



Particle Size Analysis of Nanomaterials for REACH and Beyond

Introduction

Since the complete implementation of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, it has been a requirement for companies that manufacture or import chemicals, including nanomaterials, into the European Union in quantities that exceed 1 tonne to register these substances with the European Chemicals Agency (ECHA). A major step within this process involves the physical characterisation of the material in order to determine the material's potential impact on human health and the environment.

However, the assessment of these physical properties for nanomaterials, for REACH and wider applications, is non-trivial and this is especially true for the particle size analysis of nanomaterials. In this paper we will discuss the challenges that the particle size of nanomaterials presents and how a systematic approach can be employed to overcome them.

Specifically, we will address a number of key questions:

- What techniques are available to characterise my nanomaterial?
- How should my nanomaterial be prepared for characterisation?
- How can a suite of techniques be used to give the most comprehensive characterisation?

Definition of a Nanomaterial – Implications for Particle Size Analysis

The particle size is a key property to measure for any particulate system, but especially for nanomaterials as the definition for a nanomaterial can rely so heavily on this property.

The European Commission has published a recommendation for the common definition of a 'nanomaterial' for regulatory purposes [1]. This definition includes the following:

'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate

and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm.

The first question that must be asked, having read this definition and with particle size analysis in mind, is: 'in what state are the particles present within the nanomaterial?' The definition for a nanomaterial provides three options (Figure 1):

- Particle in an **unbound state**, where 'particle means a minute piece of matter with defined physical boundaries'
- **Agglomerate**: 'a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components'
- **Aggregate**: 'a particle comprising of strongly bound or fused particles' where the resulting external surface area may be significantly lower than the sum of the surface area of the individual components.

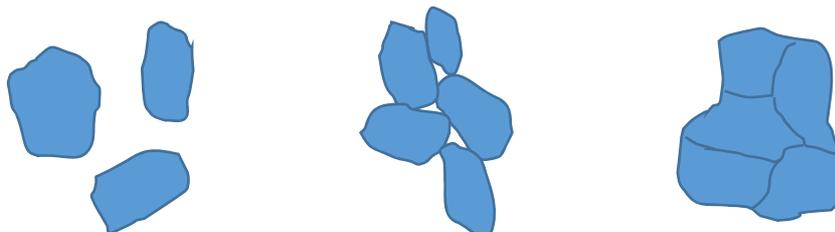


Figure 1: Particles in unbound (left), agglomerated (middle) and aggregated (right) states.



The definition for a nanomaterial requires that we measure the size of the particles in their unbound state, but with the potential for the presence of agglomerates and aggregates it cannot always be guaranteed that this will be the case when using most techniques

The agglomerates and aggregates within a sample can be quickly identified using **electron microscopy (EM)** techniques, such as **Scanning Electron Microscopy (SEM)** or **Transmission Electron Microscopy (TEM)**. Furthermore, irrespective of their involvement in agglomerates and aggregates and irrespective of the sizes of these assemblages, imaging with EM techniques allows for immediate assessment of sizes of particles in their unbound state.

For example, a sample of a commercially available TiO₂ was assessed by Escubed Limited using TEM. At low magnifications (Figure 2a, 2b), it is clear that the sample is highly agglomerated and aggregated with large assemblages observed of sizes in the order of tens of microns. As the magnification is increased, it is possible to distinguish the primary particles within these assemblages and identify that these particles are irregularly shaped and have sizes mostly smaller than 100 nm.

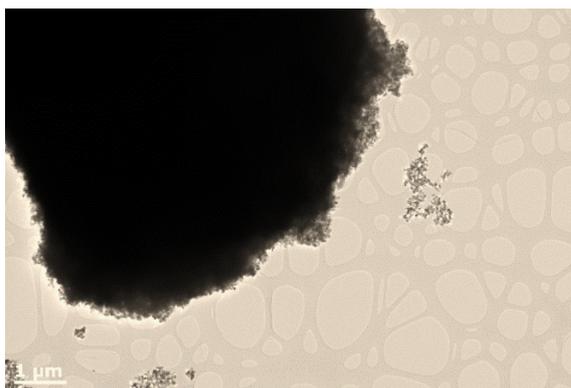


Figure 2a: TEM images of a TiO₂ nanomaterial comprising particles with diameters of less than 100 nm which are forming large agglomerates and aggregates with lower magnification.

