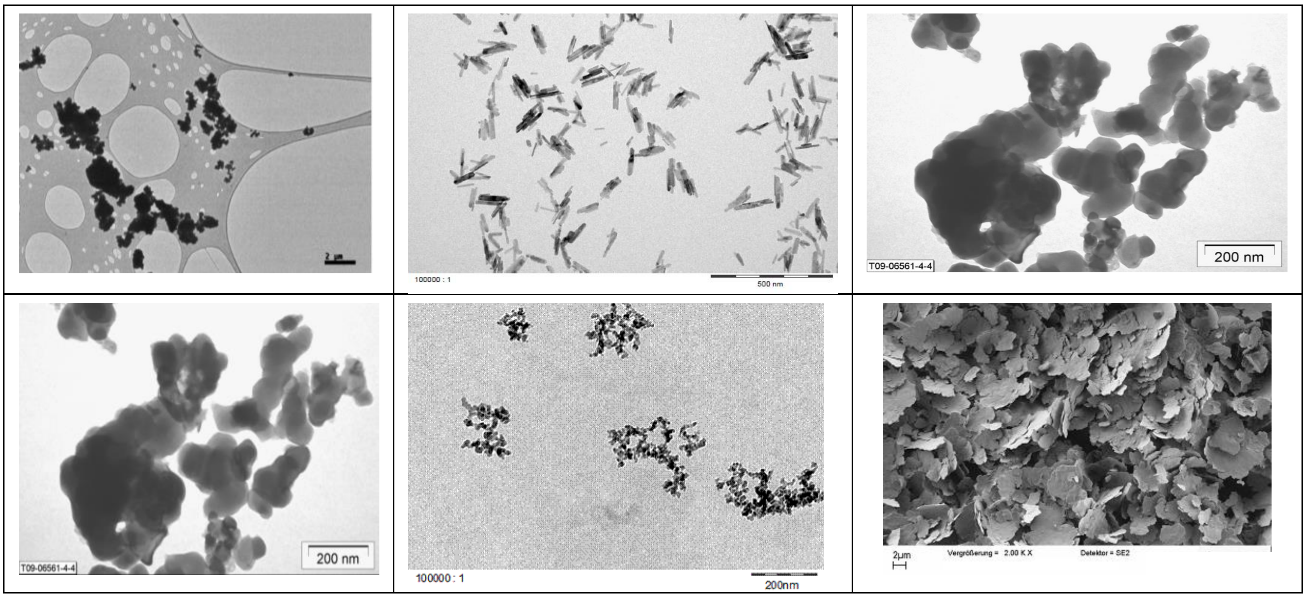
JRC TECHNICAL REPORTS

Basic comparison of particle size distribution measurements of pigments and fillers using commonly available industrial methods

Edited by
Douglas Gilliland
Neil Gibson
Uwe Hempelmann

November 2014



Report EUR 26916 EN

European Commission

Joint Research Centre
Institute for Health and Consumer Protection

Contact information

IHCP-Communication
Address: Joint Research Centre, Via Enrico Fermi 2749, TP 125, 21027 Ispra (VA), Italy
E-mail: jrc-ihcp-communication@ec.europa.eu
Tel.: +39 0332 78 5378

JRC Science Hub
https://ec.europa.eu/jrc

JRC 92531

EUR 26916 EN

ISBN 978-92-79-43974-2 (PDF)

ISSN 1831-9424 (online)

doi:10.2788/21024

Luxembourg: Publications Office of the European Union, 2014

Cover: Transmission electron micrograph (TEM) images of selected pigment particles

Abstract

The Nanobiosciences Unit of the Joint Research Centre's Institute for Health and Consumer Protection and Eurocolour, the association of European pigments, dyes and fillers industry, have carried out a program of work to evaluate a number of instrumental methods of measuring particle size distributions as required for assessing compliance versus the EU Recommendation for the definition on nanomaterials. The study has examined the use of five instrumental methods applied to a range of eight widely different but industrially relevant powder pigments. The techniques examined were Laser Diffraction (LD), Dynamic Light Scattering (DLS), Centrifugal Liquid Sedimentation (CLS), Volume Specific Surface Area (VSSA) and Electron Microscopy (EM).

This report describes the materials studied and the preparative and analytical methods used. Individual chapters provide an overview of the single analytical methods used together with a summary of the results obtained using each particular method. In considering the results of this study it is important to note that the aim was not to determine the optimum conditions for every individual sample but rather to produce and evaluate data which could be considered representative of that obtainable in industrial laboratories using existing instrumental facilities operated by experienced but not specialised operators. The report discusses the challenges of using these instrumental methods to obtain a simple unambiguous classification of the test materials according to the EC definition.

The Nanobiosciences Unit of the Joint Research Centre's Institute for Health and Consumer Protection[1] and Eurocolour[2], the association of European pigments, dyes and fillers industry, have carried out a program of work to evaluate a number of analytical methods of measuring particle size distributions as required for assessing material status with respect to the EC Recommendation for the definition of nanomaterial. The study has examined the use of methods based on instrumentation commonly used in the pigment industry and has applied them to industrially relevant powder pigments.

Authors of the report

Juan Bautista Vicent Pena	Itaca Grupo (Itaca)
Klaus Kund	Clariant Produkte (Deutschland) GmbH (Clariant)
Uwe Hempelmann	LANXESS Deutschland GmbH (LANXESS)
Wendel Wohlleben	BASF SE (BASF)
Thomas Koch	KRONOS International. Inc. (Kronos)
Alison Burke	Huntsman United Kingdom (Huntsman)
Greg McNulty	Huntsman Pigments Tioxide Europe Limited (Huntsman)
Angelika Hartl-Gunselmann	Eckart GmbH (Eckart)
Stefan Knobl	Eckart GmbH (Eckart)
Martin Reisinger	Evonik Industries AG (Evonik)
Douglas Gilliland	Joint Research Centre (JRC)
Neil Gibson	Joint Research Centre (JRC)
Birgit Sokull-Klüttgen	Joint Research Centre (JRC)
Hermann Stamm	Joint Research Centre (JRC)
Heike Liewald	EUROCOLOUR

The authors would like to thank Dr Robert Fischer for taking the initiative to start the project and Dr Claudia Cascio for her contribution to the preparation of the final manuscript text. Additional thanks go to Gert Roebben (JRC) for his valuable contribution in the reviewing and correction of the final version of this report.

DISCLAIMER

The current report does not represent the official view of the European Commission. Certain commercial equipment, instruments, brand names and materials are identified in this report as examples or to specify adequately an experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

---

[1] https://ec.europa.eu/jrc/en/research-topic/nanotechnology

[2] Eurocolour - umbrella association of European pigments, dyers and fillers industry, http://www.cefic.org/

Contents

Executive Summary

The Nanobiosciences Unit of the Joint Research Centre's Institute for Health and Consumer Protection[3] and Eurocolour[4], the association of European pigments, dyes and fillers industry, have carried out a program of work to evaluate a number of analytical methods of measuring particle size distributions as required for classification with respect to the EC Recommendation for the definition on nanomaterials. The study has examined the use of five instrumental methods applied to a range of eight very different but industrially relevant powder pigments. The materials used were chosen so as to exhibit a wide diversity of physico-chemical properties which would serve to test the general effectiveness, suitability and reliability of the measurement techniques. They included materials with different primary particle sizes and shapes (needles, platelets and more spherical forms), different levels of aggregation/agglomeration, both inorganic and organic substances, and both uncoated and uncoated pigments.

Four of the techniques, Laser Diffraction (LD), Dynamic Light Scattering (DLS), Centrifugal Liquid Sedimentation (CLS) and Volume Specific Surface Area (VSSA, assessed by the Brunauer, Emmett & Teller (BET) method) are commonly used in the pigment industry and were benchmarked against the fifth technique, Electron Microscopy (EM). The study involved nine independent laboratories composed of the eight industrial partners from Eurocolour, each of which provided one of the test materials, and the Institute for Health and Consumer Protection (IHCP) of the European Commission's Joint Research Centre (JRC). In the course of the study eight laboratories were able to undertake LD and VSSA measurements while DLS and CLS analysis were performed by four and six laboratories respectively.

This report describes the materials studied and the preparative and analytical methods used. Following a study overview, one chapter provides an overview of the materials and analytical methods used, and subsequent chapters summarise the results obtained with each method.

In considering the results of this study it is important to note that the aim was not to determine the optimum conditions for every individual sample but rather to produce and evaluate data which could be considered representative of that obtainable in industrial laboratories using existing instrumental facilities operated by experienced but not specialised operators.

From the results of this study, as well as generally available knowledge about the techniques used, the following conclusions were drawn with respect to implementation of the EC Recommendation on the nanomaterial definition:

- For those measurement techniques requiring powder samples to be first (re-)dispersed in liquids the sample state and more specifically sample preparation of the dispersions is critical to particle size distribution determination, and the results will only be valid if the materials can be dispersed

---

[3] https://ec.europa.eu/jrc/en/research-topic/nanotechnology

[4] Eurocolour - umbrella association of European pigments, dyers and fillers industry, http://www.cefic.org/

into their constituent particles, and if those constituent particles are approximately spherical in shape and (for some techniques) nearly monodisperse.

- Electron Microscopy, by directly imaging and counting particles, is probably the method which comes closest to being able to provide information on the particle size distribution as required by the EC definition. However, there are many issues related to sample preparation and counting protocols which need to be resolved and standardised before EM could provide reasonable comparability of the results between different companies as well as allowing reliable third-party testing.
- None of the simple commonly available dispersion-based methods used in this study (LD, DLS and CLS) can be recommended as a single method for classifying materials against the recommended EC definition of nanomaterial.
- Expertise and knowledge of the specific substances in question is necessary to obtain reliable results. Without such knowledge, it can be expected that third party laboratories, operating with high standards under established guidelines, would need to invest considerable time and resources in materials analysis and the optimisation of sample and technique specific preparation (dispersion) procedures in order to obtain valid results. Product-specific information supplied by manufacturers will be valuable in this respect.
- Guidelines are required regarding some aspects of the definition and its implementation (e.g. dimensional parameter/s to measure, range of sizes to take into account in median evaluation, what constitutes an aggregate or a constituent particle, etc.). Absence of such guidelines will only add to the lab-to-lab variability of results that is likely to arise from the inherent difficulties associated with all the techniques examined, including electron microscopy.

The three main methods which can be applied to the analysis of liquid dispersed materials (LD, DLS and CLS) all have particular limitations in their ability to deal with small particles in polydisperse samples. The application of any one of these techniques would need to be complemented by alternative instrumental methods which can more reliably detect particles in the lower part of the nano-size range. In the case of aggregated or agglomerated particles, these three techniques are also incapable of determining constituent particle sizes, and they are not suitable for use with particles deviating from approximately spherical shape.

