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Towards quality assessed characterization of nanomaterial: Transfer of validated protocols for size measurement by dynamic light scattering and evaluation of zeta potential by electrophoretic light scattering

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ABSTRACT

Quality control analysis of nanomaterials has been identified as a major issue to pursue their development in different industrial fields including nanomedicine. One difficulty is the lack of standardized and validated protocols suitable to achieve their characterization. In a previous work, we have developed standardized protocols for the evaluation of the size and zeta potential of nanomaterials based on methods described in the ISO standard and have performed validation of each one. The present work was aimed to transfer these protocols in three independent receiving laboratories. No official guideline was described in the literature to achieve such a transfer. A comparative study for receiving laboratories equipped with the same instrument as the sending laboratory was designed based on the Code of Federal Regulation edited by the Food and Drug Administration. For the receiving laboratory equipped with an instrument working at a different wavelength, a new validation was designed and applied. Corresponding statistical methods were used for the analysis of the results. A successful transfer of the protocols in all receiving laboratories was achieved. All laboratories recorded consistent results applying in blind the protocol of size measurements on two samples of nanomaterials from which included one reference.

1. Introduction

The interest and range of applications of nanotechnologies has rapidly expanded over the last decade. It is now about to cover all sectors of activities. For instance, applications can be found in diverse fields of the industry as energy, transports, computer sciences, cosmetics and food where nanotechnologies have the potential to confer new characteristics to manufactured products (Chaudhry et al., 2008; Jøgensen et al., 2009; Jung et al., 2005; Lu et al., 2016; Raj et al., 2012; Wissing and Müller, 2003). In medicine, there are several areas where nanotechnologies are particularly interested improving performances of drug delivery and diagnostic methods (Cormode et al., 2014; Galper et al., 2012; Liu et al., 2010;

Abbreviations: ANOVA, Analysis of variance; CRM, Certified reference material; DLS, Dynamic light scattering; ELS, Electrophoretic light scattering; ERM, European Reference Materials; FFR, Fast Field Reversal; 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; NIST, National Institute of Standard and Technology; ISO, International Organization for Standardization; OECD, Organisation for Economic Co-operation and Development; PALS, Phase analysis light scattering; PDI, Polydispersity index; r, repeatability; RL, Receiving laboratory; RM, Reference material; SFR, Slow Field Reversal; SL, Sending laboratory; t, trueness; TEM, Transmission electron microscopy.

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Neuwelt et al., 2004; Olivier, 2005; Perlman et al., 2015). Regardless the application envisaged, risk assessments imply that nanomaterials can be described in a reliable manner. However, characterization of nanomaterials was pointed out as bottleneck that was even taken as responsible to slow down development process in certain cases (Hutchison, 2016). This can be explained by at least three reasons (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, 2010,2013; 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).

Firstly, the type of relevant physicochemical parameters that needs to be evaluated is the topic of active debates. Those are generally carried out on a case by case basis thanks to research data analysed by the relevant scientific community in relation with the field of the intended application. At the cross road of all fields interested by applications involving nanotechnologies, a new discipline called nanotoxicology has emerged. It is aimed to evaluate subsequent risks due to the exposure of the human body from the understanding of potential hazards caused by nanomaterials in relation with their characteristics and properties. Several physicochemical parameters of relevance that needs to be determined to describe a nanomaterial has been identified and are mentioned in official documents including standards from International Organization for Standardization (ISO) and guidelines from Organisation for Economic Co-operation and Development (OECD) (ISO 13099-2:2012(E); ISO 13099-3:2012(E); ISO 22412:2008(E); Organization for Economic Co-operation and Development, 2010, 2013). Both texts are consistent on requirements including the evaluation of the size and surface properties of nanomaterials although they can be confusing on other parameters (Tantra et al., 2016). The particle size is obviously a fundamental parameter that defines and describes a nanomaterial. Surface characteristics were found to play an important role in the biological performance and safety of nanomaterials as they dictate their biodistribution hence potential toxicity (Clogston et al., 2016; Clogston and Patri, 2013; Moghimi et al., 2012).

