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The Role of Electron Microscopy in the Development of Monodisperse Cubic Iron Oxide Nanoparticles as Certified Reference Material for Size and Shape

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Due to their unique physico-chemical properties, nanoparticles are well established in research and industrial applications. A reliable characterization of their size, shape, and size distribution is not only mandatory to fully understand and exploit their potential and develop reproducible syntheses, but also to manage environmental and health risks related to their exposure and for regulatory requirements [1,2]. To validate and standardize methods for the accurate and reliable particle size determination nanoscale reference materials (nanoRMs) are necessary. However, there is only a very small number of nanoRMs for particle size offered by key distributors such as the National Institute of Standards and Technology (NIST) and the Joint Research Centre (JRC) and, moreover, few provide certified values [3]. In addition, these materials are currently restricted to polymers, silica, titanium dioxide, gold and silver, which have a spherical shape except for titania nanorods [4]. To expand this list with other relevant nanomaterials of different shapes and elemental composition, that can be used for more than one sizing technique, we are currently building up a platform of novel nanoRMs relying on iron oxide nanoparticles of different shape, size and surface chemistry. Iron oxide was chosen as a core material because of its relevance for the material and life sciences.

As a first candidate of this series, we present cubic iron oxide nanoparticles with a nominal edge length of 8 nm. These particles were synthesized by thermal decomposition of iron oleate in high boiling organic solvents adapting well-known literature procedures [5,6]. After dilution to a concentration suitable for EM as well as for small-angle X-ray scattering (SAXS) measurements, the candidate nanoRM was bottled and assessed for homogeneity and stability by both methods following the guidelines of ISO 17034 and ISO Guide 35 [7,8].

The focus of the present characterization study lies on particle size data obtained by SEM, which is correlated with data obtained by TEM and SAXS. An almost ideal monolayer was achieved by drop casting the iron oxide suspension on carbon coated copper grids for electron microscopy which allowed to measure individual nanoparticles with high accuracy. Representative images in Figure 1 and Figure **2** show that agglomeration and aggregation of particles are negligible and the contrast between nanoparticle and substrate is sufficient, which are crucial factors for the accurate image segmentation and reliable evaluation of the data.

For the correlative SEM imaging both operation modes with an SE Inlens detector as well as in transmission mode (STEM-in-SEM) are considered. The software package ImageJ was utilized for the automatic segmentation of the TEM images by the ISOData thresholding algorithm [9]. For the evaluation of the SEM data a STEM-in-SEM image was manually segmented in compliance with best practices regarding particle count and data quality [10].

As shown in Figure 1 and Figure **2** the particle sizes obtained by both STEM-in-SEM and TEM are in excellent agreement with a minimum Feret of 8.3 nm ± 0.7 nm. The SEM images taken with the SE InLens detector led to a significant overestimation of the particle sizes (up to 1 nm), which is due to the extreme topographical sensitivity of the SE InLens detector, and hence also to the slight surface electrical charging [11]. The aspect ratio (AR) of the iron oxide cubes were extracted from the images as the ratio of minimum Feret to Feret resulting in an AR of 1.18 for TEM to 1.25 for SEM. Alternatively, a rectangular bounding box was fitted originating from the minimum Feret and the longest distance through the particle in perpendicular direction. This led to AR values of 1.05 for TEM and 1.12 for SEM, respectively. The results confirm the almost ideal cubic shape.

To determine the accuracy and precision of the imaging data, the following major uncertainty contributions were identified: Instrument-specific contributions include instrument magnification calibration, pixel size, and the nominal resolution of the electron microscope. Sample-specific contributions are mainly due to residual oleic acid which stem from the synthesis, able to induce a drift during SEM and TEM measurement. Artifacts due to beam damage were found to be insignificant. Finally, it can be assumed that the threshold selected during segmentation has a much greater influence on the results obtained with the SEM than with TEM. Based on these contributions, the total uncertainty for SEM can be estimated to 10% (0.8 nm); for TEM this should be slightly lower.

For SAXS the bottled iron oxide suspension was measured without further preparation. The SAXS data has been evaluated according to ISO 17867 employing the model of a lognormal size distribution of spherical particles [12]. In our study, the cubes are represented by an equivalent sphere radius which is then converted into the edge length D.

The values for the minimal Feret diameter obtained by EM (corresponding to the “shorter” edge length of a non-ideal nanocube) can be compared to the cubes edge length D determined by SAXS. The overall mean resulting from the measurements of the homogeneity study of 7.9 nm ± 0.2 nm is well within the estimated uncertainties of the applied methods.

Our characterization study could show that the presented iron oxide nano cubes offer sufficient imaging contrast for EM imaging but are still a challenging sample due to their small size, cubic shape, and metal oxide nature (medium atomic number), especially for SEM measurements. The particle dimensions determined by EM as a technique sensitive to single particle size and SAXS as an ensemble method based on a completely different physical principle are in excellent agreement which makes theses nanoparticles a valuable reference material for the validation of nanoparticle sizing methods [13].