Glossary

AF4	Asymmetric-Flow-Field-Flow-Fractionation
Agglomerate	Collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components[5].
Aggregate	Particle comprising strongly bonded or fused particles
BET	Brunauer, Emmett & Teller
CLS	Centrifugal Liquid Sedimentation
D50m	The value of the particle diameter at 50 % in the cumulative mass-size distribution. Similar nomenclature (e.g. D10m and D90m) may be used for 10 % and 90 % cumulative mass-size distribution respectively
D50n	The value of the particle diameter at 50 % in the cumulative number-size distribution. Similar nomenclature (e.g. D10n and D90n) may be used for 10 % and 90 % cumulative number-size distribution respectively.
D50v	The value of the particle diameter at 50 % in the cumulative volume-size distribution. Similar nomenclature (e.g. D10v and D90v) may be used for 10 % and 90 % cumulative volume-size distribution respectively.
DLS	Dynamic Light Scattering
ECD	Equivalent circle diameter
EM	Electron Microscopy
False Negative	For the purposes of this report a false negative result occurs when the numerical output from the analysis of a sample would lead to the conclusion that the material **is not** nano-particulate in nature when in reality the sample **is** nano-particulate
False Positive	For the purposes of this report a false positive result occurs when the numerical output from the analysis of a sample would lead to the conclusion that the material **is** nano-particulate in nature when in reality the sample is **not** nano-particulate
Hydrodynamic radius	Defined as the radius of an equivalent hard sphere diffusing in a liquid at the same rate as the particle under observation
LD	Laser Diffraction
MFD	Minimum Feret Diameter ($x_{F,min}$)
Monodisperse	Consisting of particles, all of which have a very similar shape and whose sizes fall within a very narrow distribution

---

[5] European Union. Luxembourg 2011: Commission Recommendation of 18 October 2011 on the definition of nanomaterial. Publications Office of the European Union, Official Journal of the European Union (20.10.2011): L 275/38

Particle	A minute piece of matter with defined physical boundaries;
Polydisperse	Consisting of particles and not monodisperse
SEM	Scanning Electron Microscopy
Stokes Diameter	Defined as the apparent diameter of a particle sedimenting under gravitational or centrifugal force as calculated from Stokes' law
TEM	Transmission Electron Microscopy
VSSA	Volume Specific Surface Area

1 Study overview

In 2009 the European Parliament [1] called on the European Commission to review all relevant legislation to ensure that legislative provisions and instruments of implementation reflect the particular features of nanomaterials to which workers, consumers and/or the environment may be exposed. Furthermore, a call was made that a comprehensive science-based definition of the term "nanomaterial" be introduced for inclusion in Community legislation and in nano-specific amendments to relevant horizontal and sectorial legislation. In response to these requests the European Commission published a Recommendation [2] on the definition of nanomaterial which requires that materials be characterised in terms of the number size distribution of their constituent particles.

The introductory text to the Commission Recommendation (section 6) acknowledges that measuring size and size distributions of nanomaterials is challenging in many cases and different measurement methods may not provide comparable results. Furthermore it states that harmonised measurement methods must be developed with a view to ensuring that the application of the definition leads to consistent results across materials and over time, and that best available alternative methods should be applied until harmonised measurement methods are available. It is clearly in the interests of both legislators and industry that the current absence of fully validated test methods be addressed, in the short term, by examining compromise technical solutions which can be used until instrumental technology and progress in analytical research can better tackle the problem. The JRC-IHCP and Eurocolour have carried out a program of work to evaluate the practicalities of particle size measurement for a set of typical pigments and to highlight the difficulties in determining their status versus the EC recommended definition of nanomaterial. This definition, being based on the number size distribution of the particulate material, presents major technical challenges since there are few analytical methods able to directly measure both particle size and particle number [3]. Furthermore, detailed methods including recommendations for sample preparation and analysis of the instrumental data have not yet been developed into any form of validated measurement protocols.

From a purely technical point of view for dried powders electron microscopic methods could, in principle, be applied for this type of measurement, but in practice it is a relatively slow process which requires complex sample preparation, access to costly equipment and expertise which are not routinely available in many industrial facilities. In contrast to this there are a number of simpler and more economical analytical methods which can be applied to liquid dispersed materials but whose raw data output is not directly given as the desired number-size distribution. Analytical data of this type, although not directly satisfying the needs of the recommended EC definition, can theoretically be converted mathematically into a number size distribution provided that a number of assumptions and simplifications can be applied. Amongst these commonly available methods are Laser Diffraction (LD), Dynamic Light Scattering (DLS) and Centrifugal Liquid Sedimentation (CLS), all of which are already commonly used in the pigment industry. Given the already wide use of these methods in industry, it was considered a useful exercise to undertake a study to evaluate their capabilities and limitations in determining the nano-status of a range of 8 widely different but industrially relevant powder pigments. Although all samples were of pigmentary origin the wide diversity of their physico-chemical properties provides an excellent range of properties with which to test the general effectiveness, suitability and reliability of the techniques, in particular, the various pigments were characterised by different primary particle sizes (from several nm to several hundred nm), different levels of aggregation/agglomeration of the primary particles, and different primary particle shapes (needle-shaped, platelets and more spherical forms). In addition, both inorganic and organic substances were tested as well as one pigment with an applied surface coating.

Samples were also examined using electron microscopy (EM) and the surface area was assessed using the BET (Brunauer, Emmett, Teller) nitrogen adsorption method. The former is essentially considered as the reference since it is the most common technique providing direct measurement of particle size and number. Unfortunately it is not a technique which is seen by industry as desirable for routine assessment of material status versus the proposed EC definition due to its limited availability in industry, technical complexity, high operating costs and low throughput. It should also be noted that EM is not totally devoid of difficulties and careful consideration is still required in terms of sample preparation. Additionally, problems with image analysis may occur since EM is a two dimensional technique applied to a three dimensional problem. In the absence of clear guidelines regarding some aspects of the definition, and taking into account these inherent difficulties associated with sample preparation, image interpretation and counting procedures, the size parameters reported by the manufacturers were those they considered most appropriate. BET is mentioned in the EC-definition (2) as a proxy technique for identifying a nanomaterial in terms of a Volume Specific Surface Area (VSSA) limit of 60 m^2/cm^3; however the technique has limited applicability as it can only be used on powdered samples which, if porous or surface coated, or of complex shape, may produce misleading results. The threshold of 60 m^2/cm^3 corresponds to the theoretical VSSA for a sample based on particles that are spherical in shape and have a diameter of 100 nm. According to the recommended EC definition BET cannot be used to classify a material as “non-nanomaterial”.

In considering the results of this study it is important to note that the aim was not to determine the optimum measurement conditions for every individual sample but rather to produce and evaluate representative data which may be obtained within industry using existing instrumental facilities operated by experienced but not specialised operators. In particular the following points are to be considered:

1) In most cases the instrument operators were trained in the correct operation but not specialised in assessing number size distributions.
2) Instruments were operated according to best practice but with no particular optimisation of instrument or software parameters for the samples analysed.

3) Software was operated according to best practice using physico-chemical parameters supplied by the pigment manufacturer but with no particular optimisation for the data treatment of samples analysed.
4) For sample preparation of the hydrophilic materials a single standardised procedure was defined and followed wherever technically possible. A single alternative method for the hydrophobic materials was also defined.

In this work each of the eight industrial partners provided the study with a single type of pigment material considered to be representative of one of their commercial products. Sub-samples of each of these different materials were then analysed by all the participants in the comparison exercise. For those instrumental methods requiring the introduction of material-specific physico-chemical properties such as refractive index or density, a global table was assembled using input from the producers of each material. In this way it was possible to compare the results obtainable using constant, manufacturer-specified materials properties, similar types of instrumentation and preparation procedures.

The main aim of this study was to make a basic evaluation of the above-mentioned instrumental methods of measuring particle size distributions as required for assessing material status against the EC Recommendation for the definition on nanomaterials. In particular the study was conducted to assess what can reasonably be achieved using the facilities and knowledge available in the research and development or quality control laboratories within the pigments industry. The overall results have been evaluated with particular consideration being given to the following points:

1) The level of inter-laboratory reproducibility and uniformity of results achievable using the simplified procedures adopted in the study.
2) The extent to which the measured data for each material compares with the results expected by its producer.
3) Based on the results obtained for each material can a simple unambiguous classification according to the EC Recommendation on the definition be made with the types of readily available technology and generic sample preparation procedures used in this study?

In considering the eventual conclusions to be drawn from available data it should be noted that this study concentrated only on those (non-EM) methods which are most commonly available in the pigment Industry and that no attempt was made to compensate for any intrinsic limitations of these methods by using alternative complimentary techniques.

2 Methods and materials used in the study

2.1 Summary of materials studied

All samples were of pigmentary origin and taken together provide a wide diversity of physico-chemical properties with which to test the general effectiveness, suitability and reliability of the particle sizing techniques, in particular they included:

- different primary particle sizes – (i) samples essentially 100% 'nanosized', (ii) samples with particles that were mostly outside the nano size range and (iii) samples with a significant fraction of particles in the nano size range and a significant fraction with all dimensions above 100 nm;
- different levels of aggregation / agglomeration of primary particles;
- different shapes – including needles, platelets and more spherical forms;
- both inorganic and organic substances;
- both uncoated pigments and pigments with surface coatings / treatments.

Tables 2.1 and 2.2 summarise the pigment designation, supplier and a brief technical description of the materials as supplied by the manufacturers.

