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Volume determination of irregularly-shaped quasi-spherical nanoparticles

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Abstract

Nanoparticles (NPs) are widely used in diverse application areas, such as medicine, engineering, and cosmetics. The size (or volume)of NPsis one of the most important parameters for their successful application. It is relatively straightforward to determine the volume of regular NPs such as spheres and cubes from a one-dimensional or two-dimensional measurement. However, dueto the three-dimensional nature of NPs, it is challenging to determine theproper physical size ofmany types of regularly and irregularly-shaped quasi-spherical NPs at high-throughput using a single tool. Here, we present a relatively simple method that determines a better volume estimateof NPs by combining measurements from their top-down projection areas and peak heights using two tools. The proposed method is significantly faster and more economical than the electron tomography method. We demonstrate the improved accuracy of the combined methodover scanning electron microscopy (SEM) or atomic force microscopy (AFM) alone byusing modeling, simulations, and measurements. This study also exposes theexistence of inherent measurement biases for both SEM and AFM, which usually produce larger measured diameters with SEM than with AFM. However, in some cases SEM measured diameters appear to have less error compared to AFM measured diameters, especially for widely used IS-NPs such as of gold, and silver. The method provides a much needed, proper high-throughput volumetric measurement method useful for many applications.

Keywords

Nanoparticles, characterization,irregularly-shaped nanoparticles, quasi-spherical nanoparticles, spherical volume equivalentdiameter, nanotechnology

Introduction

The projected market figure for nanotechnology incorporated in manufactured goods by 2020 is approximately 3,000 billion US dollars worldwide (1, 2). Nanomaterials in the form of nanoparticles (NPs)represent an importantpart of nanotechnology. NP usage is increasing at a rapid pace with the development of new types of nanomaterials, as indicated by the existence of over 1,300 diverse listed consumer goods (3)(e.g., sunscreen, cosmetics, clothes, food, drugs, paints, varnishes, and a self-cleaning coating for floors, walls and windows) in addition to medical (4)and high technology (5)applications. Reports indicate that nanotechnologyand the use of NPs has the potential to radically change the way cancer is treated(6). Engineered NPs are a subset of nanomaterials, of which irregularly-shaped NPs (IS-NPs) are a major component. The environmental health and safety (EHS) of engineered NPs is subject to a great deal of research dueto their potential toxicity. In fact, over 10,000 publications have been written on the subject of the environmental and health effects of nanomaterials in the last 15 years (7, 8).

Several publications have highlighted the relationship between the size and toxicity of NPs(1, 6-14). They have also highlighted the lack of proper standards and characterization tools for NPs (1, 6-8, 10, 15-17). In fact, some have also complained that,“After over a decade of research, answers to the most basic questions are still lacking,” and suggest that more coherence in experimental methods and materials is needed (7, 10). One of the more pressing issues with characterization is the large size discrepancy when the same set of NPs is measured using different tools(1, 18-23). Some publicationshavedescribed variations in reported size even when NPs were measured using the same types of tool (1, 19, 20). These variationscreate confusion, as an accurate knowledge of the size of NPs is fundamental to their use and application, and shows that more accurate and reliable tools are needed.

Electron tomography is a method that has demonstrated reliable3D-shape reconstructionsof nanoparticles(24-26). But even though significant improvement has been made recently(27, 28), the technique is impractical and slow for routine and large-scalemeasurements required to statistically determineaccurate size and distribution of NPs.

At present, apart from the electron tomography, it is nearly impossible to accurately determine the size of many types of NPs(including types of IS-NPs(1, 3)). For this reason, only reference materials (a less rigorous measurement certification process) exist for IS-NPs, such as gold (29, 30). However, for some nearly spherical

nanoparticles such as polystyrene, standard reference materials (a highly rigorous and accurate measurement process) exist(31). Although several tools are currently available, more tools and methods are being sought (32, 33), mainly due to a lack of consensus in the reported sizes. One of the latest additions to this long list of tools is through-focus scanning optical microscopy (TSOM)(34, 35).

At this juncture, many critical questions remain: Is it possible to accurately measure the true volumeof NPs, especially for IS-NPsand at high-throughput? If it is, which tool or method provides the accuratespherical-volume-equivalent mean diameter at high throughput? If none exist, is there any way to determine which tool provides the smallest offsetfrom the accurate diameter? Is there any way to determine the offsetfrom the accuratemean diameter so we can estimate the errors involved in the measured values? In this paper, we attempt to provide answers to these important fundamental questions.

