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Size of monodispersed nanomaterials evaluated by dynamic light scattering: Protocol validated for measurements of 60 and 203 nm diameter nanomaterials is now extended to 100 and 400 nm

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ABSTRACT

In vivo fate of nanomaterials is influenced by the particle size among other parameters. Thus, Health Agencies have identified the size of nanomaterial as an essential physicochemical property to characterize. This parameter can be explored by dynamic light scattering (DLS) that is described in the ISO standard 22412:2008(E) and is one of the methods recognized by Health Agencies. However, no protocol of DLS size measurement has been validated over a large range of size so far. In this work, we propose an extension of validation of a protocol of size measurement by DLS previously validated with certified reference materials (CRM) at 60 and 203 nm. The present work reports robustness, precision and trueness of this protocol that were investigated using CRM at 100 and 400 nm. The protocol was robust, accurate and consistent with the ISO standard over the whole range of size that were considered. Expanded uncertainties were 4.4 and 3.6% for CRM at 100 and 400 nm respectively indicating the reliability of the protocol. The range of application of the protocol previously applied to the size measurement of liposomes and polymer nanoparticles was extended to inorganic nanomaterial including silica nanoparticles.

1. Introduction

Applications of nanomaterials cover a wide range of industrial domains improving performances of industrial products and with the possibility to introduce new properties in manufacturing products (Jung et al., 2005; Jøgensen et al., 2009; Wissing and Müller, 2003). Applications in medicine occur as therapeutic nanomedicines including drug delivery systems (Olivier, 2005), adjuvants to radiotherapy (Galper et al., 2012; Liu et al., 2010) and as medical devices including contrast agents to be used in diagnostic by imaging technique (Cormode et al., 2014; Neuwelt et al., 2004; Perlman et al., 2015). Characteristics of nanomedicines can be finely-tuned to control their in vivo fate hence their in vivo biodistribution hence their efficacy and safety. The size of nanomedicines is one of the parameters that influences the biodistribution after entry in the body. It has been identified as a parameter to include in control analysis to achieve the efficacy and safety of a nanomedicine (Draft guidance from FDA, 2011; FDA advisory committee for pharmaceutical science and clinical pharmacology meeting Topic 2 Nanotechnology—Update on FDA Activities, 2012; Gaumet et al., 2008; Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products, 2013; Organization for Economic Co-operation and Development, 2013, 2010; Reflection paper on the data

Abbreviations: ANOVA, analysis of variance; AT, ambient temperature; CRM, certified reference material; CV, coefficient of variation; DLS, dynamic light scattering; ERM, European reference materials; GUM, guide to the expression of uncertainty in measurement; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; IP, intermediate precision; IRMM, Institute for Reference Materials and Measurements; NCL, nanomedicine characterization laboratory; NIST, National Institute of Standard and Technology; ISO, International Organization for Standardization; PDI, polydispersity index; r, repeatability; t, trueness; TEM, transmission electron microscopy.