Table 2-1 Manufacturer-supplied description of the pigments types 1-4

Supplier	Pigment Identifier	Description
Itaca	**Al-Co-Blue**	The pigment Al-Co-Blue is a cobalt aluminate spinel identified with the EINECS Number 310-193-6 and with CAS Number 1345-16-0. This substance is identified in the Colour Index Constitution Number, C.I. 77346. According to the Colour Index, this pigment is produced at high temperature from a mixture of oxides of cobalt and aluminium in varying amounts to form a crystalline matrix of spinel. Its composition may include any one or a combination of the modifiers MgO, ZnO, Li_2O or TiO_2.
Clariant	**Pigment Yellow 83 Transparent**	Pigment Yellow 83 transparent is a transparent, reddish yellow organic pigment, which is mainly used in printing inks, plastics and industrial coatings. In terms of particle size Pigment Yellow 83 transparent is one of the finest organic pigments commercially available. According to a Transmission Electron Microscopic (TEM) evaluation more than 99% (number) of the primary particles have a size below 100 nm. The pigment is strongly agglomerated/aggregated. The dispersibility especially in aqueous media is very poor. The powder pigment contains below 1% (number) of free particles < 100 nm which was measured with a combination of Laser Diffraction (LD) with a Scanning Mobility Particle Sizer (SMPS).
Lanxess	**Pigment Red 101**	The pigment red 101 sample examined is an example of an iron oxide red pigment used for a wide variety of applications in building materials, paint and plastics. Its main characteristics are an elevated scattering and henceforth a high hiding power. There are a number of industrially applied methods for the synthesis of iron oxide pigments: the Laux process, the precipitation method as well as the Penniman process. The specimen in question was produced by the Laux process. No surface treatment is applied. According to TEM and SEM there are few particles below 200 nm and above 700 nm. A relatively narrow distribution of the particle sizes is necessary to produce an efficient colorant. It is a pigment that is highly agglomerated as supplied, but fairly easy to disperse in the application and in aqueous solutions.
BASF	**Pigment Yellow 42**	The material with Colour Index “Pigment Yellow 42” is a high purity iron oxide hydrate. Its strong UV absorption increases the weather resistance of binders and mixed pigments. It finds use in automotive, architectural and further applications. This product challenges measurement methods by the small size of the primary particles, which are below 100 nm in all dimensions, and also by its needle shape that strongly deviates from sphericity.

Table 2-2 Manufacturer-supplied description of the pigments types 5-8

Supplier	Pigment Identifier	Description
Kronos	**Rutile Sample**	KRONOS 2360 is a white titanium dioxide pigment specifically developed for use in high–performance coatings. The titanium dioxide core is produced by the chloride process and coated with a shell of silicon and aluminium oxides by the most modern surface treatment technology based on the “Dense Skin” method. It is equally suited to both waterborne and solvent based systems, providing ultimate hiding power and tint strength compared to similar pigment types. It also has very good dispersibility that enables production of high gloss coatings with little gloss haze. The good optical properties are supported by the particle size distribution containing only small amounts on particles less than 200 nm or greater than 600 nm. Nevertheless by using intensive dispersing procedures finer particles may occur.
Huntsman	**Anatase Sample**	This is an uncoated high purity titanium dioxide white pigment product optimised for use in fibres applications. It is of the anatase crystal form manufactured using the sulphate route process. Anatase products typically have a smaller primary particle size than the rutile crystal form and this sample was deliberately selected to represent the smallest pigmentary product available and thereby challenge the capability of the techniques to classify correctly according to the definition. The product has a mean primary particle size of approximately 130 nm based on measurement using electron microscopy.
Eckart	**Pigment Metal 2**	Pigment Metal 2 is a platelet-shaped copper/zinc alloy ("gold bronze") pigment. Pigment Metal 2 is manufactured in a variety of particle sizes. The product measured in the present study is one of the finest available. The main uses of Pigment Metal 2 are printing inks, coatings and plastics.
Evonik	**Fumed (pyrogenic) SiO_2**	Evonik Fumed (pyrogenic) SiO_2 is a white, fluffy, synthetic amorphous silica of very high purity. It is a hydrophilic fumed (pyrogenic) silica with a specific surface area of ca. 200 m^2/g. Since its development 70 years ago it is used in many applications such as silicone rubber, paints and coatings, adhesives & sealants, printing inks, polyester resins as well as in pharma, food, cosmetic and personal care products. This fumed (pyrogenic) oxide is produced in the flame hydrolysis, so-called AEROSIL® process using a silicon chloride compound, hydrogen and air as raw materials. It is hydrophilic and built up from primary particles formed in-situ which are strongly aggregated and further agglomerated in the powder state. It is important to understand that this nanostructured material* consists of aggregates and agglomerates and that there are practically no individual primary/constituent particles, neither in the dry powder nor in a dispersed state. * Definition of nanostructured material from ISO/TS 80004-4:2011(en) Nanotechnologies — Vocabulary — Part 4: Nanostructured materials https://www.iso.org/obp/ui/#iso:std:iso:ts:80004:-4:ed-1:v1:en:term:2.1

2.2 Measurement techniques, instruments and physical material parameters used

The method names are harmonised with reference [3] while abbreviations are listed immediately after the table of contents. The instrumental methods described in this section have been applied to study the previously described pigment materials. Details of the specific instruments used by the participant laboratories are included in Table 2-3 while the relevant physico-chemical material parameters are listed in Tables 2-4 and 2-5. All tables are presented at the end of the section.

LD, DLS and CLS have some characteristics in common which affect the measured results:

- all three measurement methods start from different types of raw data (volume, scattering intensity or light/X-ray extinction respectively) which must then be converted to a number distribution using mathematical algorithms – this can result in potentially large errors as discussed in more detail later in this report;
- the evaluation of particle size commonly assumes the presence of spherical particles – significant deviation from spherical geometry can render the result invalid;
- because these techniques cannot measure constituent particles within aggregates or agglomerates the presence of these will render the results invalid for assessment against the EC definition, especially for classification as “non-nanomaterial”;
- dispersions must be highly diluted to avoid errors due to multiple light scattering (DLS) or streaming (CLS).

2.2.1 LD – Laser Diffraction

The laser diffraction method is arguably the most widespread particle-sizing technique used in the pigment and filler industry. It is based on Fraunhofer diffraction, with correction for Mie scattering for the lower range of particle sizes below approximately 2 to 3 µm.

Particles suspended in a transparent medium are illuminated by a laser beam. The diffraction pattern (scattered light as a function of angle) is measured by a series of photodetectors. Large particles scatter the light mainly in the forward direction, small particles at larger angles. With a suitable fitting algorithm, the particle size distribution is adjusted so that calculated and observed scattering intensities agree. To avoid errors from multiple scattering events the sample in the measuring cell should scatter light only weakly and therefore usually requires dilution. Liquids are diluted with water or solvent. Powders can be suspended in a suitable liquid and generally need to be de-agglomerated by ultrasonication.

LD is a quick and easy method to apply since LD instruments give a particle size distribution from one single measurement. Knowledge of refractive indices and absorption coefficients are critical. The lower cut off level for size measurement is more restricted than for the other techniques, being in the range 10 nm to 50 nm depending on the instrument used. The sensitivity to particles in the range below 100 nm depends on the index of refraction of the substance in question but is relatively low since the technique is optimised for particles of 500 nm and above. Care has to be taken if the calculation of the number distribution includes a significant proportion of particles in the lower part of the instrument measurement range – poor sensitivity of detection in this range may make it difficult to distinguish between samples which may actually contain significantly different amounts of particles in the sub-100 nm range. In addition conversion of volume-based to number-based distributions can introduce large errors. Therefore LD can only be used for assessment of nanomaterial status if it is unequivocally demonstrated that there are insignificant numbers of particles outside the optimum working range of the instrument. This generally requires the use of other methods.

LD instruments typically offer an operational range from a minimum size of approximately 20 nm up to 2000 µm. Several instruments take advantage of the wavelength dependence of the scattering of light and use different light sources at different wavelengths for the measurements. The range of wavelengths corresponds roughly to the visual range.

The formula describing the scattering of light by particles correlates it with their volume based size distribution rather than the desired number-based size distribution. Consequently, to be applicable to the EC nanomaterial definition, this data must be mathematically converted to a number distribution with the related risks for error described in Chapter 8 of this report. In most cases this conversion is based on the assumption that the particles are spherical although sometimes corrections for other particle shapes can be applied by assuming certain aspect ratios.

The wide range of particle size covered by this type of instrument during one measurement is one of the advantages in contrast to the alternative techniques available. In the case of pigments coarse particles can be detected easily outside the range of the main distribution regardless of their density – something which may not be possible by centrifuges or dynamic light scattering due to premature sedimentation.

Another point of note regarding this technique is that it is not restricted to measuring only liquid dispersions but many instruments can be adapted for use directly with dry powders. However, in the case of dry powders, the measurement range may become restricted and in particular the lower size range may be more limited. For the purpose of this report only measurements of liquid dispersions are taken into account, as the nano range is only accessible this way. The measurement of dispersions is typically done in measurement chambers with continuous pumping. This ensures the virtual elimination of sedimentation and to a certain extent prevents flocculation. There is in most cases also the possibility to ultrasonicate the dispersion. On the downside it has to be checked whether artefacts like air or cavity bubbles are introduced by these methods. This can be achieved by variations on the pump speed and intensity of the ultrasonification.

For evaluation of the particle size distribution from measurements in the size range of pigments and fillers the correct index of refraction has to be used. This quantity is given as a complex number, the real component describing the phase velocity, the imaginary component describing the absorption. As pigments usually show a strong absorption at the wavelengths used for the measurement, the application of the correct index of refraction is mandatory. This may cause some difficulties as the correct values are not easily obtained in some cases. In case of uncertainty concerning the correctness of the values their influence on the size measurement must be checked by deliberately introducing small deviations in the real as well as the imaginary component of the index of refraction and evaluating the resulting changes in the calculated sizes. A more detailed description of the effect of index of refraction on the results is given in section 5.2.

Instruments based on laser diffraction, like many other particle sizing methods, cannot distinguish between single (primary/constituent) particles and agglomerates/aggregates. For the purposes of measurements with respect to the recommended EC definition of nanomaterial, it must be established that the samples being measured consist of approximately spherical primary/constituent particles that are neither aggregated nor agglomerated, and that there are practically no particles present with dimensions outside the accurate working range of the instrument. This statement applies equally to DLS and CLS as discussed later.

2.2.2 DLS – Dynamic Light Scattering

The sample, a suspension of particles in a liquid medium, is illuminated with coherent light from a laser. Brownian motion of the particles causes fluctuations in the interference pattern of the light scattered by the particles. The smaller the particles, the faster are the fluctuations in the scattered light intensity. In most DLS instruments, the autocorrelation function of the fluctuations is recorded as a function of time, after which it is analysed mathematically to determine an average diffusion coefficient for the particles (cumulants method). This diffusion coefficient can be used to calculate an average diameter of the particles via the Stokes-Einstein relationship, together with an estimate of the width of the size distribution. More advanced techniques, such as the Contin method, can be used to obtain a size distribution but are more prone to artefacts. Samples with strong scattering are normally diluted before measurement but there are also special techniques for measuring concentrated samples. Dynamic light scattering is a widely used technique for sub-micrometre particles and it thus complements laser diffraction. Its useful range for size measurement is from about 1 nm (below in some cases) to several micrometres.