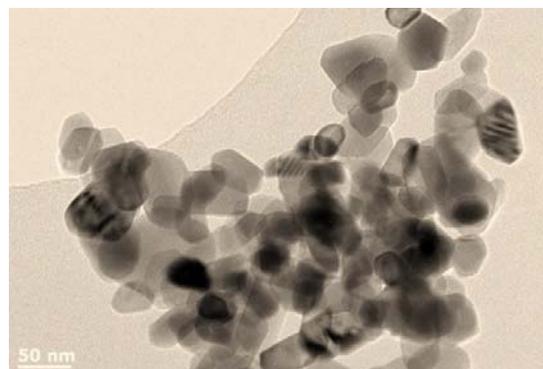


Figure 2b: TEM images of a TiO₂ nanomaterial comprising particles with diameters of less than 100 nm with higher magnification

Preparation of Samples for Particle Size Analysis

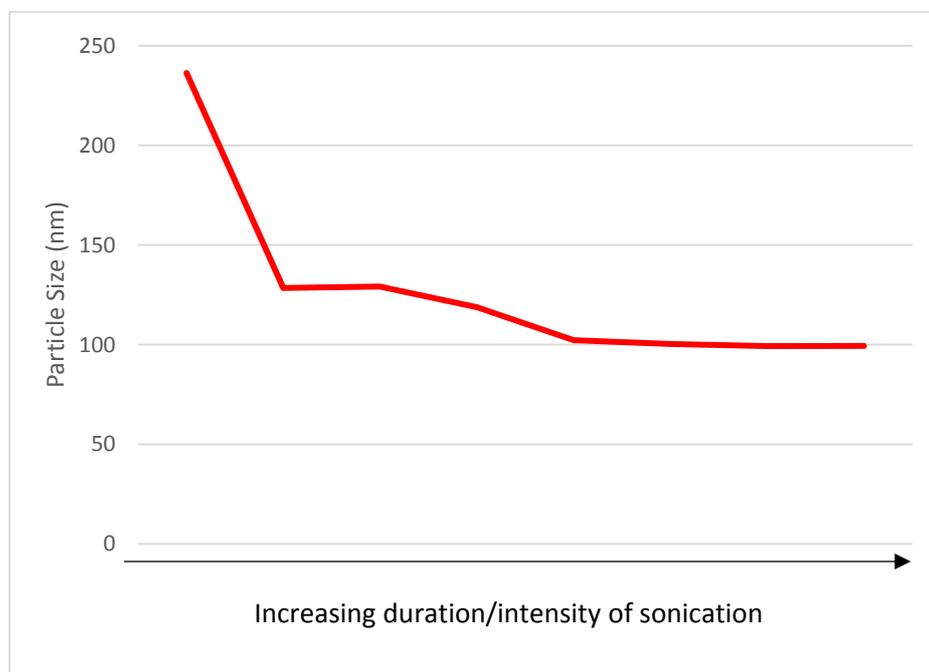
Whilst image analysis can be performed with microscope images to give a size distribution, the number of particles sampled is considerably lower than would be achieved with other conventional sizing techniques and so imaging is typically considered a qualitative source of size data rather than a quantitative one. These other techniques should be therefore be used to provide a statistically more reliable size distribution.

The main problem with other particle size techniques is that they are not capable of distinguishing between primary particles, agglomerates or aggregates. For the purposes of sizing the primary particles using these techniques sample preparation is therefore key. A physical means of decomposing the agglomerates and/or aggregates must be used and this usually involves the application of ultrasound to a dispersion of the nanomaterial with an ultrasonic bath or ultrasonic probe.



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The optimal dispersion conditions for the TiO₂ sample imaged above were investigated. The TiO₂ was dispersed in Type II water at a suitable concentration and ultrasound was applied to the dispersion for increasingly longer durations with a gradual reduction in particle size is observed with the mean particle size plateauing after 2 minutes of ultrasound with probe following 5 minutes of ultrasound with an ultrasonic bath. A combination of 5 minutes with the ultrasonic bath followed by 5 minutes with the ultrasonic probe was chosen as the best dispersion procedure. This is a compromise increasing intensity. The mean particle size was measured using Dynamic Light Scattering (see later) as a means of assessing dispersion progress. A plot of particle size versus dispersion parameters is shown in Figure 3.



Selection of Particle Size Analysis Technique

Once the sample preparation procedure for a nanomaterial has been devised, it is then necessary to choose a particle size analysis technique. In choosing the correct technique for particle size analysis of nanomaterials, there are two main considerations.

