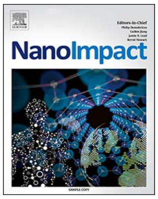
NanoImpact 10 (2018) 87–96

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.elsevier.com/locate/nanoimpact



Research paper

Identification of nanomaterials: A validation report of two laboratories using analytical ultracentrifugation with fixed and ramped speed options

Dora Mehn[a], Iria Maria Rio-Echevarria[a], Douglas Gilliland[a], Michael Kaiser[b], Klaus Vilsmeier[b], Peter Schuck[c], Wendel Wohlleben[b,*]

[a] *Joint Research Centre, Directorate F–Health, Consumers and Reference Materials F2, Ispra, Italy*
[b] *BASF SE, Dept. Material Physics, Dept. Experimental Toxicology and Ecology, Ludwigshafen, Germany*
[c] *Dynamics of Macromolecular Assembly Section, Laboratory of Cellular Imaging and Macromolecular Biophysics, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, USA*

ARTICLE INFO

Keywords:
Nanomaterial
Nanodefinition
Analytical ultracentrifugation
Method validation

ABSTRACT

The identification of nanomaterials for regulatory purposes in Europe requires a statistically significant comparison of the number metrics median diameter to the 100 nm criterion. Methods should be robust against minor imperfections of the implementation, transferable and reproducible between laboratories, and applicable to a wide range of diameters and material compositions. Sample preparation and metrics conversion are additional challenges that must be included in validation. Here we report on the validation of a protocol for analytical ultracentrifugation with fixed and ramped speed, specifically intended to serve the European Commission recommended definition, as relevant for REACH regulation, cosmetics and food labelling and national inventories. All the determined measurement uncertainties remain below 12% for volume and number metrics median diameters, in both laboratories. Traces of sub-100-nm particles with around 2% mass contribution (but > 50% number contribution) were reproducibly quantified. For powders, sample preparation is a critical step, and sonication intensities above 0.4 W/mL are recommended. The ramp operation can reduce user bias by eliminating the choice of options in data acquisition. The identification of nanomaterials and non-nano-materials by AUC in either fixed or ramp speed is consistent with TEM, excluding the platelet Kaolin material, but including the monodisperse silica, multimodal silica, a nanoform and a non-nano-form of $BaSO_4$, irregularly shaped $CaCO_3$ and coated non-nano TiO_2.

1. Introduction

Nanotechnology has significant impacts both on high-volume everyday goods and lower-volume high-tech applications. The commercial applications of nanomaterials with the highest market volume include reinforced rubber (e.g., carbon black in tires), other polymer composites (e.g., functional fillers such as amorphous silica), processing aids and components in the electronics industry (e.g., fine abrasives), cosmetics (e.g., amorphous silica), and biomedical applications (nanoformulations) (Vance et al., 2015; Stark et al., 2015; Wohlleben et al., 2017a). Additionally, forecast analysis of the market introduction of nanomaterials for novel applications suggests that the application of nanomaterials is rapidly growing, including various fields from food, cosmetic and medicine products to structural and electronic components. According to the very wide spectrum of nano-applications, various regulatory bodies are tackling the challenge of defining

---

Abbreviations: AUC, analytical ultracentrifugation; EPROM, erasable programmable read-only memory; EM, electron microscopy; IRRM, Institute for Reference Materials and Measurements; IUPAC, International Union of Pure and Applied Chemistry; JRC, Joint Research Centre; ls-g*(s), least squares fitting of sedimentation coefficient distribution of non-diffusing particles; OECD, Organisation for Economic Co-operation and Development; PSD, particle size distribution; RI, refractive index; SHMP, sodium hexametaphosphate; TEM, transmission electron microscopy; N, centrifuge speed or rotational frequency (min^{-1}); ρ, skeleton density of the material (gcm^{-3}); ρ_1, liquid density of the suspension medium (gcm^{-3}); η, viscosity of the suspension medium (Pas); s, sedimentation coefficient (Svedberg = 10^{-13} s); λ, wavelength of the RI detector (nm); l, optical path length in the AUC cell (m); C, cumulative mass concentration ($mgmL^{-1}$); C_{RI}, total mass concentration represented by the PSD ($mgmL^{-1}$); $x_{50,3}$, median diameter in volume metrics (nm); $x_{50,0}$, median diameter in number metrics (nm); s, sedimentation coefficient; ω, rotor angular velocity; r, distance from the center of rotation; r_m, meniscus position; t, time; s_{min}, minimum value of selected s range; s_{max}, maximum value of selected s range; U(s,r,t), sedimentation profile of a non-diffusing species; a(r,t), experimental scans concentration distributions; B, integration parameter; $Q_{i,3}$, cumulative mass metrics distribution; $Q_{i,0}$, cumulative number metrics distribution; $q_{i,3}$, differential mass metrics distribution; $q_{i,0}$, differential number metrics distribution; s^2_{day}, day to day variance; s^2_r, variance of repeatability; ${s_{I(T)}}^2$, time different intermediate precision; MS_{day}, between days mean of squares; MS_r, within groups mean of squares; u_x, relative measurement uncertainty

