical principles. Consequently, there is a need to develop new reproducible and metrologically traceable analytical methodologies that meet this new challenge, allowing its characterization, identification and functionalization. For this purpose, there is a need to develop complete analytical protocols, using the best measurement practices, development of standards, suitable certified reference materials, with special attention ranging from the sampling process to an assessment of uncertainties at each stage of the analysis.[12]

Metrology is the science of measurement and it takes into consideration all aspects involved in a measurement, i.e., sampling, sample preparation, physicochemical procedure and, at last, the measurement, ensuring the quality of results, traceability and reliability.[13]

One of its purposes is to guarantee the quality of products and services through the control of measurement equipment, whatever the measurement uncertainty and field of application are. The great objective of metrology, and in particular chemical metrology, is therefore to establish criteria and procedures that bring quality, comparability

*e-mail: adriana.geraldes@ipen.br
Editor handled this article: Eduardo Carasek

and traceability to chemical tests with the corresponding determined variability (uncertainty). These criteria are essential to evaluate the quality of products, develop new materials and processes, among others. For this, metrology establishes tools for reliable measurements (e.g., certified reference materials, statistical methods and calculations, among others). Moreover, metrology provides the proper validation of methods for determining nanomaterials in samples. Validation is the key activity to guarantee the traceability and reliability of the results.[2]

These principles of metrology should be applied to analytical techniques for the characterization of nanoparticles (NP). It is important to recognize that there are major differences in the metrological requirements associated with the transition from the micro-scale to the nanoscale. With the increasing advancement of nanotechnology, it is indispensable to offer means to guarantee the metrological traceability demanded by this area. Areas like pharmaceuticals, environment, materials, chemistry, food, among others, demand the need to perform dimensional measurements with precision and accuracy in accordance with the definitions of the International System of Units (SI).[14] In this scenario emerges the new science named nanometrology.[12]

Among the challenges of nanometrology is nanocharacterization with the growing need for the development of standards for the manufacture of reference materials, design of calibration methods for nanoscale measurements and determination of reproducible measurement techniques for each property of the product or material.[15,16]

There is a wide variety of techniques for characterization of NP capable of accurately and reliably determining size, size distribution, shape, agglomeration, structure, composition, surface and concentration.[2,12] Several methods are suitable to evaluate size and size distribution characteristics including the well-established and most commonly used method based on dynamic light scattering (DLS) described in the International Organization for Standardization (ISO) standard.[17] In general, results give the mean size of the dispersion expressed as the mean hydrodynamic diameter of nanomaterials. Therefore, there is a need to develop standardized protocols that can be applied to a wide range of nanomaterials. If the test result is presented without the estimated measurement uncertainty, this can be significant in the validity or application of the measurement results. Knowing the uncertainty of the particle size analysis result is important to present a complete result with quantified measurement quality.[18,19]

Goldschmidt *et al.*[18] proposed a procedure for establishing the uncertainty in measurements of the size of monodisperse nanoparticles in liquid suspension by DLS. Nominal 60 nm polystyrene nanoparticles were used in the procedure. The standard uncertainties of the input quantities viscosity, temperature, decay rate, wavelength and refractive index were evaluated. The mean effective hydrodynamic diameter (d) = 62.30 nm with standard uncertainty (u_d) = 0.34 nm was presented. Varenne *et al.*[19] proposed a standardization of two protocols to evaluate the size of nanomaterials by DLS at 20 and 25 °C and a methodology to achieve their validation by investigating their robustness, precision and trueness using appropriate certified reference materials including standards of 60 and 203 nm. Uncertainties of protocols proposed were 7.0 and 3.8% for dispersions at 60 and 203 nm, respectively, at temperature of measurement of 20 °C and 6.8 and 3.8% for dispersions at 60 and 203 nm, respectively, at temperature of measurement of 25 °C. These values attested that both protocols give reliable size measurements of diverse nanomaterials. Continuing these studies, Varenne *et al.*[20] proposed an extension of a size measurement protocol validation by DLS previously validated with certified reference materials (CRM) at 60 and 203 nm. The paper reports robustness, precision and trueness of the protocol using CRM at 100 and 400 nm. The protocol was robust, accurate and consistent with the ISO standard. The expanded uncertainties were 4.4 and 3.6% for CRM at 100 and 400 nm, respectively, indicating the reliability of the protocol. Matteucci *et al.*[21] also evaluated the uncertainty of particle size measurement by DLS. The method for estimating was based on a guide of measurement uncertainty expression, which employs the "Uncertainty Propagation Law" (GUM)[22] and identified 14 sources of uncertainty. The result of the expanded uncertainty was 4.0% with 95% coverage probability for a material of 101.8 nm in diameter (polystyrene spheres). The repeatability obtained was 1.0%, within the range of values stipulated in the reference standard (less than 5%). Monodisperse polystyrene spheres (PSL) with sizes 20, 50, 100, 300, 500 and 1000 nm were used by Pan *et al.*[23] to calculate the uncertainties. The results of repeated measures were presented with 95% confidence levels. Furthermore, it was found that the decay rate dominated the measure of uncertainty for each nanosphere size studied. Kwon *et al.*,[24] using the extrapolation procedure to obtain the size of the nanoparticles in aqueous solutions, investigated three different-sized PSL nanoparticles, with diameters of 100, 50 and 20 nm. The detailed uncertainties related to experimental procedure were evaluated. The repeatability of DLS measurements was the most important factor among the components contributing to the uncertainty. The cryo-transmission electron microscopy (Cryo-TEM) microscopic method was used to measure the size of the nanoparticles in aqueous solutions to check the reliability of

the results of DLS measurements. The results of the Cryo-TEM measurements were in good agreement with the results from the DLS measurements within the uncertainty range.