Background information

First, we briefly discuss the measurement procedures used to determine the reported diameters of NPs by the most widely used directimaging tools, such as SEM and AFM.These methods are usually considered to be the most accurate reference metrology tools, specifically when they are calibrated to an International System (SI) unit(18). At the sub-100 nm scale, it is challenging to obtain complete 3-D information about IS-NPs. For this reason, certain assumptions are typicallymade when using these tools.

The two-dimensional, top-down area of projection is used to measure the diameters of IS-NPs using SEM (and conventional transmission electron microscopy (TEM)). Information regarding the height of the NPs is ignored. The calculated projected area equivalent diameter (Dxy) is reportedas the SEM measured diameter (it is assumed that the height or the 3rd dimension is the same as the Dxy). In principle, both SEM and conventional TEM are supposed to produce the same size. In practice, however,SEM and TEM tools produce different diameters due to differences in the edge detection methods.

Accurately calibrated AFM uses a sharp tip to measure the precise peak heights of NPs. However, it is extremely challenging to obtain an accurate contour (or projection area) of NPs because of limitations in the physical interaction between the AFM tip and NPs. For this reason, only height information is usedfor the reported diameter and can be referred to as the peak-height equivalent diameter, which is the same as Dz. It is assumed that the AFM-measured height represents the diameter of perfectly spherical NPs and is reported as such.

In the case of SEM and conventional TEM, only the 2-D projection area is exploited, whereas in the case of AFM, only the 1-D height information is utilized. Because none of these three tools use complete 3-D information on IS-NPs, which are inherently 3-D in nature, they are prone to error. In fact, we should expect measurement biases in the reported diameters when SEM/TEM and AFM measurements are compared to each other. The published literature provides ample support for this observation (Table 1). We can also expect the reported diameters to include a certain offsetfrom the not yet known accuratediameters. However, with the prevailing conventional knowledge, it is challengingto routinely determine the true diameter/volume of many types of NPs. This paper deals with identifying these biases and proposes a way to minimize or eliminate them.

Table 1. Nanoparticle diameters reported in the literature. A comparison of the reported mean diameters measured from the same batch using SEM, TEM and AFM shows disagreement in their values. SEM-reported diameters are usually larger than the AFM-reported diameters. In this table, diameters reported by SEM have up to 37% offset from the diameters reported by AFM, and volumes calculated by SEM reported diameters have up to 150% offset from the volumes calculated by AFM reported diameters.

Material	Mean diameters, nm			SEM Dia.	SEM Vol.	Ref.
	SEM	TEM	AFM	Offset (%)	Offset (%)	
SiO2	39.00	35.10	30.30	28.71	113.24	[20]
SiO2	46.60	42.90	36.20	28.73	113.32	[20]
SiO2	89.80	86.30	80.20	11.97	40.38	[20]
Au	85.48		79.05	8.13	26.44	[3]
Ag	30.97		23.14	33.84	139.74	[19]
Au	9.90		7.20	37.50	159.96	[29,30]
Au	26.90		23.70	13.50	46.22	[29,30]
Au	54.90		53.90	1.86	5.67	[29,30]

Ideally, we would like to have all types of NPs to be perfectly regular in nature such as spherical, cubic, or cylindrical. In reality, however, many types of NPs are irregularor quasi-spherical as shown in Fig. 1(a). Conventionally, the IS-NPs are first spread onto a substrate (or a grid) before measuring with SEM, TEM or AFM tools. This process typically aligns the IS-NPs in the most stable orientations on the substrate such that they have a wide base and a low center of gravity (or in this case due to van der Waals'/electrostatic force)(29). If we assume the IS-NPs shown in Fig. 1(a) to be ellipsoids as shown in Figs. 1(b) to 1(d), then it is highly likely that they come to rest as shown in either Fig. 1(b) or Fig. 1(c), and highly unlikely that they come to rest in the prolate ellipsoid orientation (Fig. 1(d)). Because of this preferential orientation, measurements made using SEM/TEM and AFM tools inherently contain a systematic bias as will be shown below.

