Inspection of morphology and elemental imaging of single nanoparticles by high-resolution SEM/EDX in transmission mode†

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In the frame of the European project NanoValid, potential candidates of reference nanomaterials are manufactured and systematically characterized in particular with respect to their morphology (shape, size, and size distribution). In this study, by exploiting the transmission operation mode in a high-resolution SEM, known as transmission SEM, the potential of this methodical approach is demonstrated by means of representative examples of nanoparticles. The method enables quick and accurate morphological inspection and systematic characterization. Energy dispersive X-ray spectroscopy imaging of single nanoparticles by using the transmission mode is demonstrated as feasible, too. Copyright © 2014 John Wiley & Sons, Ltd.

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Introduction

The characterization of the morphology of nanoparticles (NPs) is becoming a task to be performed not only at a transmission electron microscope (TEM) but also more and more at modern, high-resolution SEMs. A SEM is probably the mostly widespread analytical instrument available in analytical laboratories destined to characterize physical properties such as morphology, shape, size or size distribution of materials at the microscale and nanoscale. The performance of a modern high-resolution SEM, in particular its spatial resolution, allows the identification and even accurate morphology characterization of nanoparticles down to below 10 nm.

High-priority industrial nanomaterials are being selected for manifold characterization with reliable analytical methods on a systematic basis under the frame of an ongoing European Union FP7 research project entitled NanoValid.[1] The development of certified reference nanoparticulate materials is an accompanying activity to be closely linked with. One inherent nano-morphological characterization technique is represented by electron microscopy, one of the very few techniques able to analyze individual nanoparticles. One particular analytical option, which was exploited successfully for the experiments of characterization of the nanoparticles in this project, is the transmission mode in a SEM. The methodology is not exactly new,[2,3] but in the meantime it can be successfully applied to highly relevant materials such as nanoparticles. Recently, traceable measurement of nanoparticle size and size distribution has been demonstrated with the so-called transmission SEM (T-SEM) used in conjunction with high-resolution SEM.[4,5] Furthermore, the successful extension to a more comprehensive characterization of single nanoparticles at a SEM by using also the energy dispersive X-ray spectroscopy (EDX) has been tested in our laboratory.[6–9] Hence, it can be observed that microanalysis with EDX at a SEM is more and more expanded from quantitative analysis of thin layers[10,11] to qualitative chemical analysis of single nanoparticles. This paper gives an insight over the possibilities and limitations of the multimodal methodical approach high-resolution SEM/T-SEM/EDX in transmission mode for the characterization of selected types of nanoparticles with respect to surface morphology, shape, size and size distribution, and chemical composition.

Experimental

A wide range of types of nanoparticles commercially available as well as manufactured in BAM has been considered for evaluation of their suitability to be established as a reference nanomaterial. The homogeneity of nanoparticle dispersions and their stability are principal properties to be characterized. Most of the investigated nanoparticles were basically of silica character, but metallic nanoparticles, such as silver NPs, or core-shell structures have been also systematically characterized. Selected, representative examples of nanoparticles as analyzed with high-resolution SEM/EDX employing also the transmission mode are presented in the following section.

As far as the methods and the instruments used are concerned, the new analytical techniques combined at a high-resolution SEM/EDX system are described in detail elsewhere.[7–9] The sample preparation proceeds as typically for TEM, i.e. the nanoparticles are deposited on electron transparent thin supporting foils (TEM grids), so that the transmission mode in a SEM can be operated. In the present study, in most cases, either pure carbon or carbon Formvar films have been selected as supporting foils. It should be noted that the conventional, so-called STEM detector,[12] usually applied for operating a SEM in the transmission mode by placing it directly under the samples, was not employed in this study. Instead, a
'single-unit' transmission setup, as commercially available from Carl Zeiss (Oberkochen, Germany), has been used. This special sample holder does not make necessary the use of an additional detector for the transmission mode. The transmitted electrons are being ‘guided’ via an electron multiplier, i.e. a gold layer, onto the conventional Everhart–Thornley (E–T) detector.

The SEM used was a Zeiss Supra 40 (Carl Zeiss, Oberkochen, Germany), equipped with a Schottky field emitter and having attached both a Si(Li)-EDS from Thermo Fisher Scientific (Thermo Fisher Scientific Inc., Waltham, MA, USA, energy resolution at Mn Kα of 129 eV and 10 mm² crystal active area) and a silicon drift detector energy dispersive X-ray spectrometer Quantax 400 from Bruker (Bruker Nano GmbH, Berlin, Germany, energy resolution at Mn Kα of 123 eV and 10 mm² crystal active area, see e.g. references for more details). Various beam voltages from 5 to 30 kV have been applied.

Results and discussion

Four examples of nanoparticle materials have been selected to be highlighted here as representative with respect to their accurate morphological characterization by SEM/T-SEM as well as their qualitative elemental analysis by high-resolution EDX.

The first material consists of SiO₂ nanoparticles of sizes ranging from the micrometer down to below 100 nm (Fig. 1). The attention of the reader shall be drawn in Fig. 1 on the sensitivity of the ‘top’ observation by the In-Lens detection as far as details in nanometer range on the sample surface are concerned, and on in-depth structural details, which are provided by the transmission ‘channel’, respectively. Because of its high sensitivity to even slight sample surface charging, edge effects are visible in the In-Lens imaging mode. However, operating the In-Lens mode at low beam voltages shall diminish the edge effects and improve surface topographic information in the images. This becomes challenging at very high magnifications as in the case of nanoparticles of sizes well below 100 nm. Furthermore, as systematically described in the literature, accurate and traceable dimensional results, i.e. of the nanoparticles diameter down to below 10 nm, can be gained by T-SEM.