So, knowledge of measurement uncertainty in particle size tests is important for laboratories, clients and all institutions that use these results for comparative purposes. The result of a measurement is only an approximation or estimate of the measurand value. Therefore, a test report must include a statement of measurement uncertainty estimation. However, the acceptable value of the uncertainty must be decided based on the best use and the decision must be made taking into account the needs of the client. It is sometimes possible to accept a large uncertainty, and at other times, only a small uncertainty is required.[2,25]

In our Institute, there is a high interest in the study of gold nanoparticles synthesis for many applications including cancer treatment and/or cosmetology, among others.[3-7,9-11] In this context, a general purpose of the present paper was to propose a methodology to measure the size of a broad range of nanomaterials by DLS. The quality control of analyses and results was achieved using the reference material polyvinylpyrrolidone coated silver nanoparticles (RM 8017) for size measurement in order to evaluate the uncertainty and trueness of the tests particle size analysis using DLS technique. A valid protocol is important as it assesses acceptability, reliability and adequacy of results. A detailed analysis of measurement uncertainty has to be evaluated to ensure the accuracy of the DLS technique in determining the size of nanoparticles in a solution. Due to the importance of having knowledge of the estimated measurement uncertainty, this article also aims to present the estimation and evaluation of the particle size uncertainty analysis using the DLS technique. Consequently, users of the DLS available in our Institute will have the possibility of knowing the contributions of each source of uncertainty for the control and, therefore, search for improvements in the produced nanoparticles.

Experimental

Materials

A Master System Ultra Purifiers MS3000 (GEHAKA, Kaufmann Group, São Paulo, Brazil) was used to provide ultrapure water (type I) for synthesis and dilutions of this paper.

For size measurements, quartz cuvettes with four optical faces (Qualividros, Passos, Brazil) were inspected for spotting surface scratches or coatings that could interfere with optical measurements. They were rinsed three times with filtered ultrapure water (through a 0.2 μm FILTRILO

PVDF filter) and then stored in a dust-free environment prior to be used.

For the quality control of analyses, reproducibility and reliability of DLS results, RM 8017-polyvinylpyrrolidone coated silver nanoparticles nominal diameter 75 nm sold by NIST (Gaithersburg, USA) was used. The certificate provided the hydrodynamic diameter, 105.6 ± 4.6 nm determined by dynamic light scattering technique by Malvern Zetasizer NanoZS (Malvern, United Kingdom).

Methods

DLS was carried out using Litesizer 500 Series Instrument (Anton Paar GmbH, Graz, Austria), which was equipped with a 658 nm laser and dynamic light-scattering (PCS) at 175° for particle sizing. The particle size (multimodal size distribution) was determined by measuring the angles in which an incident light beam is scattered as a function of Brownian motion of the colloidal gold particles. The average value of the hydrodynamic diameter (Z_{ave}) was calculated by Kalliope Litesizer software version 2.22.2 developed by Anton Paar. The reliability of measurements was ensured by a series of handling precautions and quality criteria for good measurements to be applied for the DLS measurements[19] performed on the multiuser device used in this paper. In the experimental protocol, approximately 1 mL of each sample was filtered in FILTRILO PVDF-0.2 μm, until measurement. The cuvettes were inspected to ensure that no bubbles are formed on the optical faces or are present in the sample after filling. The temperature of measurement (T_m) was 25 °C and room temperature 23 °C. For measurements, the diluted samples were introduced in a cuvette and placed in the apparatus for equilibration at T_m for 300 s. The time for each run was 10 s. The experimental protocol also includes turning on the device 30 min beforehand, checking the photocounting level to perform the reading, and performing five readings. The scattering intensity should be significantly greater than the dispersant alone, ideally, the intensity should be between 100 and 500 kcps. The methodology was based on the recommendation of the ISO standard 22412:2017.[17] In this protocol, for each measurement, it was also included the application of good laboratory practices, as well as diluting the samples with ultrapure water previously filtered in filter FILTRILO PVDF-0.2 μm.

The reference material (RM) was suspended in ultrapure water previously filtered in a filter FILTRILO PVDF-0.2 μm. Five samples were prepared (1:100 dilution), diluted with ultrapure water preliminarily filtered through a FILTRILO PVDF filter-0.2 μm and read these in five runs on the Litesizer 500 device. A control of the dilution

J. Braz. Chem. Soc. **2024**, *35*, 8, e-20240039

3 of 10

was achieved by weighing the introduced quantity of the solution and of the dispersant when preparing the diluted samples.