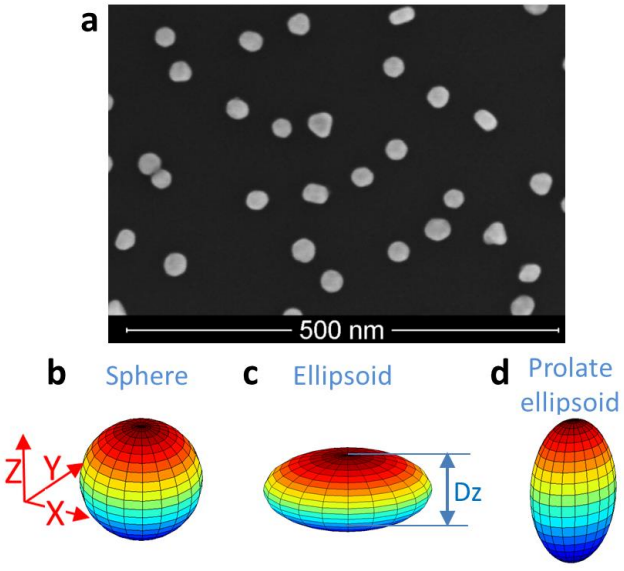

Fig. 1. Nanoparticle shapes. (a) SEM image of nominally 30 nm diameter Au nanoparticles showing their irregular shapes. (b) to (d) Simplified ellipsoid shapes assigned to IS-NPs.

Consider an IS-NP as shown in Fig. 2(a) that is in a stable position. SEM and TEM make use of the top-down projection area only to infer projection area equivalent diameter (Dxy) as shown in Figs. 2(b) and 2(c). AFM makes use of the peak height information only to infer peak height equivalent diameter (Dz) as shown in Fig. 2(d). Dxy and Dz are the routinely reported diameters for the IS-NP using SEM/TEM and AFM, respectively. Here, we propose a different method that combines top-down projection area as measured by SEM/TEM, and peak-height as measured by AFM to obtain a 3D volume from which spherical equivalent volume diameter (Dxyz) is calculated as depicted in Figs. 2(e) and 2(f), i.e. both Dxy and Dz are combined to calculate Dxyz. We theorize that the volume calculated using Dxyz has the least error compared to volumes calculated using SEM/TEM and AFM from the not-yet-known accuratevolume of the IS-NPs. In the following paper, we validate this conjecture using modeling, simulations, measurements, and the published data.

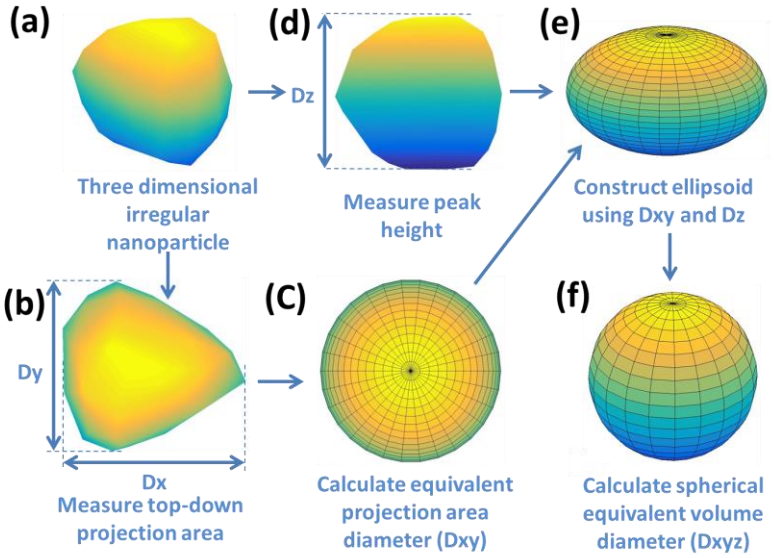

Fig. 2 Equivalent diameter calculations using the combined method. (a) A representative irregularly-shaped, three-dimensional nanoparticle. (b) Top-down irregular area of projection as measured by tools such as SEM and TEM. (c). Calculated projection area equivalent diameter (Dxy) using the measured projection area from (b). Dxy would be the reported diameter using SEM or TEM. (d)Peak-height (Dz) as measured by tools such as AFM, and would be the reported diameter (e) Constructed ellipsoid by combining SEM/TEM and AFM reported diameters, where Dx = Dy = Dxy, and Dz = peak-height. (f) Calculated spherical equivalent volume diameter (Dxyz) from the constructed ellipsoid, where Dx = Dy = Dz = Dxyz. Dx = Feret's (maximum) diameter, Dy = Feret's minimum diameter.The color of the nanoparticles transitions form dark green to yellow from the bottom of the particle to the top.