The data output from a DLS, in its standardised form, is not a particle size distribution, but a scattering-intensity-weighted average value. A well-known difficulty of DLS measurements is that the results are strongly biased in the presence of a small fraction of large particles. This is due to the fact that the intensity of the scattered light is inversely proportional to the sixth power of the radius of the nanoparticle. Thus a 50 nm particle will scatter 10^6 times as much light as a 5 nm particle. As a result, a small fraction of large particles may completely mask a large fraction of smaller particles. DLS does not distinguish between constituent particles and aggregates/agglomerates. It simply gives information about all diffusing ensembles, regardless of whether they are individual particles, agglomerates or aggregates. Even if samples are dispersed fully into their constituent particles, for size measurements related to material classification under the EC definition, these constituent particles need to be approximately spherical, since DLS cannot provide reliable and accurate dimensional information for constituent particles with complex shapes.

DLS does perform well when dealing with monodisperse samples of suspended, approximately spherical, primary nanoparticles with a known refractive index and size well within the instrumental operating range, providing they scatter light effectively and are in a suitable range of concentrations - high enough to give sufficient measurement signal but not so high as to produce excessive absorption or multiple scattering of the light. In addition to the measurement of the scattered light the final calculation of the hydrodynamic diameter requires a knowledge of the temperature and viscosity of the medium.

Attempts have been made to also produce DLS-based particle size distributions. To account for polydispersity in a measured sample, the autocorrelation function must be fitted by a sum of functions, each corresponding to monodisperse nanoparticles of the same properties. Each function is used to capture the contribution of the particles from a particular size range to the signal measured. Even if specific algorithms have been developed, the fitting is a mathematical challenge since it contains more unknowns than equations. The results of the fitting procedure are strongly algorithm and fitting parameter dependent. In these conditions, DLS can give misleading results; for example it will not resolve particles of sizes that differ by less than a factor of 3 in size. Also, it is only possible to convert the scattering-intensity–weighted size distributions to the required number size distribution in the case of spherical particles with a known refractive index. For polydisperse samples where the intensity of light scattered from populations of different sizes may vary by many orders of magnitude, conversion of intensity-weighted size distributions to number-based size distributions may introduce major errors.

With regard to the EC Recommendation it is important therefore to underline that DLS cannot determine the size of the constituent particles of aggregates or agglomerates, the presence of which will render results invalid for the purposes of implementing the definition. It cannot deal effectively with non-spherical particles, and in case of polydispersity it is very likely to give erroneous results for number size distributions. DLS reports scattering-intensity–weighted results. Conversion to number-weighted results requires strong assumptions about material shape and dispersity, which are usually not fulfilled in real samples.

DLS could be improved for the implementation of the EC definition, for non-aggregated/non-agglomerated spherical primary particles, if the measurement step is preceded by a fractionation step in which particles of different sizes are separated into a series of narrower size fractions. In these conditions of measuring almost monodisperse material the DLS cumulants method can give more reliable measured values.

2.2.3 Centrifugal Liquid Sedimentation (CLS) using Disc Sedimentation instrumentation

Centrifugal Liquid Sedimentation (CLS) operates on the principle of separating particles by size using centrifugal sedimentation in a liquid medium. Overall, the term CLS can refer to methods based on the use of instrument of analytical ultracentrifuge

In the disc-sedimentation version of CLS the instrumentation is based on a hollow transparent rotating disc containing a liquid in which the particles undergo forced sedimentation through centrifugal force. By the use of suitable detectors it is possible to measure the speed of this sedimentation within the rotating disc and (assuming constant particle density) use this information to calculate a particle size distribution.

The basic technique exists in two main forms known as line-start and homogenous start (HOST) depending on the way samples are introduced in to the instrument. In this study four laboratories operated instruments designed for line start operation (CPS DC2000 and CPS DC24000) while the remaining two performed homogenous start measurements (Brookhaven XDC).

In the version of the CLS method more correctly known as “Line-start CLS”, particles are injected into the centre of a liquid medium contained within an optically clear, rotating disc. This minimises settling/sedimentation at the start of the measurement run. In order to stabilise sedimentation and avoid streaming, the spin fluid should have a small density gradient. Once the particles enter the rotating liquid media they sediment radially outwards at a speed which is a function of their density and Stokes diameter. At a certain point in time the particles pass through a narrow beam of light which shines through a region near the outside edge of the rotating disc. As the particles pass through the light beam, the amount of light transmitted to the detector decreases due to absorption and scattering by the particles.

The determination of particle size is based on the sedimentation speed which is then used to determine the equivalent Stokes diameter of the particle assuming spherical geometry. To establish the relationship between sedimentation time and particle size each sample analysis is preceded by a calibration run performed using a narrowly distributed size standard. The variation in light intensity is continuously recorded as a function of sedimentation time and at the end of the experiment converted by the operating software into a mass/volume based particle size distribution.

In the alternative method, homogeneous start, the instrument disc is firstly loaded with a homogenous solution of the particles which begin to sediment once the disc is spun. By measuring the temporal variation in the particle concentration at either a fixed point across the disk or at variable points along the radius of the disk with a scanning detector it is possible to determine the sedimentation velocity of the particles which can then be used to calculate a particle size distribution. The particle detection may be based on attenuation of an optical beam as previously described or alternatively by measuring the decrease in intensity of a weak beam of x-rays depending on the instrument used.

In some instruments equipped with optical detectors their design and software permit the operation in either mode while others are dedicated to one or the other of the two variants. In those instruments where both methods are possible the line start method provides the highest resolution in terms of particle size at the expense of longer analysis times while the HOST method may have inferior resolution but provides faster analysis time. The use of x-ray detection has the advantage of providing a more direct measure of particle concentration then the optical system but has the disadvantage that it is poorly adapted to use with organic or polymeric particles due to their low absorption coefficients for x-rays.

For a more detailed description of the basic principles of the CLS methods reference may be made to ISO 13318-1:2001 [7] while the different available CLS methods are described in ISO 13318-2:2007 [8] and ISO 13318-3:2004 [9].

CLS has the potential advantage over LD and DLS that it provides a degree of size separation (based on the application of centrifugal force) prior to particle detection. Thus problems associated with the measured signal from large particles dominating the signal from the smallest particles are reduced but the risk of errors from the conversion to number-based size distributions is still a weakness of the technique if the relative concentration of smaller particles is too low. Furthermore for CLS, as for LD and DLS, aggregates/agglomerates cannot be distinguished from primary particles and achieving a valid result for classification against the EC definition relies on achieving complete dispersion into constituent particles as part of the sample preparation. It should also be noted that the applicability of CLS, even when used with fully dispersed samples is limited by the smallest size of particle which can be sedimented by the particular instrument being used. This limit depends, in part, on the maximum centrifugal force achievable by the instrument but also on the relative density of the particles themselves. In practice high density particles such as gold can be analysed even with diameters as small as 3-4 nm while low density material such as SiO_2 may not be separable unless they have a minimum size in the range 15 nm to 20 nm. This technical limitation means that CLS may be applied for valid classification against the EC definition only when verification by other methods has been done to ensure that no significant numbers of particles below this limit are present.

CLS is performed in liquids and this limits the use of CLS to the analysis of particles which do not dissolve in the analysis media. Also, the particles generally need to be of a higher density than the liquid which can be a problem for organic particles although some manufacturers have proposed technical solutions to this limitation. In addition, the density of the particles must be known and be uniform, which may pose a problem for particles with relatively thick surface coatings, for porous particles and irregular particles such as aggregates and agglomerates where the liquid penetrates the particle.

In theory, CLS can deal with polydispersity, because the sedimentation process naturally results in a fractionation of particles of different sizes. However, the dynamic range (1:50 to 1:70)) in a single measurement cycle imposes some limitations on the ability to deal with polydisperse materials with large particle size ranges: for materials with a broad size distribution it is difficult to select a rotational velocity that is suitable for both the larger and the smaller particles. This problem, although general to the technique, can be addressed using variable speed centrifugation or by making several measurements at different speeds in static mode as proposed by some instrument manufacturers.

Deviation of particle shape from a spherical or equiaxial shape is another problem as the measured diameters are based on the assumption of a spherical shape. The sedimentation velocity of particles can be influenced by shape, potentially leading to over- or under-estimations of the size. Furthermore, the use of CLS instruments in which detection is based on light absorption/scattering may be problematic as conversion of the optical signal to a quantified mass/volume/number of particles depends on accurately knowing the light scattering properties of the particles as a function of size. In the case of spherical particles this relationship is generally well understood and can be mathematically modelled with sufficient accuracy for quantification, but the use of this technology with non-spherical particles may introduce errors which are not easily evaluated. Furthermore, for non-spherical particles CLS will determine a mean hydrodynamic size which is not appropriate for particle classification with respect to the recommended EC definition of nanomaterial.

Although a very few laboratories have performed a full validation of the disc-centrifuge as a technique to measure the sizes of nano-materials, these studies have been limited to spherical particles only [6]. In the case of materials where near spherical geometry cannot be assumed it is often not possible to reliably assign an estimation of measurement uncertainties to the measured size values.

In summary, the disc centrifuge technique is relatively simple to apply in the laboratory with a number of different instruments being commercially available at moderate costs comparable with those of DLS and LD. Sample preparation, as with LD and DLS, will depend on the type of sample under investigation and, for implementation of the EC definition, the validity of the results will depend on whether a full dispersion into stable primary particles can be easily achieved. The time required for analysis is strongly dependant on the instrument used (rotation speed), the density of the material being examined and the minimum size to be determined –in practice sedimentation times may be as long as several hours for small (<20nm) low density particles such as silica.

Key technical limitations mean that it unlikely that the CLS method will develop into a general method that can reliably satisfy the EC definitions requirement of measuring the smallest external dimension of particles. However, with improved detection systems, temperature control and increased rotational speeds, CLS may provide method-defined estimates of the apparent Stokes' diameter in increasingly broad size and concentration ranges [3].

2.2.4 BET – Volume Specific Surface Area according to Brunauer, Emmett & Teller

The BET method may be used to determine the specific surface area, in m²/g, of a solid by quantifying the surface adsorption of nitrogen gas at the temperature of liquid nitrogen (according to ISO 9277). There are several equivalent measurement methods available (volumetric or gravimetric, or with different carrier gases). The standard describes also multipoint and single point techniques to calculate the results.

Conversion of the specific surface area to volume specific surface area (VSSA) requires correct knowledge of the material density.