Quantification of uncertainty

To estimate the uncertainty in particle size measurements by DLS, some measurement parameters must be established, such as quantification of hydrodynamic diameter by means of the mathematical model (Stokes equation). In addition, uncertainty evaluation in the scattering angle (θ), wavelength (λ), temperature (T), viscosity function and uncertainty of factor (f_μ), refractive index factor (u_n) and uncertainty of decay rate (Γ). The relative standard uncertainty (u_d/d), relative uncertainty of trueness ($u_{trueness}$), relative uncertainty of repeatability (u_{repe}), combined uncertainty, and expanded uncertainty (U) were also estimated. All sources of uncertainty considered were identified in the Ishikawa diagram (Figure 1).
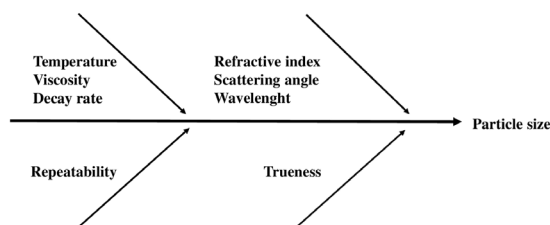

Figure 1. Ishikawa diagram. Sources of uncertainty considered in this paper.

Quantification of hydrodynamic diameter

According to the ISO 22412: 2017[17] the basic equation for calculating the hydrodynamic diameter in DLS measurements is the Stokes-Einstein (equation 1):

$$d = \frac{k(T)}{3\pi\eta\left(\dfrac{\Gamma}{q^2}\right)} \qquad (1)$$

where d is the hydrodynamic diameter (nm), k is the Boltzmann constant (J K^{-1}), T is the temperature (K), η is the viscosity of the dispersing medium (Pa s), Γ is the decay rate (s^{-1}) and **q** is the dispersion vector (m^{-1}), equation 2.

$$q = \frac{(4\pi\eta)}{\lambda} \times \sin\left(\frac{\theta}{2}\right) \qquad (2)$$

where **q** is the dispersion vector (m^{-1}), η is the index of refraction of the dispersion medium (dimensionless), θ is the scattering angle (rad) and λ is the wavelength (m).

Uncertainty evaluation

To estimate the uncertainty in a DLS measurement is needed to estimate the sources of uncertainty and quantify them using the bottom-up approach based on the Guide to the Expression of Uncertainty in Measurement (GUM).[22]

Uncertainty of scattering angle θ

In our measurements, the angle was fixed at $\theta = 175°$. According to Goldschmidt *et al.*,[18] in DLS instruments it is not possible to evaluate the uncertainty of this angle, it is necessary to adopt a conservative estimate for the maximum error $\theta = 1°$. Then, based on a rectangular probability distribution over the interval $\theta \pm e_\theta$, in the GUM.[22]

$$u_\theta = \frac{e_\theta}{\sqrt{3}} = 0.0101 \text{ rad} \qquad (3)^{[17,24]}$$

Uncertainty of wavelength λ

The standard uncertainty of the wavelength was calculated according to equation 4, considering the resolution (0.1 nm) of the DLS instrument with a probability rectangular distribution.

$$u_\lambda = \sqrt{\left(\frac{\dfrac{\text{resolution}}{2}}{\sqrt{3}}\right)^2} \qquad (4)^{[21]}$$

The estimated wavelength is $\lambda = 658$ nm with a relative standard uncertainty $\dfrac{u_\lambda}{\lambda} = 1.3 \times 10^{-6}$.

Uncertainty of temperature T

Most DLS instrument makers will have adopted precautions to keep temperature stability while measuring. In fact, ISO standard 22412:2017[17] specifies that one of the components of the apparatus should be a test sample holder, allowing fluctuations of the sample temperature to be controlled to within ± 0.3 °C.[18] To determine the standard temperature uncertainty, it was used the value informed in DLS instrument 0.2 °C. The temperature established for the measures was 25 °C, giving an estimated value, T = 298.15 K and a maximum error 0.2 K, so:

$$u_T = \frac{e_T}{\sqrt{3}} = 0.115 \text{ K} \qquad (5)$$

Viscosity function and uncertainty of factor f_μ

The standard uncertainty of the viscosity of the dispersing medium (solvent) at 25 °C was calculated according to equation 6. The equation combines the water uncertainty at 20 °C defined by ISO/TR 3666,[26] as being 1.0016 mPa s[21] and the difference between the value read at 25 °C (0.890308 mPa s) and the value from the DLS Litesizer 500 Series Instrument database (0.89 mPa s) at 25 °C. The probability distribution was normal (k = 2) for the normalized value and rectangular for the difference between the values.

$$u_{f_{\mu\,25\,°C}} = \sqrt{\left(\frac{u_{\mu_{20°C}}}{2}\right)^2 + \left(\frac{Error_{\mu_{25°C}}}{\sqrt{3}}\right)^2} \qquad (6)$$

Refractive index factor u_η

The standard uncertainty of the refractive index of the dispersion medium (water) was calculated according to equation 7, considering the difference between the theoretical value and the read value. The distribution probability used was rectangular.

$$u_\eta = \sqrt{\left(\frac{n_{theoretical} - n_{read}}{\sqrt{3}}\right)^2} = 0.000002 \qquad (7)^{[21]}$$

This result shows that, since the standard uncertainty of the wavelength is so small, its effect on the uncertainty of the refractive index is minimal.