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requirements for intravenous liposomal products developed with reference to an innovator liposomal product, 2013; Report of the Joint Regulator—Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials, 2011; Shekunov et al., 2006). Measurements of size of nanomedicines performed in the framework of quality control measurements require the application of a recognized method to achieve the measurement and the existence of validated protocol to perform the measurements (Shekunov et al., 2006). According to recommendations given be Health Agencies, size of nanomedicines can be accessed by dynamic light scattering (DLS) (Draft guidance from FDA, 2011; FDA advisory committee for pharmaceutical science and clinical pharmacology meeting Topic 2 Nanotechnology—Update on FDA Activities, 2012; Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products, 2013; Organization for Economic Co-operation and Development, 2013, 2010; Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product, 2013; Report of the Joint Regulator—Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials, 2011). The method described in the standard of the International Organization for Standardization (ISO) (ISO 22412:2008(E)) provides with the hydrodynamic diameter or radius of the nanomedecine assuming a spherical shape (Cao, 2003; Braun et al., 2011; ISO 22412:2008 (E)). Despite the wide application of DLS, only two papers report the validation of an operating protocol to assess nanomaterial size measurements by this method (Braun et al., 2011; Varenne et al., 2015a). The protocol validated by Braun et al. addressed the determination of nanomaterials having diameters in the range of 35–50 nm (Braun et al., 2011). In a previous work, we have proposed a protocol to carry out size measurement of unknown dispersions of nanomaterials by DLS. This protocol was standardized to be applied to a wide range of nanomaterials including handling precautions and a methodology to prepare samples. We have developed a procedure to achieve the validation of a protocol for the measurement of nanomaterial diameters based on the use of certified reference materials (CRM) at 60 and 203 nm. The methodology was based on the recommendation of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines (ICH) Q2(R1) (ICH Harmonized Tripartite Guideline, Validation of analytical procedures, 1994), the ISO standard (ISO 22412:2008 (E)) and the Guide to the expression of uncertainty in measurement (GUM) (Guide to the expression of uncertainty in measurement, 1995). The validation is necessary to provide proof that the protocol is sufficiently acceptable, reliable and adequate for the different elements of its scope using reference material (ICH Harmonized Tripartite Guideline, Validation of analytical procedures, 1994; ISO 5725-3:1994; ISO 22412:2008(E); NIST-NCL Joint Assay Protocol, PCC-1 Version 1.1, Measuring the Size of Nanoparticles in Aqueous Media Using Batch-Mode Dynamic Light Scattering, 2010; Shekunov et al., 2006). Statistical analyses of the data were developed based on analysis of variance, ANOVA, using nested design, mixed design and pooled variance approaches. This validation permitted to define the reliability of the protocole for normal usage and the limits of use (robustness study) and to provide expanded uncertainties (precision and trueness studies). The expanded uncertainties correspond to the quantitative expression of the reliability of the size results of a validated size measurement protocol. Results from the evaluation of the expanded uncertainties have revealed that it differed between measurements performed on CRM of small (diameter 60 nm) and large (diameter 203 nm) diameters (Varenne et al., 2015a). For unknown dispersions of nanomaterials, the developed measurement approach included (i) the control of the absence of an absorption band of the nanomaterial dispersion at the wavelength of the laser source that equipped the DLS instrument, (ii) the determination of the optimal concentration to carry out size measurements, (iii) the operational qualification of the instrument using the proposed protocol and (iv) the determination of size of the nanomaterials taking account all handling precautions, key factors revealed by the validation and defined quality criteria for good size measurements. It is noteworthy that the validation of a size measurement method should be distinguished from the operational qualification of the instrument which consists in establishing confidence that the instrument operates consistently with its operating characteristics within stated tolerances for a given environment with the CRM. For operational qualification, criteria were defined including (i) a diameter, for a given CRM, within the range of size defined by the validation and (ii) polydispersity index (PDI) inferior to 0.1. As DLS provides direct size measurements, it is not required to perform calibration considering the commonly sense of this term. However, validation of size measurement protocols and operational qualification of the instrument are recommended (ISO 22412:2008(E); NIST-NCL Joint Assay Protocol, PCC-1 Version 1.1, Measuring the Size of Nanoparticles in Aqueous Media Using Batch-Mode Dynamic Light Scattering, 2010).

Therefore, the aim of the present work was to extend the validation of the previously developed protocol over a larger range of the sizes including CRM with diameters of 100 and 400 nm and providing with the corresponding expanded uncertainties. Additionally, it was also aimed to extend the range of nanomaterials for which the protocol can be applied. While it was shown that the validated protocol can be applied to the determination of the size of polymer nanoparticles and liposomes, here the DLS protocol for size measurement developed in our previous work was challenged with inorganic nanoparticles.

2. Materials and methods

2.1. Materials

A Millipore water system was used to provide ultrapure water (MilliQ®). Size measurements were only performed using perfectly clean disposable macrocuvettes with four optical faces (VWR) that showed no defects. For validation, Nanosphere Size Standards NIST (National Institute of Standards and Technology) Traceable Mean Diameter of 100 and 400 nm composed of polystyrene nanospheres with calibrated mean diameters traceable to the Standard Meter through the NIST were provided by Thermo Scientific. These CRM were calibrated by Transmission Electron Microscopy (TEM) and were supplied as an aqueous dispersion in dropper-tipped vials. Their characteristics are certified for operating conditions ranging from 2 to 30 °C, their density were 1.05, their refractive index at 589 nm were 1.59 and the approximate concentrations were 1% solid weight. The certified mean diameter was certified by TEM at 100 ± 3 nm for the CRM at 100 nm and 400 ± 9 nm for the CRM at 400 nm with a coverage factor $k = 2$. References are given in Supplementary material Appendix A. Sodium chloride (purity $\geq$ 99.5%) was supplied by Sigma.

Non-Functionalized NanoXactTM Silica nanoparticles of 100 and 400 nm were purchased from Nanocomposix. Ultrapure water and all solutions used for dilutions were filtered through a 0.22 μm filter (Roth). All glassware and measurement macrocuvettes were rinsed three times with filtered water and stored in a dust free environment prior to be used.

A procedure based on recommendation given in the protocol proposed by the Nanomedicine Characterization Laboratory, Frederick, MD, USA (NIST-NCL Joint Assay Protocol, PCC-1 Version