Results and discussion

Model

Let us consider a simplistic case of a 30 nm sphere for this initial validation. This sphere can be distorted into several ellipsoidal shapes while keeping the volume constant. The equivalent diameters that would be inferred for the different-shaped nanoparticles using SEM/TEM (Dxy), AFM (Dz) and the combined method (Dxyz) are then evaluated as described in Fig. 2 (after aligning them into the most stable orientation) and presented in Table 2. Comparing the calculated Dz and Dxyin Table 2, we can see that the diameters determined by SEM/TEM are larger

while the diameters determined by AFM are smaller than the accuratediameter of 30 nm (except for the perfect spherical particle). The same trend can be observed in the published literature (Table 1) where SEM/TEM reported diameters are larger than AFM reported diameters, indicating that the simplistic model we assumed so far points in the right direction.

Table 2. Inferred equivalent diameter evaluation by distorting a 30 nm diameter spherical NP.

S.No.	Dx	Dy	Dz	Dxy (SEM/TEM)	Dz (AFM)	Dxyz	Dxy Offset	Dz Offset
	nm	nm	nm	nm	nm	nm	%	%
1	30.00	30.00	30.00	30.00	30.00	30.00	0.00	0.00
2	31.00	29.50	29.52	30.24	29.52	30.00	0.80	-1.59
3	36.00	27.00	27.78	31.18	27.78	30.00	3.92	-7.41
4	50.00	20.00	27.00	31.62	27.00	30.00	5.41	-10.00
5	31.00	31.00	28.10	31.00	28.10	30.00	3.33	-6.35
6	35.00	35.00	22.04	35.00	22.04	30.00	16.67	-26.53
7	37.00	32.00	22.80	34.41	22.80	30.00	14.70	-23.99

Dxy = projection area equivalent diameter as measured by SEM/TEM
Dz = peak-height equivalent diameter as measured by AFM
Dxyz = the diameter evaluated by combining the Dxy and Dz diameters

Thesemeasurement biases for SEMs and AFMscan be explained based on (i) the way particles orient themselves and come to rest on a smooth substrate, and (ii) the way SEM/TEM and AFM measurements are made. SEM and AFM attempt to measure 3D volume based purely on 2D top-down projected area and 1D peak height, respectively. Even if the particles do not orient in any particular way, these measurement methods could give rise to some biases. The AFM measured Dz is usually smaller than the largest of the measured Dx (Feret's maximum diameter)and Dy (Feret's minimum diameter) and hence often results in a smaller AFM measured diameter than SEM measured diameter. We can infer another minor point: the difference in the diameters measured by SEM/TEM and AFM is proportional to the deviation of the NP from the perfect spherical shape, whereas the accuratespherical-equivalent-volume (SEV) diameter equals the proposed combined diameter of Dxyz (shows the least error). The following important inferences can be made based on the simplistic model.

I. It is possible to obtain the SEV diameter of IS-NPs by combining SEM/TEM (projection-area) and AFM (peak-height) measurements with thelowest error.
II. The SEM/TEM-measured mean diameters are usually larger than the AFM-measured mean diameters.
III. The accurateSEV diameters are usually in between the SEM/TEM- and the AFM-measured diameters.
IV. The SEM/TEM-measured diameters may have asmaller error (or are more accurate) than the AFM-measured diameters.

Simulations

Here we test the combined method approach by simulating a more realistic irregularly shaped particles and also test if the above inferences hold. A key benefit of this approach is that we know the precise volume of the computer-generated particles. From a geometric point of view, the computer generated irregularly shaped particles are similar to IS-NPs. The computer program generates irregularly shaped particles, rotates and aligns them as if they are laid onto a smooth substrate in the most stable orientation similar to the process when NPs are spread onto a substrate (using the minimum centroid height principle), and determines the diameters as if they are measured using SEM (Dxy), AFM (Dz) and the combined method (Dxyz). The volume (from Dxyz) that is calculated using the combined method can then be compared with the accurateknown volume from the simulation for validation. Even though the program is capable of generating many types of irregularly-shaped particles,here we present results for three types of irregularly shaped particles dominated by (i) nearly smooth

round surfaces (Fig. 3(a)), (ii) faceted and angled surfaces (Fig. 3(b)), and (iii) faceted and angled surfaces deviating from quasi-spherical shape (Fig. 3(c)). Simulated results for 50 NPs each are presented in the Electronic Supplementary Material Table S1and a summary of the results is presented in Table. 3. Nearly identical inferences (as presented above) can be made from these simulated results as well, with one minor difference. For particles dominated with asmooth surface, Dxyz has an error of less than one percent from the precise diameter. However, for the particles dominated by faceted surfaces, Dxyz has a relatively larger error (in this case about 2.11% and 2.49 %), indicating that the error in Dxyz increases as the particle surface turns from a smooth to a faceted surface. This increase in error makes sense, considering that the shape being used as a model for these measurements (an ellipsoid) has a smooth surface. As the particle deviates from the model, the error increases. The combined method still shows significantly less error compared to SEM or AFM measured diameters.