Uncertainty of decay rate Γ

The uncertainty of the decay rate (equation 8) is influenced by the repeatability of measurements, the particle size distribution, the analytical methods dependency, the occurrence of multiple scattering effects, and perhaps other factors.[18,24,26] Except from the first, the uncertainty due to the other components of uncertainty is difficult to evaluate. The standard uncertainty of the decay rate was calculated considering the difference between the highest and lowest value (amplitude) of the decay rate, these values were recorded in the particle size analyzer software. The distribution of probability used was rectangular.

$$u_\Gamma = \sqrt{\left(\frac{Bigger_\Gamma - Smaller_\Gamma}{\sqrt{3}}\right)^2} \qquad (8)^{[21]}$$

Uncertainty contribution and relative standard uncertainty (u_d/d)

The uncertainty contribution for the scattering angle, wavelength, temperature, viscosity of the dispersing medium, refractive index and the decay rate are calculated by equations 9 to 14.[18,21]

Scattering angle	$(u_\theta/(\tan\theta/2))^2$	(9)
Wavelength	$4(u_\lambda/\lambda)^2$	(10)
Temperature	$(u_T/T)^2$	(11)
Viscosity	$(u_{f_\mu}/f_\mu)^2$	(12)
Refractive index	$4(u_{f_\eta}/f_\eta)^2$	(13)
Decay rate	$(u_\Gamma/\Gamma)^2$	(14)

The square root of the addition[18] of these components gives the relative standard uncertainty (u_d/d) of the mean effective diameter of the nanoparticles in the sample (equation 15), concerning the input data in the equipment.

$$\frac{u_d^2}{d^2} = (u_\theta/(\tan\theta/2))^2 + 4(u_\lambda/\lambda)^2 + (u_T/T)^2 \qquad (15)$$
$$+ (u_{f_\mu}/f_\mu)^2 + 4(u_{f_\eta}/f_\eta)^2 + (u_\Gamma/\Gamma)^2$$

Evaluation of relative standard uncertainty of trueness

To evaluate the trueness and to control whether there was a significant difference between the measurement results and the certified value, the same approach applied by Varenne *et al.*[19,20] and in concordance to ISO Guide 5725-1/1994.[27]

The difference between the average measured value (d_m) and the certified value (d_{RM}) represented by the Δ_m, was calculated according to equation 16.

$$\Delta_m = |d_m - d_{RM}| \qquad (16)$$

From the certified value uncertainty (u_{RM}) and the experimental measurement uncertainty (u_m) was calculated by using equation 17.

$$u_\Delta = \sqrt{u_m^2 + u_{RM}^2} \qquad (17)$$

To obtain the uncertainty u_m according to equation 18, the standard deviation (s) was divided by the square root of the number of measurements (n).

$$u_m = \frac{s_{intermediate\ precision}}{\sqrt{n}} \qquad (18)$$

The expanded uncertainty (U_{RM}) of the RM was given on

J. Braz. Chem. Soc. **2024**, *35*, 8, e-20240039

5 of 10

the certificate. The uncertainty of the certified value (u_{RM}) was obtained using equation 19 where k is the coverage factor, 2 for the confidence level of 95%.

$$u_{RM} = \frac{U_{RM}}{k} \tag{19}$$

The expanded uncertainty (U_Δ) with a level of confidence of 95% was calculated according to equation 20.

$$U_\Delta = 2u_\Delta \tag{20}$$

Finally, Δ_m is compared with U_Δ. If $\Delta_m \leq U_\Delta$, it indicates that there is no significant difference between the measured value and the certified value. Then, the relative standard uncertainty of trueness ($u_{trueness}$) can be estimated using equation 21.

$$u_{trueness} = \frac{u_\Delta}{d_m} \tag{21}$$

Evaluation of relative uncertainty of repeatability (u_{repe})

The relative uncertainty of repeatability (u_{repe}), i.e., in the condition of a single operator, using the same measurement procedure, in the same place (Instituto de Pesquisas Energéticas e Nucleares (IPEN) laboratory), with a single equipment (Litesizer 500 Anton Paar, Graz, Austria) and five repetition in the five samples of RM (25 readings). The repe value also includes dilution control by means of weighing on a semi-analytical balance and the operator competence. Some uncertainty components can be evaluated experimentally from the dispersion of repeated measurements. The repe was estimated from equation 22.

$$u_{repe} = \frac{Sreplicates}{dm} \tag{22}$$

Combined uncertainty ($u_{c_{size}}$)

The combined uncertainty ($u_{c_{size}}$) was estimated by combining the standard uncertainties of relative standard uncertainty (u_d), concerning the input data in equipment, relative uncertainty of trueness ($u_{trueness}$) and relative uncertainty of repeatability (u_{repe}) according to equation 23.

$$u_{c_{size}} = \sqrt{(u_d)^2 + (u_{trueness})^2 + (u_{repe})^2} \tag{23}$$

Coverage factor (k) and expanded uncertainty (U)

The coverage factor (k) is a numerical factor used as a

multiplier of combined uncertainty ($u_{c_{size}}$) in hydrodynamic diameter measurement, in order to obtain an expanded uncertainty of measurement (equation 24). For the confidence level of 95%, the coverage factor is k = 2.[28]

$$U_{measurement} = u_{c_{size}} k \tag{24}$$

Results and Discussion

To evaluate if the results of the DLS instrument are reproducible and reliable, some details must be established. First, the evaluation is carried out according to the procedure applied to a reference material. Second, the dilute standards must be stable during analysis. Particles should be only subjected to Brownian motion and no interference movements such as sedimentation or convection should disturb DLS measurements. The evaluation of the stability of the standard during analysis was monitored following the signal intensity recorded for five consecutive measurements for each of the five samples (total 25 runs). Ideally, the intensity should be between 100 and 500 kcps.[29,30] The mean count rate for dilute dispersions of RM was 293.3 ± 7.5 kcps and coefficient of variation 2.5%.