[*] Corresponding author at: BASF SE, RAA/OR - B7, Carl-Bosch-Strasse 38, 67056 Ludwigshafen am Rhein, Germany.
E-mail address: wendel.wohlleben@basf.com (W. Wohlleben).

https://doi.org/10.1016/j.impact.2017.12.005
Received 23 August 2017; Received in revised form 29 November 2017; Accepted 16 December 2017

nanomaterials and establishing frameworks that ensure their safe production and use (Stark et al., 2015; Wohlleben et al., 2017a; Bawa, 2016; Boverhof et al., 2015). Among them, the European Commission published its recommendation for nanoparticle definition that, ultimately, requires the number metrics size distribution analysis of materials for their identification as nano or non-nano (European Commission, 2011). Reporting requirements with this criterion as key element of nanomaterial identification are now legally required in several EU countries, and the results published by the French Ministry of Environment support the above prioritization of substance classes (Ministère de l'Environnement and d.l.É.e.d.l.M., 2015).

However, the quality and enforceability of the reporting critically depends on valid methodology, which was not available for the specific number metrics criterion when the definition recommendation was published. Proper size distribution analysis of polydispersed materials with or without fractions in the nanoscale is often much more challenging than the characterisation of selected size distribution standards. Apart of the complications introduced in the analysis process by the presence of aggregates or irregular shaped particles, some widely used particle size measurement techniques provide unrealistic results because of their limited working range, and/or because of inadequate performance for polydispersed samples (Filipe et al., 2010; Mehn et al., 2017; Varenne et al., 2016; Anderson et al., 2013). The number metrics pose an additional challenge, because most established methods intrinsically use volume or other metrics, so that metrics conversion challenges the quality of the (nano) material identification (Brown et al., 2013; Anderson et al., 2013).

To address this methodological challenge, the recently established NanoDefine project evaluating particle sizing techniques for the characterisation of nano-materials (NanoDefine, 2017). Centrifugal methods (including analytical ultracentrifugation, AUC) showed excellent performance not only in the trueness of sizing of quality control samples (Anderson et al., 2013; Kestens et al., 2016; Planken and Colfen, 2010), but also in the correct identification of representative test materials. Generally, centrifugal methods determine the particle size (Stokes diameter of an equivalent sphere) from sedimentation speed applying the Stokes' law. While disc centrifuges and cuvette centrifuges typically detect light extinction and provide extinction based size distributions (needing further conversion to mass metric distribution using refractive index and absorption as input parameters and the Mie theory for the transformation), AUC instruments used in this study are able to detect refractive index changes and thus to generate sedimentation coefficient distributions from interference measurements (Ullmann et al., 2017). This can be transformed directly to mass metrics distribution requiring particle density as input parameter. In a recent study it was found that the simultaneous separation and detection in the AUC instrument allows to generate number weighted medians ($x_{50,0}$) that differ by < 50% from the EM results for 10 of 15 tested materials (Babick et al., 2016). Nevertheless, the covered working size range for a certain (density of) material depends strongly on the selected speed in a conventional, fixed speed AUC experiment. In order to cover a wider sedimentation coefficient (and thus mass) range during the analysis the concept of variable-field sedimentation was introduced and tested for macromolecules (proteins) and gold nanoparticles by Ma et al. (Ma et al., 2016). By applying a sequence of increasing centrifugal fields to a polydisperse sample, particles of decresing size are forced to migrate. The "gravitational sweep" strategy has originally been devised for the sizing of latex suspensions with a custom-built fixed-radius detector (Scholtan and Lange, 1972). The recent implementation is based on sequential concentration distributions acquired in current commercial AUC instrumentation, and exploits modern whole-boundary data analysis approaches in the context of sedimentation coefficient distribution analyses (Schuck, 2016a).