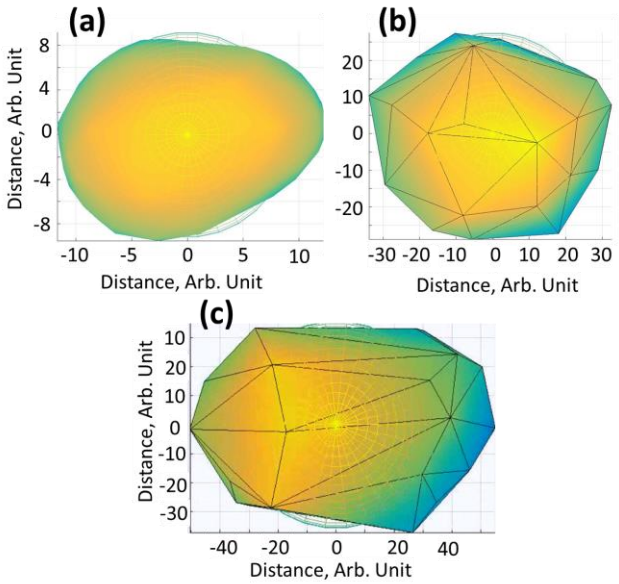

Fig. 3. Top-down images of computer generated particles dominated by (a) smooth, and (b) and (c) faceted surfaces. Particle in (c) is farther away from a quasi-spherical shape. Spheres with equivalent volumes are superimposed on the particles (Online resource: Multimedia showing 3D view of particles as: Fig3a.mov, Fig3b.mov and Fig3c.mov).

Table 3. Average inferred equivalent diameterscalculated using simulated irregularly shaped particles.

Surface Type	Do	Dz (AFM)	Dxy (SEM)	Dxyz	Dz Offset	Dxy Offset	Dxyz Offset
	AU	AU	AU	AU	%	%	%
Smooth	60.8	53.7	64.8	60.8	**-11.8**	**6.8**	**0.1**
Rough	57.6	51.4	63.0	58.8	**-10.8**	**9.4**	**2.1**
RoughLong	77.1	59.2	91.3	79.0	**-23.2**	**18.6**	**2.5**

AU = Arbitrary Unit, Do = spherical equivalent diameter calculated using volume from simulation, Dz = peak height, Dxy = calculated projection area equivalent diameter. Dxyz = spherical equivalent volume diameter calculated using Dxy and Dz, Dz Offset = percentage offset of Dz from Do, DxyOffset = percentage offset of Dxy from Do, Dxyz Offset = percentage offset of Dxyz from Do.

Measurements

Here we apply the combined method to experimentally measure volume (or Dxyz) of irregularly-shaped macroparticles. As with the simulation, a key benefit of this approach is that the spherical equivalent volume obtained using the combined method can be corroborated by measuring the actual volume of the particle. This approach provides a definitive experimental validation of the combined method presented in this paper. To perform this, we selected irregularly shaped aquarium glass pebbles weighing approximately 15 g to 21 g (Fig. 4). Each pebble was measured for its projection area, peak height and volume. We obtained a mean Dxyz of 23.8 mm using the projection area and the height of the 54 pebbles (Electronic Supplementary Material Table S2). The independently calculated Dxyz using the measured volume (obtained from mass and density) is 23.96 mm. We can observe that the Dxyz measurement has less than 1% error compared to the accuratespherical equivalent diameter calculated using the actual measured volume,validating the proposed combined method. Identical inferences (as presented above) can be made from these measurements as well. This measurement result also validates the simulation results presented earlier for particles with thenearlysmooth surface (Fig. 3(a)).

The modeling, simulations and measurement results presented so far strongly support and appear to confirm the accuracy of the proposed combined method (and the four associated inferences presented earlier). Here we proceed to apply the combined method to real SEM and AFM measurement data. This application requires identification of the same NP during SEM and AFM measurements. Such an experimental data for Au IS-NPs are presented in Fig. 15 in Ref. (3). We extracted the pairs of SEM- and AFM-measured diameters for each Au NP,calculated their Dxyzs,and tabulatedthe results in Electronic Supplementary Material Table S3 for the reported 68 NPs.