Firstly, the size technique must be capable of providing a number-based distribution so as to conform to the definition of a nanomaterial. Not all size techniques provide a number-based distribution and not all size techniques provide a number based distribution as the primary result. Other possible distribution types include volume, surface area and intensity based distributions.

Secondly, the size technique must be capable of sizing at such a small scale. The size capabilities of a range of particle size techniques offered by escubed limited is provided in Figure 4

Figure 3: Assessing the optimal dispersion conditions for a sample of TiO₂ in water – a gradual decrease in particle size is observed as the duration of sonication is increased and more intense sonication is applied. 5 minutes of sonication with an ultrasonic bath followed by 5 minutes with an ultrasonic probe was selected as the best dispersion procedure.

| | Size Range | | | | | | | |
|---|--|--|---|--|--------------|----------------|---------------|--------------|
| | 0.1 nm – 1 nm | 1 nm – 10 nm | 10 nm – 100 nm | 100 nm – 1 µm | 1 µm – 10 µm | 10 µm – 100 µm | 100 µm – 1 mm | 1 mm – 10 mm |
| Laser Diffraction | | | [Red bar spanning from 10 nm to 1 mm] | | | | | |
| Optical Microscopy/Image Analysis | | | | [Blue bar spanning from 1 µm to 100 µm] | | | | |
| Light Obscuration/Electrical Sensing Zone | | | | [Green bar spanning from 100 nm to 100 µm] | | | | |
| Dynamic Light Scattering | [Yellow bar spanning from 0.1 nm to 10 µm] | | | | | | | |
| Differential Centrifugal Sedimentation | | | [Brown bar spanning from 10 nm to 100 µm] | | | | | |
| Nanoparticle Tracking | | [Cyan bar spanning from 10 nm to 100 nm] | | | | | | |
| SEM/Image Analysis | | | [Pink bar spanning from 10 nm to 1 mm] | | | | | |
| TEM/Image Analysis | [Black bar spanning from 0.1 nm to 10 nm] | | | | | | | |

Figure 4: Comparison of size capabilities of some common particle size analysis techniques – a wide range of sizes can be measured by escubed limited



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In addition to SEM and TEM, it is possible to obtain size information for nanomaterials from laser diffraction, dynamic light scattering, centrifugal differential sedimentation and nanoparticle tracking analyses. Each of these techniques has its own advantages and disadvantages depending on the sample under test. These are summarised in Table I.

| Technique | Advantages | Disadvantages |
|--|--|---|
| Laser diffraction e.g. Malvern Mastersizer 2000 or 3000 | <ul style="list-style-type: none"> Quick measurements Application flexibility – wet or dry systems Wide size range – 10 nm to 3000 μm | <ul style="list-style-type: none"> Generates a volume distribution – cannot measure according to nanomaterial definition Relatively large concentrations required |
| Dynamic Light Scattering e.g. Malvern Zetasizer Nano ZS | <ul style="list-style-type: none"> Quick measurements Performs well when measuring monodisperse samples with known refractive index | <ul style="list-style-type: none"> In standardised form, this does not generate a size distribution Distributions are generated by fitting procedure, which can be prone to error, and are intensity based – bias from larger particles |
| Differential Centrifugal Sedimentation e.g. CPS DC24000 | <ul style="list-style-type: none"> Very high resolution Capable of reliably measuring polydisperse samples | <ul style="list-style-type: none"> Generates a number distribution Small band-width with respect to size Relies on particle density being known and uniform |
| Nanoparticle tracking analysis e.g. Malvern Nanosight LM10 | <ul style="list-style-type: none"> Generates a number based distribution Particle size on a particle-by-particle basis – no bias from larger particles | <ul style="list-style-type: none"> Analysis of captured videos involves more operator input than other techniques Mostly limited to size analysis in solvent for which instrument is calibrated |

Table I: Comparison of size techniques for nanomaterial particle size analysis – each technique has advantages and disadvantages that make it suited for certain types of analysis

The TiO_2 sample prepared above was tested using these four techniques and the number-based distribution is provided in Figure 5. These techniques appear to be generating distributions that are mostly in agreement, with median sizes of 90 to 100 nm and minimum sizes of 40 to 50 nm. The sizes also agree closely with the sizes of particles expected from TEM images – in this way electron microscopy is very useful in helping to validate the size measurements.