The second example illustrates the practical performance of a modern SEM able to operate with both high-resolution SEM and T-SEM modes to determine mean size and size distributions for a SiO₂ nanoparticulate material, which has been developed at BAM. This material is considered as a suitable candidate for certification. Even in a qualitative way, it can be definitely stated from the T-SEM micrograph in Fig. 2a that the material consists of quite spherical-shaped nanoparticles. It is rather monodisperse, i.e. having an almost monomodal size distribution, and the mean size of the nanoparticles is close to 15 nm. All these findings could be confirmed by the inspection with the TEM (Fig. 2b). As expected, T-SEM imaging mode provides a distribution that is slightly broader than that obtained by TEM. For such small nanoparticles, the accurate delimitation of the nanoparticles in the T-SEM mode is definitely constrained by the inferior spatial resolution attainable in comparison with that of a conventional TEM, where much higher beam voltages and aberration...
corrections are available. A further systematic data reduction procedure involving the accurate determination of the size distribution of the SiO₂ nanoparticles on a metrological basis is in progress. The traceability of the size distribution ‘back’ to an International System of Units (SI) unit, here the length, as measured with an electron microscope, is carefully taken into consideration. Thus, the two main sources generating significant measurement uncertainties are evaluated: (i) the calibration of the image magnification of the electron microscope and (ii) the calibration of the processing software, including the setup of the threshold necessary to delimitate the nanoparticles in the acquired images. In the addressed size range of about 15 nm, these particular aspects are more challenging than for bigger nanoparticles (of several tens of nanometer and more) or consisting of materials with a higher atomic number where a higher material contrast can be obtained.

The third example of accompanying morphological characterization of engineered nanoparticles manufactured in the frame of the NanoValid project by electron microscopy is represented by silver nanoparticles. After a proper preparation of the particles on a carbon Formvar TEM grid, it could be unambiguously observed by means of T-SEM (Fig. 3) that the nanomaterial consists of particles having a size ranging from below 10 nm up to about 50 nm, which are mostly of non-spherical shape. The example was selected in order to demonstrate how effective such a ‘quick’ T-SEM characterization measurement can be for the manufacturers of the nanoparticles.

The last example is a so-called hyper-spectral map obtained by EDX as additional information on the elemental distribution over the scanned area. In each image pixel, an X-ray spectrum is acquired, so that reconstructions of elemental maps are easily possible with a standard personal computer (PC). The SEM/T-SEM/high-resolution EDX multi-method approach applied on the SiO₂ nanoparticles selected in Fig. 4 shows manifold details, which are of relevance for the nanoparticle’s characterization:

(i) The surface-sensitive In-Lens observation in Fig. 4a shows unambiguously a defect in the surface/shell of the bottom nanoparticle;
(ii) The in-depth sensitive T-SEM mode confirms that the bottom nanoparticle in Fig. 4b is ‘empty’, in contrast to the other two upper particles (‘Mickey-Mouse’ structure);
(iii) The metrological advantages of T-SEM can be demonstrated also by comparing the apparently two smaller upper nanoparticles in the T-SEM mode as in Fig. 4b relative to their size in the In-Lens mode as in Fig. 4a. Saturation effects in the In-Lens imaging mode resulting in apparent overestimated sizes are in the meantime well known and reported by more...
The In-Lens detector is particularly sensitive to charging of the particles surface. On the other side, the EDX map of Si in Fig. 4c may suggest that the two upper particles are provided with a thin overlayer, which is not detected by EDX. Also, the T-SEM imaging mode does not detect clearly this surface layer. A reduction of the beam voltage may possibly help to diminish the apparent edge/border effects in the In-Lens mode and enhance the sensitivity to surface topography. However, because of a significant worsening of the image resolution, the decrease of the beam voltage has not led to a successful result in this particular case of such fine nano-structures.

(iv) The EDX hyper-spectral maps of Si K and O K X-ray lines in Fig. 4c and 4d confirm the main composition of the SiO$_2$ nanoparticles and, in particular, reveal the fact that the bottom particle is ‘broken’ (Fig. 4a) and is constituted mainly of a SiO$_2$ shell. It is not yet clear if this latter artifact either originates from the synthesis or occurs because of sampling and preparation onto TEM grid or because of transfer into vacuum, or, respectively, during the electron bombardment.

**Conclusion**

By means of four examples of nanoparticle materials selected as representative from the NanoValid project, it was demonstrated that a modern SEM is capable to provide a reliable characterization of the morphology of nanoparticles both i) as a screening method for accompanying characterization ‘close’ to the nanoparticles manufacturer and ii) as a metrological tool for the evaluation of shape and size distribution. The exploitation of the rather rarely used option of the transmission mode at a SEM (T-SEM) confers a significant enhancement of the quality of the results. Moreover, in combination with EDX applied to samples prepared specially on electron transparent supporting foils for the transmission mode, it is demonstrated how elemental imaging of high spatial resolution can be used to complete the characterization of individual nanoparticles.

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**References**