Secondly, suitable and relevant methods are needed to perform the characterization of nanomaterial. There is a consensus that the characterization of nanomaterial is not an easy task. At present, there are only few methods that can be used in routine to characterize nanomaterials while some parameters can be accessible only at the expense of use of highly sophisticated methods or are still even not accessible experimentally. Regarding the determination of the size and surface characteristics of nanomaterials that are considered as the two major parameters that needs to be determined, relevant routine methods are existed. The size can be measured by different techniques that are described in ISO standard and in several documents published by governmental and health agencies (FDA advisory committee for pharmaceutical science and clinical pharmacology meeting Topic 2 Nanotechnology-Update on FDA Activities, 2012; ISO 22412:2008 (E); Organization for Economic Co-operation and Development, 2010; Report of the Joint Regulator-Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials, 2011). Among the different methods, the one based on dynamic light scattering (DLS) is generally used thanks to the ease to perform the analysis with affordable commercial instruments. This method evaluates the Brownian motion of the nanomaterials that is dispersed in a liquid phase measuring its diffusion coefficient. The hydrodynamic size is then calculated from the diffusion coefficient from the well-established Stokes and Einstein law. The method that is described in ISO standard (ISO 22412:2008(E)) and recommended by health agencies (FDA advisory committee for pharmaceutical science and clinical pharmacology meeting Topic 2 Nanotechnology-Update on FDA Activities, 2012; Organization for Economic Co-operation and Development, 2010; Report of the Joint Regulator-Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials, 2011) is quite precise providing that the size distribution of the sample is homogenously distributed (Varenne et al., 2015a). Regarding the characterization of surface properties, the most easily accessible parameter is the zeta potential that is related to the surface charge of the particles. It is defined as the potential difference between the bulk solution and the slipping plane located at the boundary between ions strongly associated with the particle and moving with the particles and ions which movements are independent of those of the particle. It is noteworthy that the evaluation of the zeta potential is not straightforward and that the value generally obtained corresponds to an apparent zeta potential. This parameter can be evaluated from the measurement of the electrophoretic mobility of the nanomaterial that is accessible by different techniques described in ISO standard (ISO 13099-2:2012(E); ISO 13099-3:2012(E)). Then, an apparent zeta potential is calculated from the electrophoretic mobility applying suitable mathematical models. The value that is finally obtained greatly depends on the model of calculation and on the composition of the liquid phase in which the nanomaterial is dispersed during the analysis (Adamczyk et al., 2010; Bouhaik et al., 2013; ISO 13099-1:2012(E); ISO 13099-2:2012(E); ISO 13099-3:2012(E); Leroy et al., 2013).

The third reason that makes critical the characterization of nanomaterials is the lack of standardized and validated methods to ensure quality assessed measurements and traceability (Manfield et al., 2017; Tantra et al., 2016). Yet this is paramount to ensure that different measures made at different times and different locations can be compared objectively and reliably and provide with comparable results (Linsinger et al., 2012). Although developing standardized and validated protocols are needed, the lack of appropriate reference samples is another problem found to develop relevant protocols to perform the characterization. Considering the characterization of the size and zeta potential that were mentioned above, certified reference materials (CRM) or reference materials (RM) are existing as marketed compounds. This makes possible the validation of protocols for the evaluation of the size and zeta potential of nanomaterials with suitable methods. However, there is still a limited number of papers reporting the validation of protocols that characterize these parameters (Braun et al., 2011; Dudkiewicz et al., 2015; Loeschner et al., 2015; Varenne et al., 2015a,b).

In the efforts of developing quality ensured characterization of nanomedicines, we have started the validation of a series of protocols evaluating size and zeta potential of nanomedicines based on methods described in ISO standards and health agency guidelines (FDA advisory committee for pharmaceutical science and clinical pharmacology meeting Topic 2 Nanotechnology-Update on FDA Activities, 2012; ISO 13099-2:2012(E); ISO 22412:2008(E)). The protocols described conditions to measure the hydrodynamic size of nanomaterials by DLS and to evaluate zeta potential by electrophoresis light scattering (ELS) using phase analysis light scattering (PALS). They were standardized to be applied on various types of nanomedicines including organic and inorganic nanoparticles (Varenne et al., 2015a) of a wide range of size (hydrodynamic diameter 60 to 400 nm (Varenne et al., 2016) and having negative or positive surface charges (Varenne et al., 2015b). No specific guideline could be found in the literature to