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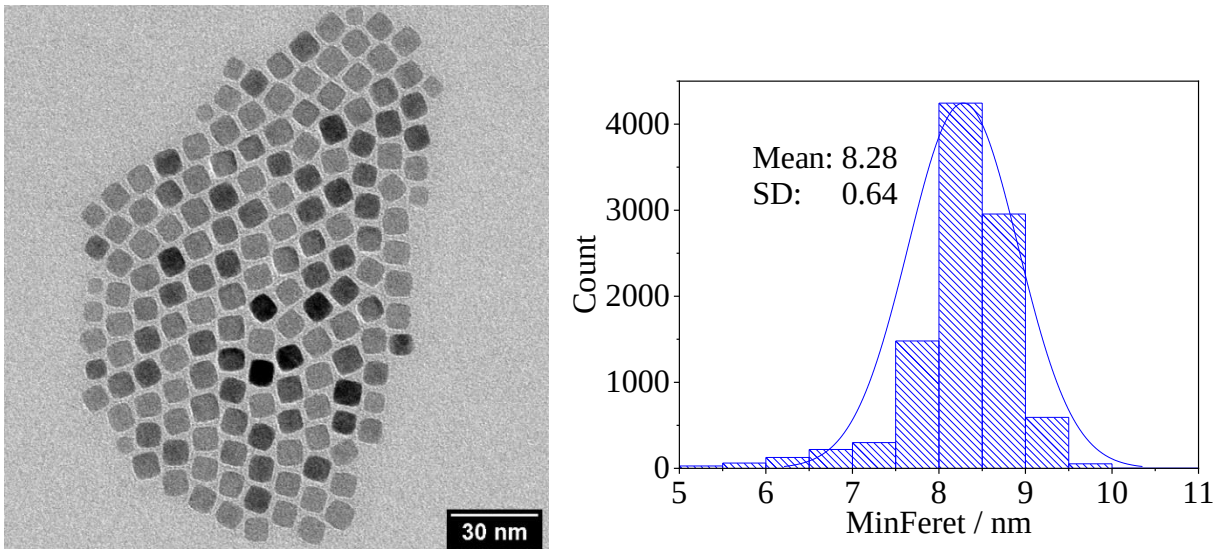

Figure 1 TEM image of iron oxide nanoparticles together with the corresponding particle size distribution of the Minimum Feret.

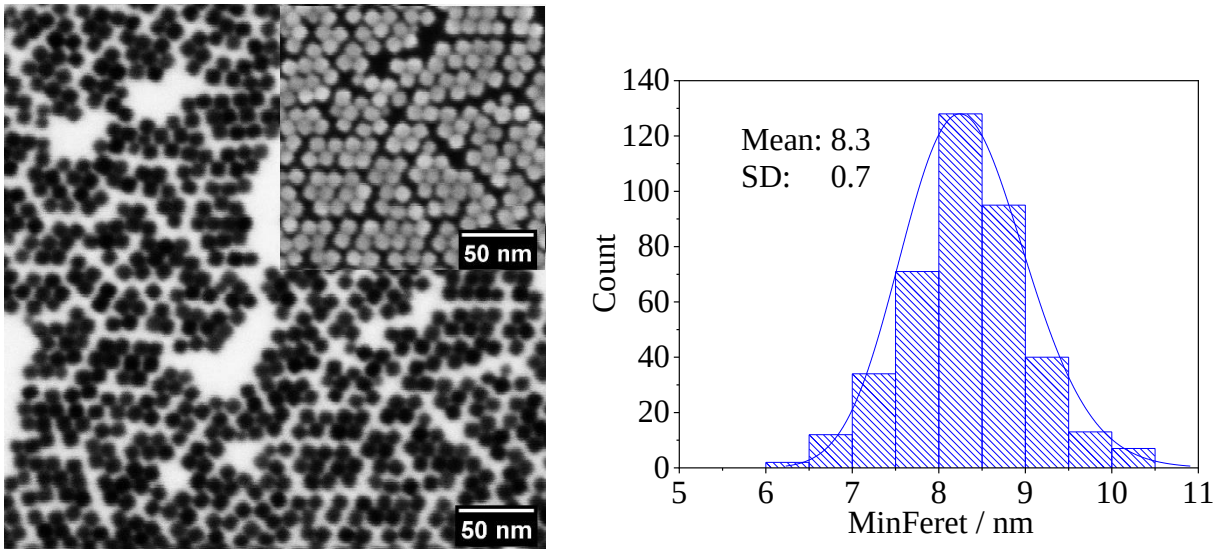

Figure 2 STEM-in-SEM image as well as the SE Inlens mode (top-right) together with the corresponding particle size distribution of the Minimum Feret extracted from the STEM-in SEM image.

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