Since our instrument does not furnish the corresponding decay rates, we calculated them using equation 25.

$$\Gamma = D\, q^2 \tag{25}$$

where q is the modulus of the scattering vector and D is the diffusion coefficient. The mean effective nanoparticle diameter from the five samples and five independent analyses for each sample as well as decay rate are d = 109.14 ± 1.58 nm and $\Gamma_{calc} = 3937.16$ s⁻¹, respectively.

Uncertainty results are summarized in Tables 1 and 2. In Table 1, the values of the standard uncertainties of the input quantities are estimated; while Table 2 presents the values of the uncertainty components in equation 15 calculated using equations 9 to 14 for scattering angle, wavelength, temperature, viscosity factor, refractive index factor and decay rate.

Table 1. Estimated values and standard uncertainties of input quantities

Quantity	Symbol	Estimated value	Standard uncertainty
Scattering angle / rad	θ	3.0543	0.0101
Wavelength / nm	λ	658	0.00083
Temperature / K	T	298.15	0.115
Viscosity factor / (Pa s)	f_μ	1	0.005
Refractive index factor	f_n	1	0.000002
Decay rate / s⁻¹	Γ	3937.16	74.47

6 of 10

J. Braz. Chem. Soc. **2024**, *35*, 8, e-20240039

Table 2. Relative uncertainty components in equation 15

Quantity	Expression	Std. unc. contribution
Scattering angle	$(u_\theta/\tan(\theta/2))^2$	0.143
Wavelength	$4(u_\lambda/\lambda)^2$	ca. 0
Temperature	$(u_T/T)^2$	0.149
Viscosity	$(u_{f_\mu}/f_\mu)^2$	25
Refractive index	$4(u_{f_\eta}/f_\eta)^2$	ca. 0
Decay rate	$(u_\Gamma/\Gamma)^2$	357.8
Sum	383.05	

Std. unc. contribution: standard uncertainty contribution = value $\times 10^{-6}$.

In accordance with equation 15, the square root of the addition of these components (383.05×10^{-6}) gives the relative standard uncertainty of the mean effective diameter of the nanoparticles in the sample. The result is $u_d/d = 0.0196$. As the mean hydrodynamic diameter d = 109.1 nm, the standard uncertainty is u_d = 2.14 nm (equipment). The corresponding relative value as a percentage was 1.9% of the diameter.

The obtained results suggest that the standard uncertainty is strongly dominated by the uncertainty associated with the magnitudes of viscosity and decay rate. However, the uncertainties associated with temperature, wavelength and refractive index proved to be irrelevant. Goldschmidt *et al.*[18] also observed the uncertainty associated with the viscosity, followed by temperature and decay rate dominated the standard uncertainty value. In contrast, Pan *et al.*[23] observed that the decay rate was the main source of the measurement uncertainty, whereas the Boltzmann constant and viscosity have the smallest contributions. The authors claim that the error source of the decay rate consists of measurement repeatability, the quality of the particle dispersion, fluctuations in laser intensity, unwanted laser light interference with scattering light, and the possibility of sedimentation or agglomeration, all of which contribute to the uncertainty. Therefore, the measurement uncertainty of the DLS method can be reduced by using a stabilized laser source or by measuring high-quality monodispersing particles.

In this paper, this means that any necessary improvement, regarding the uncertainties related to the input data in the DLS equipment to improve the expanded uncertainty of the method, requires a reduction in the standard uncertainty values of the decay rate and viscosity.

The trueness of a method indicates the closeness between the average value, which is obtained by a large series of measurements using a method in specific conditions, and the reference value. The report of RM informs the hydrodynamic diameter of 105.6 nm and expanded uncertainty of 4.6 nm, coverage factor k = 2 with 95% of uncertainty interval.

The data used for the evaluation of relative standard uncertainty of trueness are given in Table 3 and equations 16 to 21 were used to calculate some variables.

Table 3. Used data for the evaluation of relative standard uncertainty of trueness

d_m / nm	109.1	
s / nm	1.6	
n	25	
u_m / nm	0.32	equation 18
d_{RM} / nm	105.6	
U_{RM} / nm	4.6	
k	2	
u_{RM} / nm	2.3	equation 19
Δ_m	3.5	equation 16
u_Δ / nm	2.3	equation 17
U_Δ / nm	4.6	equation 20
$u_{trueness}$	0.021 (2.1%)	equation 21

d_m: measured average diameter; s: standard deviation; n: number of measurements; u_m: uncertainty; d_{RM}: measured average diameter of RM; U_{RM}: expanded uncertainty; k: coverage factor; Δ_m: difference between the average measured value and the certified value; U_Δ: expanded uncertainty; $u_{trueness}$: relative standard uncertainty of trueness.