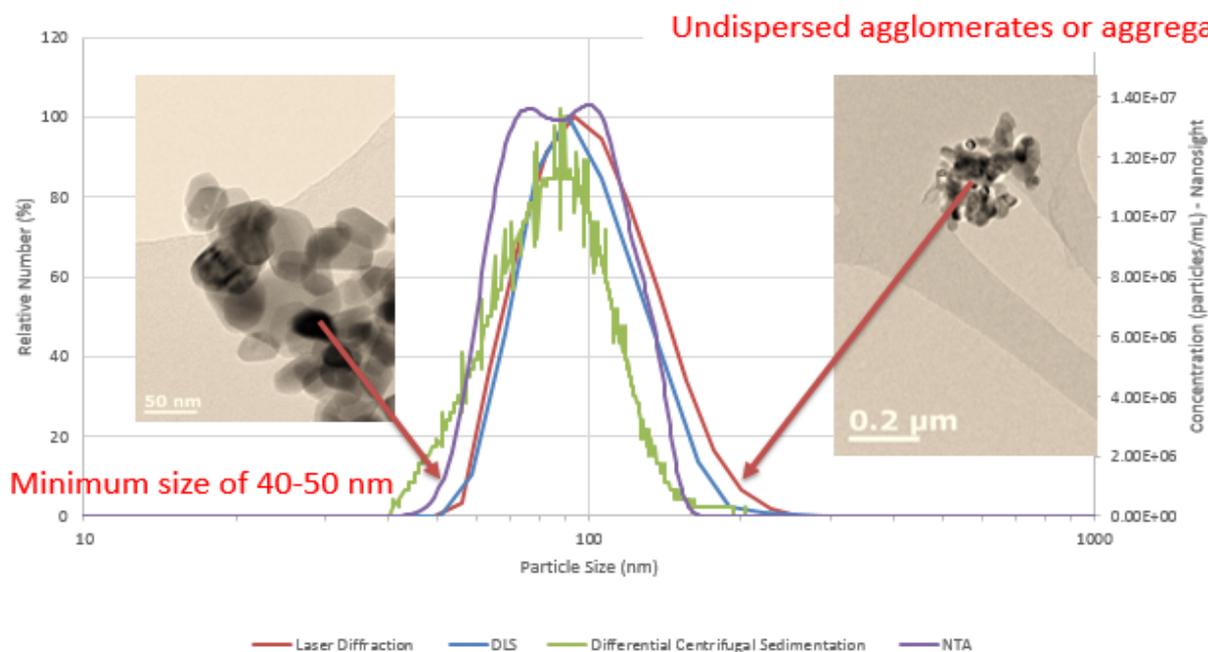


Figure 5: Comparing number-based size distributions for a sample of TiO_2 – TEM images are used to validate and provide context for the size distributions.



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The largest discrepancy, however, between the distributions is at the larger sizes - nanoparticle tracking gives a maximum of size of 187.5 nm whilst laser diffraction gives a maximum size of 259.3 nm. As discussed, these four techniques are not capable of distinguishing between primary particles, agglomerates and aggregates, so it is not initially clear what the nature of these particles is.

Further assessment of the TEM images for this TiO₂ sample helps to shed some light on this matter and help to validate our assessment of the maximum particle size. At higher magnifications, strongly bound agglomerates or aggregates with sizes of approximately 200 nm can be observed and it is possible that the different techniques have different sensitivities for these assemblages. Without the use of the TEM images, it would not have been possible to gain this insight and contextualise the size results generated.

Conclusions

- Particle size analysis of nanomaterials is non-trivial and presents a number of challenges.
- Electron microscopy (SEM or TEM) can be used to give qualitative information about the size, shape and state of the particles within a nanomaterial.
- Other techniques must be used to produce more statistically reliable distributions e.g laser diffraction, dynamic light scattering, differential centrifugal sedimentation and nanoparticle tracking analysis. Each technique has its own advantages and disadvantages depending on the sample under test.

- Sample preparation is essential to ensure adequate dispersion of the particles and that the primary particles are being sized rather than agglomerates or aggregates. This is achieved through application of ultrasound to particle dispersions.
- To achieve the best outcome from the characterisation of a nanomaterial, electron microscopy and sizing with another complementary technique must be used co-operatively.

escubed limited can provide a comprehensive size analysis of your nanomaterial. We work with our customers to understand their requirements and ensure that the most appropriate testing is selected.

For more information

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[1] Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU)

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