A pre-treatment is necessary to remove the adsorbed water on the particles which would adversely affect the measured specific surface. An example of how this may be achieved is as follows: the sample is firstly held under reduced pressure at 50 °C for 15 minutes after which it is flushed with a flow of dry nitrogen gas for 2 minutes. This procedure is then repeated 3 times before finally cooling the samples to ambient temperature under a flow of dry inert gas ready for testing. The exact pre-treatment required may vary depending on the specific instrument used and the type of material under examination.

Determination of VSSA is identified as a proxy measurement method within the proposed EC definition of nanomaterial [2]. Measurement by BET has the advantage over LD, DLS and CLS (and EM) in that it does not involve dispersion protocols. However, BET analysis is only directly applicable to the measurement of dry powders. For the purpose of assessing materials against the recommended EC definition, aggregation (and to some extent agglomeration) will lead to a reduced VSSA and subsequent overestimation of constituent particle size. In addition, the presence of surface treatments or porosity can alter the relationship between VSSA and particle size, as can non-spherical particle shapes [13]. For polydisperse samples, BET cannot be relied on to deliver an accurate estimate of median particle size except in some very specific cases. However, if a VSSA > 60 m^2/m^3 is determined for a dry powdered material consisting of non-porous, smooth particles, this will often be a reliable indication that the material would also be classified as a nanomaterial according to the particle size criteria of the recommended EC definition.

2.2.5 Electron Microscopy

Electron microscopy (EM) is a technique which uses an electron beam to illuminate a specimen and produce a magnified image. The use of an electron beam rather than light permits the visualisation of objects and structures with dimensions much below that which is physically possible by any optical microscopy technique. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are the most common methods in use, the first being capable of atomic resolution, the latter having a resolution limit of roughly 1 nm. In general, both techniques give a two-dimensional projection of the three-dimensional object/s of interest. When attempting to apply EM methods to the determination of particle size distribution this represents one of the main challenges for extracting appropriate dimensional information. EM based methods have advantages over all the previously discussed methods, in that they can provide information about the size and shape of individual particles and are able (sometimes with some limitations) to distinguish and characterise primary (constituent) particles within larger aggregates/agglomerates (especially high resolution TEM). The major disadvantage of EM methods is their limited availability in industry, the high purchase and running costs and slow throughput.

For SEM analysis, the sample surface is scanned line by line with a focussed electron beam. Part of the beam is scattered back by the sample (backscattering electrons). Also, sample electrons are ejected (secondary electrons). These two signals are registered by special detectors. The resolution of the image obtained from the secondary electrons is somewhat better; the light and shadow effects give a three dimensional impression of the sample surface. The backscattering electrons provide material contrast. The sample surface must be electrically conducting, and samples of non-conductive material may need to be coated in advance with a thin (< 20 nm) gold film. For analysis of materials such as powder pigments, a small amount of material may be deposited onto a conducting surface or else the material may be embedded into a conducting resin that is then polished to obtain a cross section through a large number of particles at random orientations.

For TEM analysis, nanoparticle samples are usually dispersed in a volatile liquid which can be spotted and dried on a special TEM grid that allows transmission of the electron beam through the specimen. Analysis of the shape and size of constituent particles within aggregates/agglomerates is easier to achieve with the higher resolution TEM technique, particularly since aggregation/agglomeration is more likely with primary/constituent particles towards the lower end of the nano size range where the higher resolution of TEM is of great value. With special preparation techniques it may be possible to obtain a reasonably reliable constituent particle size distribution by appropriate evaluation of a series of TEM images, although the choice of which objects constitute “primary/constituent” particles may be rather subjective. Evaluation of constituent particle size is only practical with SEM if the particles are large enough and well separated in the electron micrographs. Section 3 provides a more in-depth description of particle characterisation with EM methods.

Table 2-3 Instruments[1] used in the study

Participant	Laser Diffraction (LD)	Dynamic Light Scattering (DLS)	Centrifugal Liquid Sedimentation (CLS)	Specific Surface Area Measurement (BET)	Electron Microscopy
Itaca	Malvern Mastersizer 2000	Malvern Zetasizer Nano ZS	---		
Clariant	Malvern Mastersizer 2000	Malvern Zetasizer Nano S	CPS DC 20000	Ströhlein Areameter II	
Lanxess	Coulter LS 13320	---	---		
BASF	Malvern Mastersizer 3000 Coulter LS 13320 (part.)	Nanotrac UPA253	Brookhaven XDC	Micromeritics Tristar (DIN 66131)	FEI, Strata 400 DB
Kronos	Malvern Mastersizer 2000	Malvern Zetasizer 3000	CPS DC 20000	Micromeritcs Tristar Analyzer 3000	FEI, Tecnai 20. LEO 1530 VP
Huntsman	Malvern Mastersizer S	---	Brookhaven XDC	Micrometrics Gemini 2365	JEOL 1200EX
Eckart	Malvern Mastersizer 2000	---	---	BELSORP Mini II, BEL Japan Inc.	
Evonik	Beckman Coulter LS 13320 780 nm Laser	Malvern Zetasizer Nano ZS	CPS DC 24000	Micromeritics Tristar 3000	Hitachi H 7500. 125 kV and JEOL 2010 F, 200 kV (incl. EDX)
JRC-IHCP		Malvern Zetasizer Nano Z S	CPS DC 24000		

(1) Disclaimer: The instruments noted in Table 2-3 are mentioned for technical information only and do not constitute a recommendation by either JRC or the European Commission

Table 2-4 List of physical material properties* used for LD/DLS/CLS measurements

Sample No	1	2	3	4	5	6	7	8
Producer Pigment Identifier	**Itaca Al-Co-Blue**	**Clariant Pigment Yellow 83 transparent**	**Lanxess Pigment Red 101**	**BASF Pigment Yellow 42**	**Kronos Rutile Sample**	**Huntsman Anatase Sample**	**Eckart Pigment Metal 2**	**Evonik Industries Fumed(pyrogenic) SiO2**
Particle Density [g/cm³]	4.26	1.46	5	3.7	4.15	3.84	7.6	2.2**
Particle Absorption (λ1)	0.1	0.52	0.9 (450 nm)	0.1	0.08 (633 nm)		2.5 (488 nm)	0.01
Particle Absorption (λ2)	0.1	0.03	0.17 (600 nm)		0.08 (450 nm)		2.6 (514.5 nm)	
Particle Absorption (λ3)			0.05 (780 nm)				3.4 (632 nm)	
Particle Absorption (λ4)			0.05 (900 nm)					
Particle Absorption (λ5)			----					
Index of refraction (λ1)	1.74	1.78	2.8 (450 nm)	2.3	2.741 (633 nm)	2.55	1.14 (488 nm)	1.45
Index of refraction (λ2)	1.74	1.75	2.9 (600 nm)		2.96 (450 nm)		1.12 (514.5 nm)	
Index of refraction (λ3)			2.65 (780 nm)				0.25 (632 nm)	
Index of refraction (λ4)			2.8 (900 nm)					
Index of refraction ((λ5)								
Liquid Viscosity (Temperature)	0..8872 cP (25ºC)	0..8872 cP (25ºC)	0..8872 cP (25ºC)	0..8872 cP (25ºC)	0.8872 [mPas] (25°C)	0.8872 cP (25ºC)		0.8872 (25°C)

* Data supplied by material manufacturer

** It should be noted that this material is highly structured and branched, consisting of aggregates of primary particles. Therefore the specific material density of 2.2 g/cm³ does not provide the appropriate density value for the measurement of the aggregate dimensions. In fact, publications and calculation have shown that the filling volume is approximately 25 – 30 %; thereby the density value to be used should be considerably lower.

Table 2-5 List of experimental parameters reported for centrifugal liquid sedimentation (CLS)

Partner No.	**2**	**4**	**5**	**6**	**8**	**9**
	Clariant	**BASF**	**Kronos**	**Huntsman**	**Evonik Industries**	**JRC**
Liquid Gradient Used	---	None (H2O)	8%-24% sucrose	Water	8%-24% sucrose	8%-24% sucrose
Liquid Density/ (g/cm³)	---	0.997	1.045	0.998	1.045	1.045
Refractive Index (Liquid)	---	Not required	1.344	1.344	1.344	1.344
Liquid Viscosity/(cP)	---	0.89	1.2	0.990;0.978;0.955	1.2	1.2

2.3 Sample preparation protocol

2.3.1 Factors relevant to the design of an effective dispersion procedure

The dispersion procedure is a pivotal step in the process of making measurements of the particle size distribution since the three primary techniques under assessment (LD, DLS and CLS) require the generation of a stable dispersion of particles. It is therefore necessary to outline the basic concepts leading to the definition of a proper procedure.

With respect to the EC definition, it is important to note that without confirmed de-agglomeration into constituent particles (which must be approximately spherical and proven to be completely within the operating range of the instrument used), and verification of the absence of persistent aggregates, the results of LD, DLS or CLS cannot be considered as a valid measurement of constituent particle sizes.

In the industrial applications of pigments and fillers a broad variety of different dispersion techniques are used, e. g. pearl mills, roll mills, extruders, high speed stirrers etc. As these many variations cannot be taken into account, and in any case they may not be suitable for sample preparation for LD, DLS or CLS, in the current study a compromise method had to be agreed upon. Ultrasonic dispersion is the standard technique used in many laboratories due to the ease of applicability to a very wide range of particulate materials. The details of the method must be fixed taking into account the basic demands for the dispersion of pigments and fillers. The dispersion process can be described as consisting of several steps:

- Wetting: The solvent must come into intimate contact with the surface of the particle and air must be displaced.
- De-agglomeration: The breaking up of agglomerates by the application of externally supplied energy.
- Stabilisation: Establishing a repulsive force between the particles to avoid re-agglomeration and maintain the dispersion state achieved.

It has to be verified for each examined specimen whether a wetting agent is needed and whether water as the simplest solvent can be used. It must be determined whether the dispersion obtained is sufficiently stable to ensure a reproducible measurement. This must be done taking into consideration the requirements of the measurement instrument being used. Additives used to achieve dispersion and stability need to be appropriate to the surface chemistry of the sample and in particular whether the substance is hydrophilic or hydrophobic. Filtration of samples (33mm diameter PVDF syringe filter with 0.45 µm pore-size) to remove very large particles which might dominate the measured results was attempted but proved unsuccessful due to blocking of the filters. The input of energy by the dispersing equipment is determined by two factors: the probe power and the time. For measurements aimed at determining whether a material is a nanomaterial according to the EC definition, the objective of a dispersing procedure is to fully break up all of the agglomerates and to achieve a stable suspension of free constituent particles.

It is important to examine the effect of both the probe power and the time on the particle size measurement results. Typically the extent of agglomeration will vary in an exponential manner with both time and power tending towards a stable value (Figures 2-1 and 2-2).