The relative standard of uncertainty of trueness ($u_{trueness}$) can be estimated using equation 21 and was estimated at $u_{trueness}$ = 0.021 nm. The uncertainty of trueness was thus 2.1%. The measured mean value was therefore not significantly different from the certified value. Varenne *et al.*[19,20] believe that a limit set at 10% is an acceptable value for trueness. Therefore, the relative standard of uncertainty of trueness of 2.1% is within the acceptable value.

The precision of a method is defined by the closeness among independent test results performed under experimental defined conditions. Repeatability and intermediate precision are to be distinguished. Repeatability indicates the closeness among independent test results obtained by the same analyst using the same method and the same instrument in the same laboratory for a short period, i.e., typically when measurements are performed on the same day.[19,21] However, intermediate precision conditions explore the variation of factors as the instrument and/or the analyst, or over a longer period of time (i.e., typically on different days) but within the same laboratory, while repeatability conditions have to hold all these factors constant.[19] The intermediate precision was not evaluated in this paper. The repeatability (repe) in each sample is quantified by means standard deviation of diameters. The mean diameter (d) calculated from the five samples and five runs of each sample, therefore, 25 readings,

J. Braz. Chem. Soc. **2024**, *35*, 8, e-20240039

7 of 10

was d = 109.14 ± 1.58 nm. The relative uncertainty of repeatability (u_{repe}) was estimated from equation 22 and was, therefore, u_{repe} = 0.014 nm (1.4%). The expected parameter defined in the ISO standard for repe was 5%,[17] and so RM agrees.

The combined uncertainty ($u_{c_{size}}$) = 2.14 nm, was estimated by combining the relative standard uncertainty (u_d = 2.14 nm), concerning the input data in equipment, relative uncertainty of trueness ($u_{trueness}$ = 0.021 nm) and relative uncertainty of repeatability, u_{repe} = 0.014 nm, according to equation 23.

Finally, the expanded uncertainty (U), i.e., the quantitative expression of the reliability of the results of a measurement method, equation 24, with a confidence level of 95% and coverage factor k = 2 was calculated. The value was U = 4.28 nm (corresponding to an expanded uncertainty of 3.9%). Consequently, the mean hydrodynamic diameter (d) determined by DLS, following the experimental protocol proposed in this paper was (109.1 ± 4.3) nm.

Figure 2 compiles the uncertainty estimated from the validation data (equipment, trueness and repeatability) as well as expanded uncertainty.
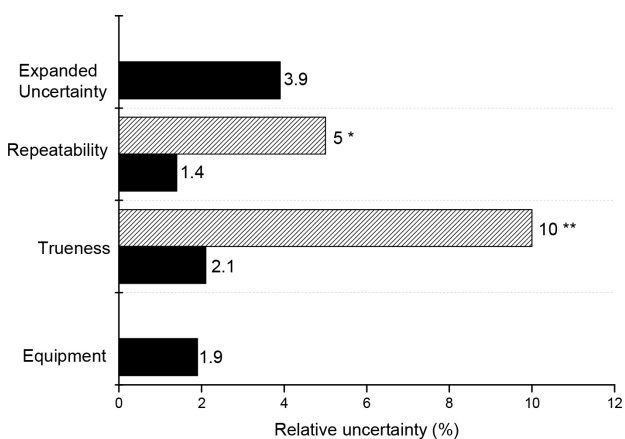

Figure 2. Relative standard and expanded measurement uncertainties (in percentage) estimated for size measurements performed by DLS (black). Striped: *from the ISO standard[17] and **according to Varenne *et al.*[19]

The quality of uncertainty presented for the result of a measurement depends on the understanding, critical analysis and reliability of those that contributed to assigning the value to it. Therefore, the result of a measurement should not be composed of only a number and a unit of measure, but of a range of values and unit of measure. In such a way that the true value of the measurand is contained within this interval. So, according to Figure 2, the relative uncertainty inherent to the input data in the equipment was 1.9%. The uncertainty of trueness estimate (2.1%) was below the limit defined by Varenne *et al.*[19] (10%).

The relative standard uncertainties related to repeatability (1.4%) were below the limit defined by ISO (5%)[17] and can be associated with the sample preparation protocol carried out by weighing and the well-trained researcher and his accuracy. The same observations were found by Varenne *et al.*[18,19,31] which attributed to the control of different factors associated with good laboratory practices, such as rinsing the cuvettes with filtered water, storing in a dust-free environment, prior filtering of the solutions, etc. These precautions were also taken in this paper.

When comparing the expanded uncertainty value (U_{95}) obtained in this paper (3.9%) to the main data presented in the literature (Table 4), it can be observed that the data is in good agreement with the present in the literature.

