Role of Sample Preparation for Accurate and Automated Morphological Analysis of Nanoparticles proven in Interlaboratory Comparison Exercises

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1. Introduction

The accurate analysis of size and shape distribution of nanoparticles is a challenging task, which is strongly dependent on the complexity of the morphology of the particles and the type of material. Simple, model nanoparticles with spherical shape and narrow/monodisperse size distribution can be easily and accurately analysed basically by any sizing method (ensemble and counting). Nanoparticles which have a complex shape, are polydisperse in size, have a complex chemistry (including defined and unexpected outer shells/coatings), or provide a certain level of agglomeration and/or aggregation, are mostly prone to a faulty quantification regarding their particle size and shape distribution. Guidance for accurate measurement by electron microscopy techniques has been recently developed under ISO [1,2], however, the large variety of morphologies and chemistries of commercial nanoparticles cannot be covered with uniform guidance. Furthermore, one crucial point for an accurate determination of the size and shape nanoparticle distributions by imaging methods is the sample preparation, such that the individual particles are finally prepared as isolated particles on a suited substrate, not touching or overlapping each other.

In this contribution three examples of nanomaterials with different challenges for the morphological analysis will be highlighted: i) TiO_2 nano-bipyramides, ii) SiO_2 nanoparticles of spherical shape and a bimodal distribution with different relative concentrations, and iii) graphene oxide flakes.

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2. Sample Preparation

The single-particle preparation of nanoparticles on a substrate for imaging guarantees not only an accurate dimensional measurement, but also enables the use of automated segmentation of the images. Hence, a more representative quantitative analysis of a statistically large number of nanoparticles by imaging methods becomes possible as a routine procedure.

Another parameter of high relevance for nanoparticles is the number concentration. Here again, the more complex in morphology and chemistry the particles are, the more complex the accurate measurement of their number concentration is, regardless by the analysis method employed. Once suited sample preparation procedures resulting in single particles deposited on a substrate are available, the accurate counting by imaging methods becomes routine, too.

Various sample preparation procedures such as the substrate pre-treatment (with ozone, glow discharge or deposition of poly-L-lysine, etc), electrospray deposition, spin coating, etc., are available mostly depending on the type of nanoparticle material.

The alternative to the laborious, 'perfect' single-particles sample preparation for analysis would be the imaging of the nanoparticles as they are, directly deposited on a substrate, and application of machine learning approaches after appropriate manual training. First studies have showed success [3].

3. Measurements

Figure 1 illustrates the successful sample preparation as isolated particles for TiO_2 as a nanoparticulate material which usually tends to agglomerate and is difficult to completely deagglomerate [1,5].

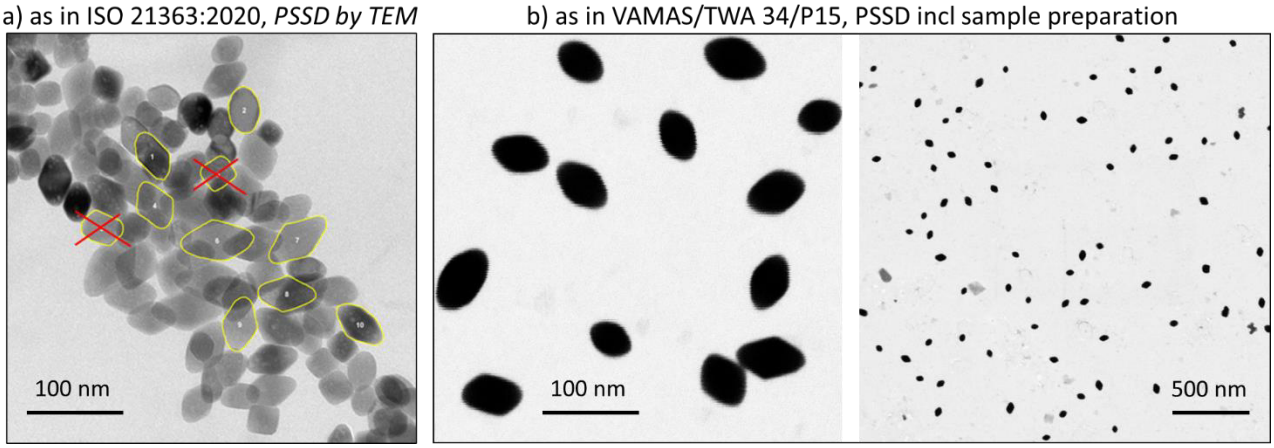

Figure 1: Result of the improved sample preparation of TiO_2 nanoparticles for more accurate imaging analysis, i.e. with almost only isolated nanoparticles, homogeneously deposited on the substrate

Whilst 2020 the sample preparation for standardized imaging analysis was not yet optimised (Fig. 1 left), [1] recently, progress in sample preparation of the same nanoparticles has been reached within the pre-standardisation platform of VAMAS [5]. Similar progress has been achieved in a parallel VAMAS interlaboratory comparison on SiO_2 bimodal nanoparticles [6], where the homogeneous and single-particle deposition

on substrate has resulted in a significantly more accurate particle relative number concentration as measured by imaging methods (SEM, TEM, AFM).

A third case study, namely a graphene oxide 2D material in form of µm-large flakes of single monolayers, demonstrates the power of good sample preparation, see Figure 2 after optimisation of the sample preparation procedure. The study is conducted also as a (ongoing) VAMAS interlaboratory comparison, under TWA 41, project #13 [7].

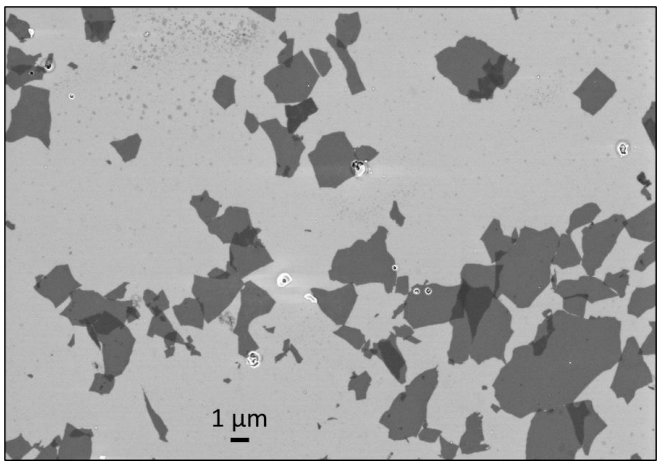

Figure 2: Result of improved sample preparation of graphene oxide monolayer flakes as measured with an SEM with an SE InLens-type detector

4. Results and Conclusions

After imaging of the particles deposited according to the optimised sample preparation protocols, the particle size and shape descriptors (minimum Feret, maximum Feret, ECD, aspects ratio) and the relative number concentration of the SiO_2 bimodal nanoparticles in the three interlaboratory comparisons described above have been measured. The image analysis approaches used and the final data will be presented, with a comparative discussion of the results obtained by individual laboratories and methods, and of the sources of measurement uncertainties observed. Relative deviations between laboratory mean values for the size and shape descriptors of max 10% could be reported particularly due to the improved sample preparation protocols. Significantly larger deviations have been reported for the (relative) particle number concentration by imaging and ensemble methods, the latter ones measuring the nanoparticles in suspension. The reasons for these deviations have been identified and will be also discussed.

5. Acknowledgements

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