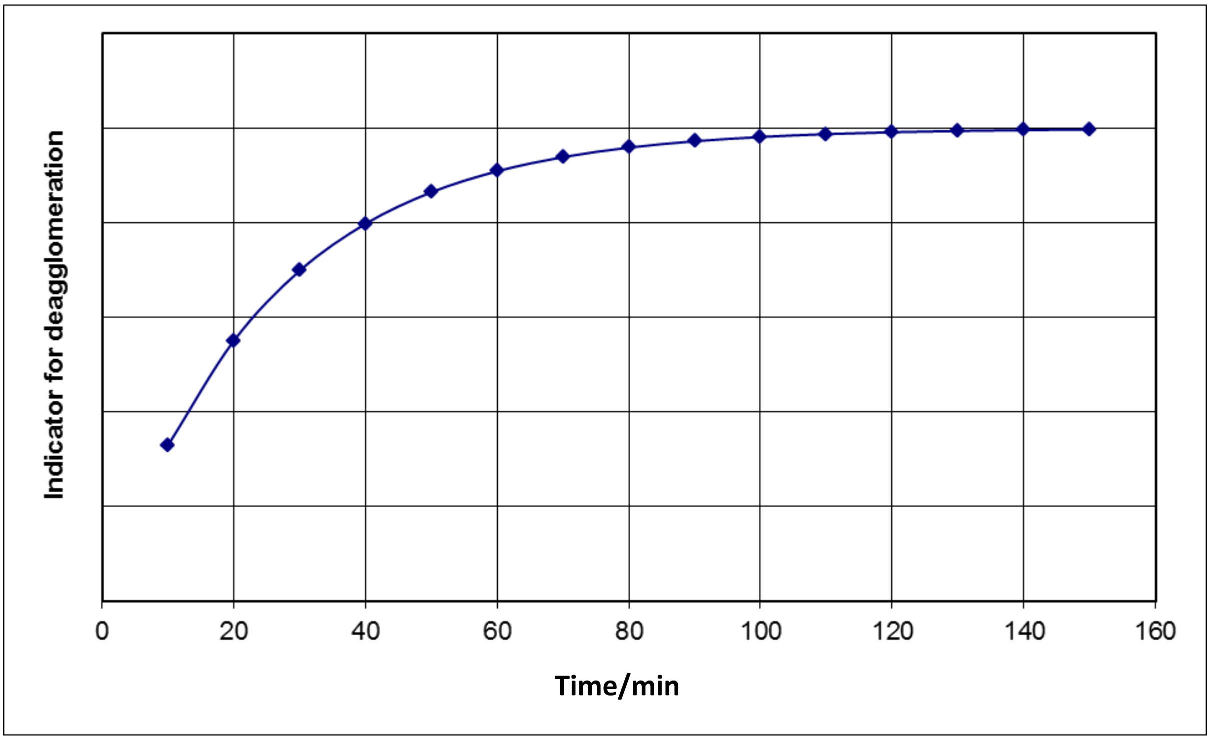


Figure 2-1 Effect of sonication time on de-agglomeration

For a reproducible particle size measurement it is necessary to choose the power and time to maximise the dissipation of agglomerates as seen in the region of the graph where the gradient is shallow.

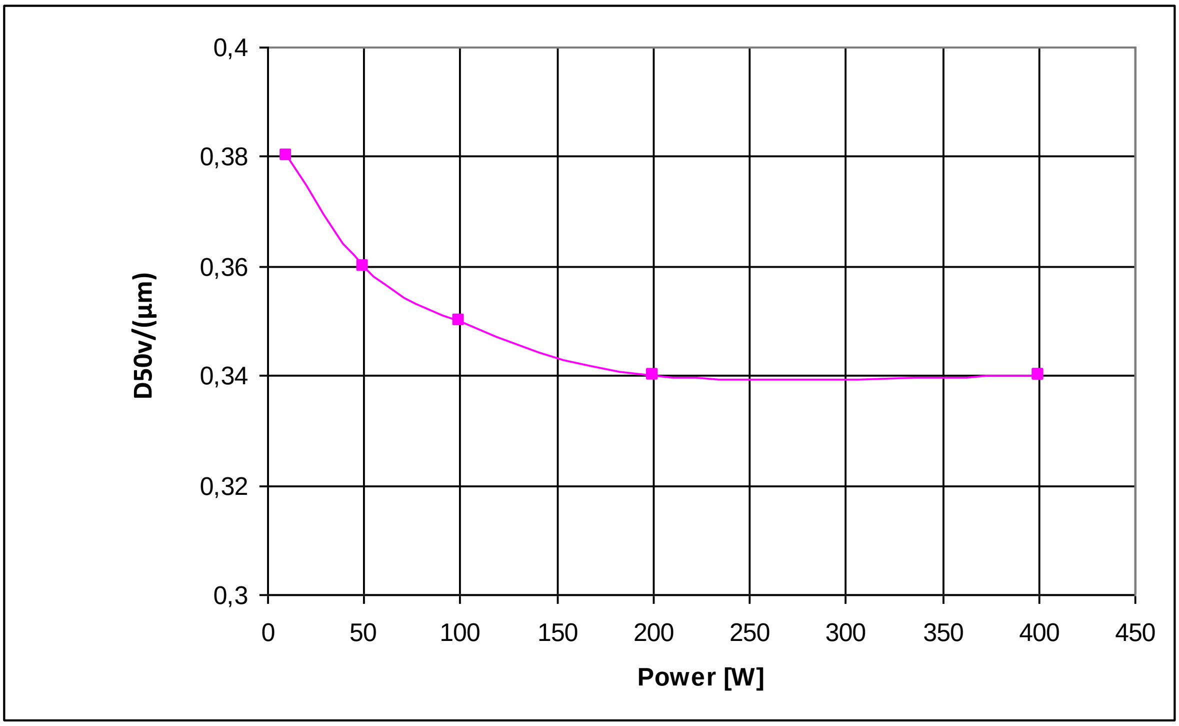


Figure 2-2 Effect of sonication power input on de-agglomeration

The probe power is the principal factor in determining whether the agglomerates can be separated into the primary particles. Insufficient power will not only lead to a misleading particle size distribution but also to a worse reproducibility as the inevitable variations with different types of dispersing equipment will produce very different final results. The treatment time has to be chosen in a way that all agglomerates present can be broken up. It is important to note that insufficient power input cannot be substituted by dispersing for longer times.

Excessive power also has to be avoided as it not only causes excessive wear at the equipment but also might result in artefacts by introducing particulate contamination from the ultrasonic probe. Additionally, some materials can show partial re-agglomeration with excessive ultrasonic energy input. To minimise this possibility it is recommended that particle size measurement be made on dispersions across a range of sonication powers and times so as to determine the conditions necessary to achieve minimum stable particle size measurement values i.e. that neither an increase of power nor more time leads to a significant change in the particle size detected. In order to verify whether the dispersion procedure has resulted in a stable suspension of constituent particles, a comparison of the results of the size determination with the constituent particle size range expected from TEM analysis may be employed.

If the above noted basic principles are taken into consideration, and verification of full de-agglomeration and absence of aggregates is carried out, then meaningful results might sometimes be obtainable for specific sample types even when using different instruments and dispersion techniques which differ in some details.

Normally the industry uses dispersion equipment which operates by treating the products to high shear forces (e.g. pearl mills) in order to reach the optimal dispersion status in the respective application. Nevertheless it has to be mentioned that for certain materials (e.g. some organic pigments) the use of other dispersion techniques with an extremely high power may result in a destruction of even the "primary particles", although this will not normally be the case for sonication techniques. In industrial processes this is strictly avoided due to the fact that in these cases the intended application properties would be lost. If full de-agglomeration cannot be achieved without destruction of the primary/constituent particles then, for classification against the EC definition, constituent particle size distributions have to be determined using other techniques.

2.3.2 Methods used

In this study, the original intent was to adopt a single, identical dispersion protocol for all eight different materials but in practice this could not be achieved due to the widely varying physico-chemical properties of the test materials. Based on the experience of the materials manufacturers it was possible to adopt two main dispersion protocols, the first for seven of the materials and the second for the poorly water dispersible Pigment Yellow 83 by Clariant. It should be noted that some partners had to further adapt these basic protocols to make them compatible with their own specific instruments and techniques.

All samples (except Pigment Yellow 83 transparent)

1. Take 100 mg powder pigment
2. Add to 50 ml sodium polyphosphate solution (1 g/l)
3. Homogenise with vortex or similar
4. Treat with ultrasonic finger at 85% maximum instrument power for 3 minutes in ice-bath.

An alternative specific sample preparation for Pigment Yellow 83 transparent was necessary due to the poor dispersibility of the pigment in water.

Pigment Yellow 83 by Clariant

1. Take 20 mg powder pigment
2. Add 5 ml ethanol as wetting agent
3. Add mix to 20 ml Nekal BX (0.5 g/l)
4. Treat 10 minutes with ultrasonic finger at 85% maximum instrument power with ice bath cooling.

2.3.3 Deviations and problems

The previously detailed dispersion procedures were initially adopted as standard for all the laboratories but in practice some laboratories found it necessary to adopt modified dispersion procedures for a limited number of materials. Any such modifications were done only when necessary to ensure for the correct operation of the particular instrumentation available in the partner laboratory. The main modifications to the dispersion protocols were as follows.

Partner Evonik for preparation of fumed (pyrogenic) SiO_2 in laboratory

1. Take 750 mg powder pigment
2. Add to 15 ml water
3. Treat with ultrasonic finger for 15 minutes with ice-bath cooling.

Partners Clariant and Eckart for preparation of Pigment Metal 2

1. Take 20 mg powder pigment
2. Add 5 ml of ethanol as wetting agent
3. Add to 20 ml of Nekal BX (0.5 g/L)
4. Treat with ultrasonic finger for 10 minutes with ice-bath cooling.

A specific instrument related problem was encountered by one partner when attempting to analyse Pigment Yellow 83 transparent prepared by the standard dispersion protocol using a LD instrument equipped with a sample recirculation pump. In this case the use of the Nekal BX led to an increased foaming tendency of the dispersion which, in combination with equipment specific parameters may have resulted in problems with the measurability. At the time of the study one partner did not have access to an ultrasonic finger but used an ultrasonic vial tweeter whose dispersion performance has been found to be similar to that of a typical 200 W probe sonicator equipped with immersion probe.