In the present work we further examined AUC methods for the characterisation of representative nanoparticle samples (nano and non-nano $BaSO_4$ and SiO_2). The analysis included also sample preparation from powders. First, we carried out an evaluation of the conventional fixed speed AUC, using a nested design to evaluate repeatability and measurement uncertainty. One of the critical experimental parameters for the study of macromolecular samples is the temperature at high centrifugal fields (Zhao et al., 2015). We tested the hypothesis that lower centrifugal fields required for nanoparticle characterisation would permit greater robustness with regard to temperature changes, by deliberately introducing small initial temperature differences. Next, the application of a rotor speed ramp on the working range was analysed for both quality control and representative test materials. The results generated in 2 separate laboratories (Lab1: BASF, Lab2: JRC) are compared, to allow verifying the transferability of the method and to investigate the effect of sample preparation.

In order to evaluate the repeatability of the determined number metrics median ($x_{50,0}$) values, time-different independent precision variances ($s_{I(T)}^2$), sum of the day to day variance and variance of reproducibility and relative measurement uncertainties (u_x) were calculated from the results of nested design experiments, performing 3 repeats on 5 separate days. Effect of sample preparation (as possible source of systematic error) is also discussed focusing on differences between the analysis of powder materials and suspensions, as well as different sonication devices available in the two test laboratories.

2. Materials and methods

2.1. Materials

For the evaluation of the performance of the AUC method in the identification of nanomaterials, three type of samples were chosen from the quality control material and representative test material selection of the NanoDefine project (Source: JRC, Institute of Reference Materials and Measurements) (Babick et al., 2016). The sample from the quality control material group, a trimodal silica suspension, represents a polydisperse, wide size distribution sample in the nano size range. It is composed of spherical, non-aggregated particles, and has been assessed by various microscopic, scattering and colloidal techniques (Fig. SI.1) with TEM modes around 20 nm to 32 nm and 117 nm to 134 nm. As these particles are already in (stable) suspension and the sample was analysed without dilution, sample preparation effects are expected to have negligible effect on the method performance. Additionally, two representative test materials with the same ($BaSO_4$) chemical composition but different particle size distribution range were selected. The fine and ultra-fine grade $BaSO_4$ samples containing particle aggregates in solid form were characterized earlier by two different TEM labs, providing $x_{50,0}$ values of about 280 nm or 253 nm (fine) and 21 nm or 33 nm (ultrafine), respectively (Babick et al., 2016). For these materials, our evaluation of the AUC method performance includes also the steps of sample preparation. The ultra-fine $BaSO_4$ IRMM387 is the same grade as the JRC repository material OECD NM220.

For the comparison of fixed speed and ramp measurements additional four representative test materials were selected, including solid samples (TiO_2, IRMM 388; kaolin, IRMM 385; $CaCO_3$, IRMM 384) and another suspension in the nano-size range (Silica Nanolyse02). The following parameters were independently determined for the test materials: $BaSO_4$: $\rho = 4.4\ \mathrm{gmL}^{-1}$; $dn/dc = 0.066\ \mathrm{mL/g}$. SiO_2: $\rho = 2.305\ \mathrm{gmL}^{-1}$; $dn/dc = 0.063\ \mathrm{mL/g}$; TEM median = 22 nm (Nanolyse02). TiO_2: $\rho = 3.99\ \mathrm{gmL}^{-1}$; $dn/dc = 0.376\ \mathrm{mL/g}$; TEM median = 185 nm or 180 nm. $CaCO_3$: $\rho = 2.657\ \mathrm{gmL}^{-1}$; $dn/dc = 0.11\ \mathrm{mL/g}$; TEM median = 160 nm or 153 nm. Kaolin: $\rho = 2.61\ \mathrm{gmL}^{-1}$; $dn/dc = 0.087\ \mathrm{mL/g}$; TEM median lateral diameter = 121 nm or 128 nm (Babick et al., 2016). High purity (MilliQ) water was used as solvent for suspension medium. All other reagents and materials were purchased from Sigma Aldrich and used without further modification.