Table 4. Overview of some results for expanded uncertainty values established in the literature for some reference materials determined by DLS

Reference material	U_{95} / %	Reference
Polystyrene nanospheres - 60 nm	1.0	Goldschmidt *et al.*[18]
Polystyrene nanospheres - 60 nm	7.0	
Polystyrene nanospheres - 203 nm	3.8	Varenne *et al.*[19,20,31]
Polystyrene nanospheres - 100 nm	4.4	
Polystyrene nanospheres - 400 nm	3.6	
NIST SRM 1963 - 100 nm	4.0	Matteucci *et al.*[21]
Polystyrene nanospheres - 20 nm	17	
Polystyrene nanospheres - 50 nm	8.5	Pan *et al.*[23]
Polystyrene nanospheres - 100 nm	5.4	
IRMM-304 silica nanoparticles - 40 nm	5.4	Braun *et al.*[32]
NIST RM 8011 - 10 nm	5.0	
NIST RM 8012 - 30 nm	6.8	Melli *et al.*[33]
NIST RM 8013 - 60 nm	3.6	
NIST RM 8017 - 75 nm	3.9	this paper

U_{95}: expanded uncertainty value.

As a last observation, the hydrodynamic diameter result presented with the measurement uncertainty value, 109.1 ± 4.3 nm, proved to be fit for purpose, giving a measure of the confidence that can be placed on the result.

Conclusions

Reliability of measurement results is an important prerequisite for materials science. Adequate characterization of NPs is of paramount importance to develop well-defined nanoformulations of therapeutic relevance. The accuracy and reliability of measuring your properties become essential. In our Institute, the DLS technique is widely used in the characterization of the produced nanoparticles, for having a simple fast and easy response, and yet for being a reproducible tool to determine particle size.

8 of 10

J. Braz. Chem. Soc. **2024**, 35, 8, e-20240039

The calculation of the estimated uncertainty associated with the measurement process is of great importance for understanding the analytical procedure, allowing improvements or adjustments in the measurement protocol.

In this paper, a reasoned, reliable and traceable protocol was presented to measure the real size of nanoparticles in aqueous solution using the DLS technique and to estimate the reliability of the results.

The relative uncertainty of the input quantities was determined and it was found that the uncertainties associated with the magnitudes of decay rate and viscosity had greater relevance in the standard uncertainty value determined. The relative standard uncertainty of trueness was below the limit defined by the ISO standard indicating the proximity between the mean value and the reference value. The relative standard uncertainty of repeatability was also below the limits defined by the ISO standard. An equilibration time of 300 s chosen for the protocol was long enough to reach thermal equilibrium of the dispersion that is required to record only Brownian motion of the particle and not movements that can result from a thermal gradient occurring in the sample.

Relevant results were therefore obtained for robustness and accuracy studies leading to low expanded uncertainty. The hydrodynamic diameter determined was 109.1 ± 4.3 nm. The expanded uncertainty was lower than 4% with a confidence level of 95%. In conclusion, this methodology is acceptable for the analyses of nanoparticle dispersions stability and the instrument reports robust and reliable results. So, our laboratory is able to present the result with a statement about the uncertainty measurement of the DLS device. Based on the uncertainty estimate, the researcher is able to decide, with confidence, whether your sample is suitable for the desired application.

Acknowledgments

References

1. Soriano, M. L.; Zougagh, M.; Valcárcel, M.; Ríos, A.; *Talanta* **2018**, *177*, 104. [Crossref]

2. López-Sanz, S.; Bernardo, F. J. G.; Doimeadios, R. C. R. M.; Ríos, Á.; *Anal. Chim. Acta* **2019**, *1059*, 1. [Crossref]

3. Geraldes, A. N.; da Silva, A. A.; Leal, J.; Estrada-Villegas, G. M.; Lincopan, N.; Katti, K. V.; Lugão, A. B.; *Adv. Nanopart.* **2016**, *5*, 176. [Crossref]

4. Geraldes, A. N.; da Silva, D. F.; Pino, E. S.; da Silva, J. C. M.; de Souza, R. F. B.; Hammer, P.; Spinacé, E. V.; Oliveira Neto, A.; Linardi, M.; dos Santos, M. C.; *Electrochim. Acta* **2013**, *111*, 455. [Crossref]

5. Geraldes, A. N.; Silva, D. F.; Silva, J. C. M.; Souza, R. F. B.; Spinacé, E. V.; Neto, A. O.; Linardi, M.; Santos, M. C.; *J. Braz. Chem. Soc.* **2014**, *25*, 831. [Crossref]

6. Geraldes, A. N.; da Silva, D. F.; e Silva, L. G. A.; Spinacé, E. V.; Neto, A. O.; dos Santos, M. C.; *J. Power Sources* **2015**, *293*, 823. [Crossref]

7. de Souza, C. D.; Barbezan, A. B.; Rosero, W. A. A.; dos Santos, S. N.; Carvalho, D. V. S.; Zeituni, C. A.; Bernardes, E. S.; Vieira, D. P.; Spencer, P. J.; Ribeiro, M. S.; Rostelato, M. E. C.; *Nanomaterials* **2022**, *12*, 187. [Crossref]

8. Toma, H. E.; Zamarion, V. M.; Toma, S. H.; Araki, K.; *J. Braz. Chem. Soc.* **2010**, *21*, 1158. [Crossref]

9. Daruich de Souza, C.; Barbezan, A. B.; Rosero W. A. A.; Santos, S. N.; Carvalho, D. V. S.; Zeituni, C. A.; Bernardes, E. S.; Vieira, D. P.; Spencer, P. J.; Ribeiro, M. S.; Rostelato, M. E. C. M.; *Nanomaterials* **2022**, *12*, 187. [Crossref]