Real experimental measurement data on Au NPs producesidentical inferences. Based on the above-presented results and the proposed combined method, we can expect the mean Dxyz of 55.1 nm would have the least error from the accurate SEV diameter(Inference I), which is challenging to measure. The SEM-measured meandiameter of 58.2 nm is larger than the AFM-measured mean diameter of 49.7 nm (Inference II). The mean Dxyz of 55.1 nm is in between the SEM- and the AFM-measured diameters (Inference III), similar to the accurateSEV diameter. The SEM-measured diameter has less error of 5.3% compared to the AFM-measured diameter error of 9.9% (Inference IV). The experimental data appear to confirm all the important inferences made above.

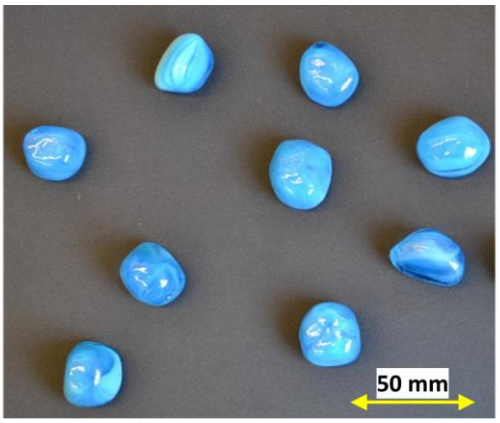

Fig.4. Macroscale pebbles used to compare the spherical equivalent volume diameters (Dxyz) calculated based on Dxy and Dz and from mass and density.

There is some degree of uncertainty associated with the SEM data presented in Ref. (3), whichstems from the fact that no accurate edge detection method has been developed for SEM images of Au nanoparticles. Due to this uncertainty, the reported diameters of Au NPs were extracted at the full-width-half-max of the SEM intensity profiles (3). This selection is a convenience based on the general nature of SEM imaging, but is not based on a rigorous model for imaging of Au NPs. Hence, we can expect an unknown measurement bias in the SEM measured diameters. However, the AFM measured peak-heights are less prone to error and hence these can be considered fixed. Availability of the accurate edge detection methods of NPs using SEM could further improve SEM results and hence the calculation of Dxyz. At present, we can expect a more accurate Dxyz by combining TEM and AFM measurements, as it is possible to identify the end of atoms at the edges in TEM images more precisely than any non-model-based assignment of the physical edge location within the intensity profile of the SEM image.

For regularly-shaped symmetric NPs such as spheres and cubes, the proposed combined method may not be of much use, as either SEM or AFM can provide a one-dimensional measurement which will be same inother directions also. For perfect cylindrical-shaped NPs (withthe axis along the substrate), SEM alone could provide all the necessary dimensions to calculate the volume; however, AFM cannot provide both necessary dimensions. For some other types of regularly-shaped NPs such as prisms, cuboids, and cylinders (resting on the end), the height information cannot be automatically obtained using SEM measurements alone, and lateral dimensions cannot be obtained using AFM alone. In such cases, again, combining SEM and AFM measurements will provide a more accurate volume determination compared to using either SEM or AFM alone; however, for these regular shapes, it is unnecessary and in fact preferable not to assume a spheroidal shape. Becauseof their regular shapes, basic geometric formulas can be used to determine their volumes from the two or three dimensions measured using SEM and/or AFM.

Conclusions

In this paper, we have proposed a method to determine the volume of certain types of irregularlyshaped, and regularly-shaped NPs by combining measurements obtained using top-down projection areas (as measured by tools such as SEM/TEM), and peak heights (as measured by tools such as AFM). Modeling, simulations and experimental data provided appear to show that NP diameters measured by the combined method has the least error, and is more accurate than the diameter measurements made by using either SEM or AFM alone. Based on themore accurate volumetric measurement proposed, we demonstrate that for certain types of NPsthere is a high probability that SEM/TEM measured diameters are more close to the accuratediameter compared to AFM measured diameters. We expect this method to facilitate the certification of accurate reference materials for IS-NPs, which willincrease the reliability and enhance trust in the results of studies of size-dependent properties of NPs, such as in toxicology.

Supplementary material

See the Electronic Supplementary Material for the detailed diameter measurement data.

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Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

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