3 Descriptive summary of electron microscopy investigations

In this study only a limited number of partners had direct access to EM facilities and so measurements using this family of techniques were not obligatorily undertaken as part of the comparative study. Instead, EM data previously acquired by the producers of each material were made available and served as a benchmark against which the results of the other measurement methods could be compared. It must be stressed that for particle size determination EM is not devoid of difficulties and careful consideration is required for sample preparation. Fundamental issues may arise since EM is a two dimensional technique applied to a three dimensional problem, thus image interpretation and particle counting may often be rather subjective. Taking this into account, and in the absence of clear guidelines regarding some basic aspects of the recommended EC definition, the size parameters reported by the manufacturers were those they considered most appropriate. The following section will firstly provide a brief introduction to the use of EM for particle characterisation before presenting a summary Table 3-2 of the EM data supplied by the individual producers of each type of pigment.

3.1 General remarks regarding particle characterisation with EM

In general, both SEM and TEM give a two-dimensional projection of the three-dimensional object/s of interest. This two-dimensional projection is the origin of the main challenges involved with the method for particle size measurement, which vary in complexity depending on the properties of the material/(nano-)objects being examined. A few examples of the more common cases are briefly discussed below.

Quasi-Spherical Particles: The case of quasi-spherical (nano-)objects is the one which represents the easiest case in particle-sizing. Their projection is similar, independent of the viewing direction.

Straight Rods: Rods have one distinguished (long) dimension and two short dimensions. The length of the projection depends on the elevation angle of the axial direction to the beam direction and the projection plane.[6]

Platelets: Platelets, e.g. circular discs, have one short and two long dimensions. As for the rods, the size and shape of the projection depend on the elevation angle of the plate-normal direction towards the beam. In the simplified case of the circular disc, the long axis of the projected ellipse corresponds to the diameter of the disc, whereas the short axis depends on the elevation angle. **Arbitrary Shapes**: For objects with arbitrary shapes, the sizing with electron microscopy can in general give useful results, yet the set of parameters evaluated needs to be chosen appropriately. This is discussed below.

Aggregates and Agglomerates: In the EC recommended definition the nanoparticle size distribution must consider constituent (primary) particles even in the case where these form part of larger aggregates and agglomerates. For these entities EM based methods are critical as none of the common optical or

---

[6] In the general case the angle between the beam direction and the projection plane is 90 degrees, so that no additional considerations need to be taken into account and will not be discussed in this document.

centrifugal methods can distinguish them from single particles. Taking into account the great variation of shapes and the associated projections, a given size-distribution based on methods of electron microscopy needs to be accompanied by a set of representative images that allows the quality of the dataset to be judged.

3.2 Strategies for the identification of constituent/primary particles

One of the main challenges in relation to the EC definition is the identification of individual particles for sizing – consideration must be given not only to free primary particles but also the constituent particles of aggregates and agglomerates. A number of different strategies, each with advantages and disadvantages, are in use. The methods described in the following paragraphs are, to the best of our knowledge, the most common.

Strategy based of the availability of an "Ideal" sample: this strategy assumes that the material under examination has been prepared by methods which produce samples composed of well distributed and isolated constituent particles. When applied to such ideal samples both SEM and TEM can produce images well adapted to the use of computer based image analysis for automated particle detection. Such software is able to identify, characterise and count the individual objects (particles) based on the contrast between background and particle. This method offers the great advantage that user based decisions are minimised and a maximum reproducibility is achieved. However, automated detection requires a subsequent image filtering in many cases, which can either be based on grey-values or shape. The filtering process can involve the separation of adjacent objects or the discrimination of suboptimal detected objects. In all cases, but especially in cases of automated detection and subsequent filtering, adequate statistics must be achieved with counting of several thousand particles being crucial. With a fully automated process this can be done with reasonably low effort. The main source of errors with this strategy is introduced by an insufficient contrast for the automated detection and by overlapping particles but in an ideal sample, the number of suboptimal detected particles should be irrelevant.

Counting strategy for "Non-Ideal" samples: If an ideal sample is not available, automated procedures for particles sizing are not suitable. Therefore, the particles need to be detected manually, with the operator deciding where the object boundaries are located and using a suitable interface to feed them into the evaluation software. Constituent particle sizing of non-ideal samples with SEM is generally not possible, and high-resolution TEM is required. It has to be assured that the operator doing the manual particle counting is well trained so that identification and detection errors are minimised. These may occur for the detection of particle boundaries when the physical separation of individual objects is not sufficient to make them clearly separable on the resulting micrograph images. This can result from inadequate sample preparation, or with agglomerated or aggregated samples. In these cases, it is the decision of the operator as to whether the overlapping objects are counted as individual (separated) objects or as one (single) object.

For determination of a particle size distribution, the minimum number of particles which has to be counted depends on the width of the particle-size distribution, the size binning and the need to ensure that these are considered in a statistically relevant manner. The importance of this issue increases with the inhomogeneity of shape and size of the material under investigation. With respect to material classification against the EC definition, a suitable software package must be set up to produce an appropriate size parameter for the EC definition, and the required number of particles for a reliable decision will depend on how close the median value of this parameter is to the 100 nm threshold.

Influence of Sample preparation: Samples may be directly deposited onto an appropriate support or prepared as a cross-section. It needs to be noted that, at this time, no universally accepted or validated methods exist for either the counting procedures or for the sample preparation methods.

Direct deposition onto a support: The objects are placed on a commercially available carbon, SiO_2, or Si_3N_4 support film (TEM-Grid) in case of TEM, STEM[7] or TSEM[8] imaging. In the case of SEM imaging, carbon pads or silicon wafers are commonly used. The sample is either applied from the dry state or from spotting and drying of a drop of dispersion. Samples of anisotropic shape will be found to have a preferred orientation, which may be desired (rods) or not (plates). An example for the preparation of rod-shape (nano-)objects is shown (Figure 3.1) for Pigment Yellow 83. The objects are reasonably well separated, have a sufficient contrast and the axial direction is within the plane of the support film, perpendicular to the beam.

Preparation of a cross-section: An alternative route for preparation is the embedding of the sample. Based on a representative, polished section of the embedded sample, a statistically relevant number of objects can be evaluated.

For sizing based on either type of preparation, a stereological correction of the obtained size distribution may be necessary. The stereological correction may be of minor importance for samples deposited on a support but, depending on the size and shape of the sample, may be of major importance for cross-sections. In general (for equiaxial particles), stereological corrections calculate larger particles sizes than the uncorrected data, as it is unlikely that a cross-section of a particle reveals the relevant diameter or external dimension of the sectioned particle.

A crucial aspect for all preparation methods is that the final sample needs to be truly representative of the starting material. It needs to be ensured that the preparation method does not suppress any size fraction of the material, e.g. due to sedimentation, clustering or mobility reasons.

Size parameters to be assessed: In general terms, in order to achieve comparable and meaningful size distributions for a specific application the measurement applied to evaluate the size distribution needs to be chosen properly. In the case of quasi-spherical objects, the choice of the parameter is of minor importance – ECD ((area-)equivalent circle diameter), area and Feret diameter are all examples which can be used. In practical cases, ECD and Feret diameter are the most commonly chosen ones. Additionally, for

---

[7] Scanning Transmission Electron Microscopy, used with modern TEMs

[8] Transmission Scanning Electron Microscopy, used with modern SEMs

rod-shaped objects, length and aspect ratio are useful, in case of discs, the diameter and thickness. For pigments where the quasi-spherical shape is a reasonable approximation, further information can be deduced from shape parameters, such as roundness, convexity or the shape-factor. These parameters may help to estimate the reliability and error of the size distribution. This is especially relevant to many industrially produced products in which the wide variety of object shapes present mean that no stereological correction is performed.

The choice of the parameter is strongly related to the question of interest, therefore a "best choice" for all materials and for all applications does not exist. In industrial situations, reproducibility and sensitivity of the parameter with respect to variation in the production process may be ranked higher than the absence of minor systematic errors. However, with respect to the recommended EC definition of nanomaterial, the Minimum Feret Diameter (MFD) appears to be the most suitable parameter to be assessed. Specific guidance is required in this respect in order to remove uncertainty and reduce at least one source of lab-to-lab variability of TEM results.

3.3 EM as applied to aggregates/agglomerates - a brief consideration

In the strict application of the recommended EC definition the measured particle size distribution must include not only free single primary particles but also those which are contained in aggregates and agglomerates. When compared to the other methods for measuring particle size distributions EM based methods have, in theory, the advantage of being able to visualise particles either as individual entities or as part of a larger assembly of particles – i.e. aggregates or agglomerates. This ability makes it a powerful tool for application to the recommended EC definition. However, in cases where the analysed sample has very pronounced aggregation/agglomeration it may become impossible to realise the advantages of EM due to the difficulties of analysing complex three dimensional structures using a method which produces a 2 dimensional projection. Recent developments in EM hardware and software have led to the development of Electron Tomography [4], an extension of traditional TEM which is able to produce detailed 3-dimensional representations of complex microscopic structures. Currently this type of technology can achieve a resolution of 5 nm to 20 nm which, although not sufficient for the requirements of the EC definition is likely to improve with future development of EM equipment. Such technology may greatly assist operators in the future for defining and correctly measuring appropriate objects for assessing materials against the EC definition.

3.4 Results obtained with the pigments used in the study

As a result of EM analysis, information on size, shape, homogeneity, aggregation and agglomeration of particulate materials can be obtained. In order to obtain reliable results, a suitable sample preparation and a sophisticated counting procedure are required as described above. If both are available, statistically relevant results based on the 2D projected images of most samples can be obtained. Figures 3-1 and 3-2 show representative EM images of the pigments used for the present study.