10. Silva, J. T.; Daruich de Souza, C.; Angelocci, L. V.; Rosero, W. A. A.; Nogueira, B. R.; Correia, R. W.; Zeituni, C. A.; Rostelato, M. E. C. M.; *Appl. Radiat. Isot.* **2021**, *178*, 109952. [Crossref]

11. de Souza, C. D.; Nogueira, B. R.; Rostelato, M. E. C. M.; *J. Alloys Compd.* **2019**, *798*, 714. [Crossref]

12. Bogue, R.; *Sens. Rev*. **2007**, *27*, 189. [Crossref]

13. González, A. G.; Herrador, M. Á.; *TrAC, Trends Anal. Chem.* **2007**, *26*, 227. [https://doi.org/10.1016/j.trac.2007.01.009]

14. Torres, F.; *Revista IPT Tecnologia e Inovação* **2021**, *5*, 66. [Link] accessed in March 2024.

15. Gómez, E. R.; Giraldo, L. F.; Jaramillo, G.; *Cien. Ing. Neogranadina* **2016**, *226*, 49. [Crossref]

16. Li, G.; Wang, Z.; *IEEE Nanotechnol. Mag*. **2021**, *15*, 3. [Crossref]

17. ISO Guide 22412: *Particle Size Analysis-Dynamic Light Scattering (DLS)*, ISO: Geneva, 2017.

18. Goldschmidt, G.; Hotza, D.; Plentz, F.; Camarano, D.; Lira, I.; *Meas. Sci. Technol.* **2020**, *31*, 075005. [Crossref]

19. Varenne, F.; Botton, J.; Merlet, C.; Beck-Broichsitter, M.; *Colloids Surf., A* **2015**, *486*, 124. [Crossref]

20. Varenne, F.; Botton, J.; Merlet, C.; Hillaireau, H.; Lerand, F. X.; Barrat, G.; Vauthier, C.; *Int. J. Pharm.* **2016**, *515*, 245. [Crossref]

21. Matteucci, C.; Dinis, A. C.; Guimarães, K. L.; *Revista IPT: Tecnologia e Inovação* **2017**, *5*, 67. [Link] accessed in March 2024

22. Baratto, A. C.; Damasceno, J. C.; Alves, J. A. P.; Filho, J. T.; Couto, P. R. G.; Oliveira, S. P.; *Guia para a Expressão de Incerteza de Medição-GUM-Avaliação de Dados de Medição*; INMETRO: Rio de Janeiro, 2012. [Link] accessed in March 2024.

23. Pan, S.-P.; Weng, H.-F.; Lin, C.-M.; Liu, T.-S.; *Jpn. J. Appl. Phys.* **2010**, *49*, 06GK05. [Crossref]

J. Braz. Chem. Soc. **2024**, *35*, 8, e-20240039

9 of 10

24. Kwon, S.-Y.; Kim, Y.-G.; Lee, S.-H.; Moon, J.-H.; *Metrologia* **2011**, *48*, 417. [Crossref]

25. Cunha, F.; *Metrologia e Incerteza em sua Essência: Tudo o que Nunca te Contaram sobre Incerteza de Medição*; Atena: Ponta Grossa, Brazil, 2022.

26. ISO/TR Guide 3666: *Viscosity of Water*, ISO: Geneva, 1998.

27. ISO Guide 5725-1:1994: *Accuracy (Trueness and Precision) of Measurement Methods and Results-Part 1: General Principles and Definitions*, ISO: Geneva, 1994.

28. Takahashi, K.; Kato, H.; Saito, T.; Matsuyama, S.; Kinugasa, S.; *Part. Part. Syst. Charact.* **2008**, *25*, 31. [Crossref]

29. Light scattering ISO standard anyone [ISO13321, ISO22412]?, https://www.materials-talks.com/light-scattering-iso-standard-anyone-iso13321-iso22412/, accessed in March 2024.

30. Hackley, V. A.; Clogston, J. D.; *NIST Special Publication 1200-6: Measuring the Size of Nanoparticles in Aqueous Media Using Batch-Mode Dynamic Light Scattering*, NIST-NCL Joint Assay Protocol, PCC-1 version 1.2, 2015. [Crossref]

31. Varenne, F.; Hillaireau, H.; Bataille, J.; Smadja, C.; Barratt, G.; Vauthier, C.; *Colloids Surf., A* **2019**, *560*, 418. [Crossref]

32. Braun, A.; Couteau, O.; Kestens, K.; Roebben, G.; Lamberty, A.; Linsinger, T. P. J.; *Adv. Powder Technol.* **2011**, *22*, 766. [Crossref]

33. Meli, F.; Klein, T.; Buhr, E.; Frase, C. G.; Gleber, G.; Krumrey, M.; Duta, A.; Duta, S.; Korpelainen, V.; Bellotti, R.; Picotto, G. B.; Boyd, R. D.; Cuenat, A.; *Meas. Sci. Technol.* **2012**, *23*, 125005. [Crossref]