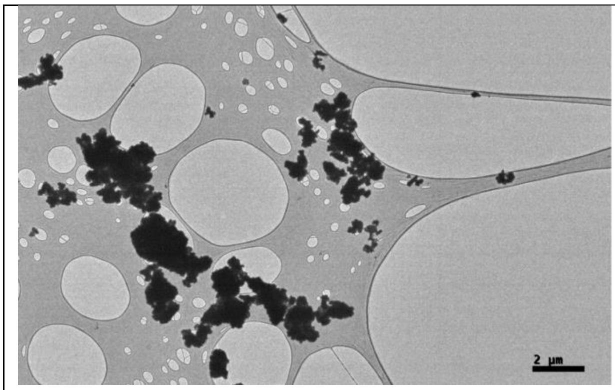


Image 1: Al-Co Blue Pigment (ITACA)

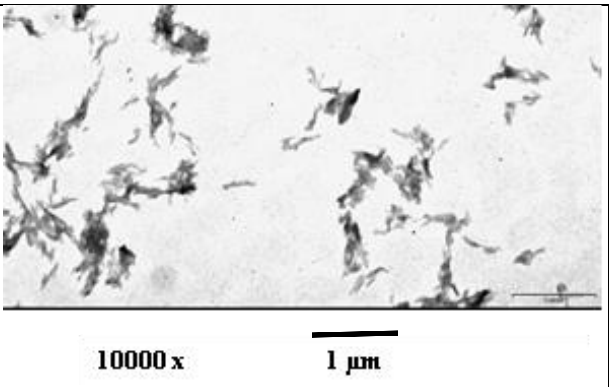


Image 2: Pigment Yellow 83 transparent (Clariant)

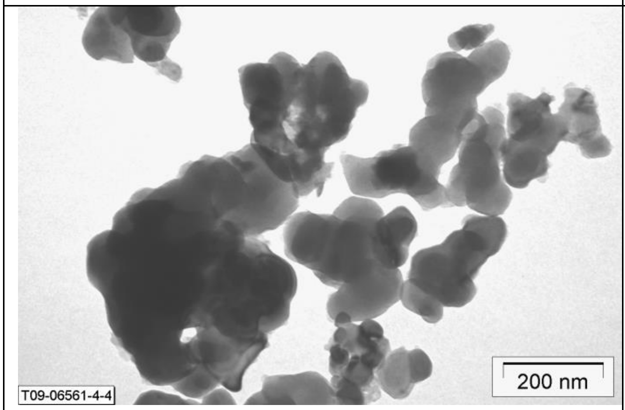


Image 3: Pigment Red 101 (Lanxess)

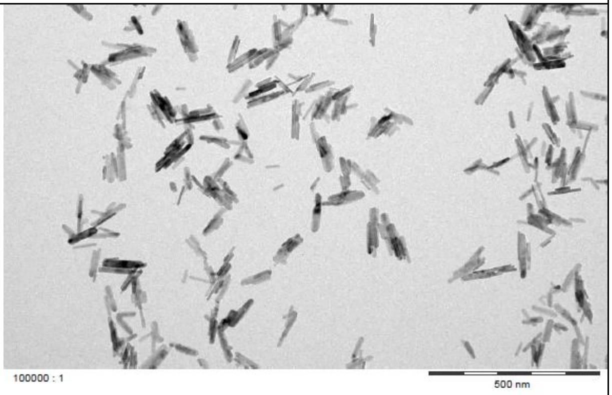


Image 4: Pigment Yellow 42 (BASF)

Figure 3-1 Electron Microscopy images from the investigated pigments 1-4

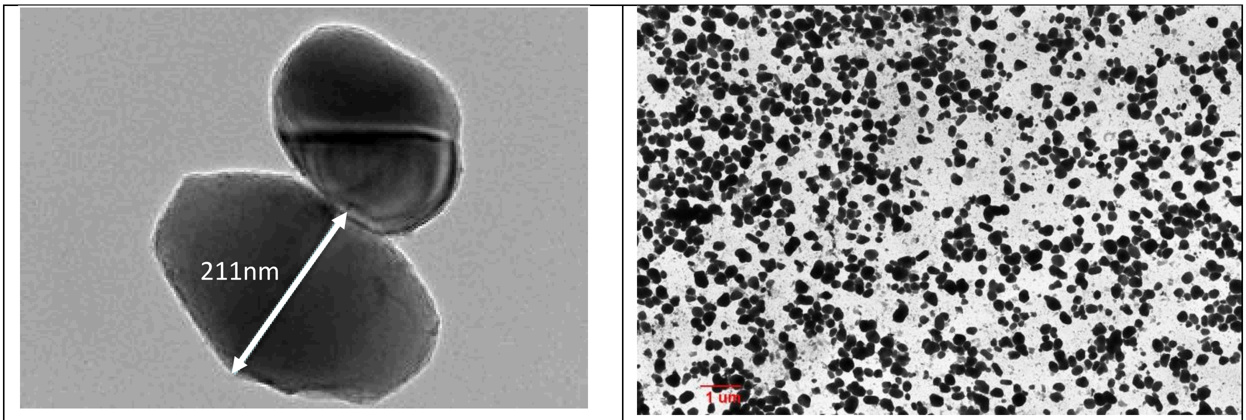


Image 5: Rutile Sample (Kronos)

Image 6: Anatase Sample (Huntsman) 1µm

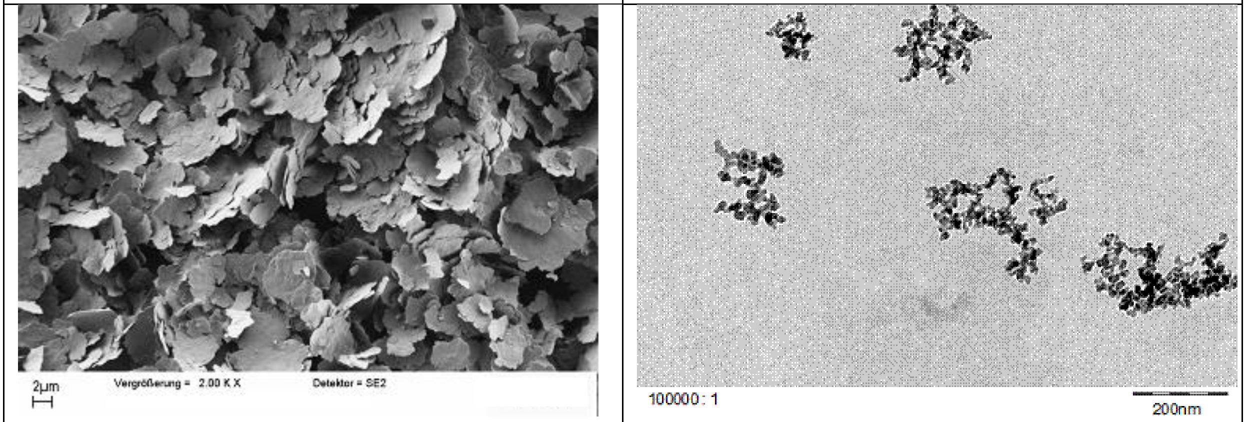


Image 7: Pigment Metal 2 (Eckart)

Image 8: Fumed Silica (Evonik)

Figure 3-2 Electron Microscopy images from the investigated pigments 5-8

A wide variety of sample preparation and sizing/counting methods have been used and each company has used procedures it considered to be appropriate and specific to the product under investigation. Some details are reported in Table 3-1. As a consequence, neither the sample preparation nor the sizing/counting methods can easily be harmonised as one standard procedure or reference method for characterising nanomaterials.

Due to the uncertainties associated with the recommended EC definition and the inherent difficulties with sample preparation and TEM analysis, it can be expected that preparation and investigation of each company's material by other (third-party) laboratories may (in some cases) lead to different size distribution and classification results.

Table 3-1 Summary of the EM sizing/counting methods provided by the participating partners

Company	EM	Method	Measure	Preparation
P2. Clariant	TEM	manual	ECD	dispersion
P3. Lanxess	TEM	not specified	not specified	dispersion
P4. BASF	TEM	not specified	short and long axes	dispersion
P5. KRONOS	SEM	automated	ECD	cross-section
P6. Huntsman	TEM	semi-automated	ECD	dispersion
P7. Eckart	SEM	manual	morphology	dry
P8. Evonik	TEM	Automated(ASTM D3849)	aggregates	dispersion

The development of a set of standards that is suitable to quantify the characteristics of a product by methods of electron microscopy has mostly been driven by the need to provide measures for product quality and customer requests. It is obvious, that these highly specialised and product specific methods of electron microscopy cannot be simply converged in order to match the requirements proposed by the recommended EC definition of nanomaterial. For these requirements, as stated above, it is necessary to establish an agreement on a set of preparation and counting protocols for electron microscopy, which allows reasonable comparability of the results of different companies and also allows reliable third-party testing. It may be expected however that for some industrial materials significant variability of results will remain due to basic difficulties with electron microscopy being used as a technique for constituent particle sizing.

Table 3-2 Manufacturer-supplied descriptions and classifications of the test materials (not validated by any other laboratories)

Material	Producer	Type or composition	Dimensions of primary particles	D50n/nm*	Agglomeration state in solid	Nano-status
1 Al-Co-Blue	Itaca	$CoAl_2O_4$	Not specified	527	Not specified	Non-nano
2 Pigment Yellow 83 transparent	Clariant	Azopigment	>90 % below 100 nm D50n ≈ 50 nm	47	Aggregation/agglomeration	Nano
3 Pigment Red 101	Lanxess	Fe_2O_3	Mean 270 nm, Range 3 nm to 1 µm	249	Aggregation/agglomeration	Non-nano
4 Pigment Yellow 42	BASF	FeOOH	<100 nm all dimensions	20	Aggregation/agglomeration	Nano
5 Rutile Sample	Kronos	TiO_2	D50n ≈ 210 nm	250	Aggregation/agglomeration	Non-nano
6 Anatase Sample	Huntsman	TiO_2	D50n ≈ 130 nm	130	Aggregation/agglomeration	Non-nano
7 Pigment Metal 2	Eckart	Cu/Zn alloy	In-plane diameter ~ 4000 nm	4000**	Aggregation/agglomeration	**
8 Fumed (pyrogenic) silicon dioxide	Evonik Industries	SiO_2	10 nm to 20 nm	12	Aggregation/agglomeration	Nano

* Prior knowledge data based on EM analysis by material manufacturer.

** Electron microscopy studies of this material show it to be platelet in form with the major axis being around 4000 nm. Due to the complexity of the material the thickness of the platelets has not been accurately determined and in the absence of a verified value the nano-status has not been assigned to this material.

4 Descriptive summary of BET data obtained by partners

4.1 The BET Method as applied in the present study

The BET **(**Brunauer, Emmett, Teller) method uses physical adsorption of gas molecules on the solid surface of a material to obtain information about the surface area of that material (according to ISO 9277). Several approaches are possible, such as single point or multipoint measurements. For the present study, the latter was used. Samples were cooled, under vacuum, to cryogenic temperature (77.3 K). Nitrogen (purity 99.999%), as analysis gas, was admitted into the vacuum chamber in controlled increments. After each dose of gas the pressure was allowed to equilibrate. The equilibrated pressure (P) is compared to the saturation pressure (P_0) and their relative pressure ratio (P/P_0) is recorded along with the quantity of gas adsorbed by the sample at each equilibrated pressure within the range of 0.05 to 0.3 P/P_0 (according to ISO 9277). The gas volume adsorbed at each pressure at one constant temperature defines an adsorption isotherm from which the quantity of analysis gas required to form a monolayer over the external surface of the solid and its accessible inner pores (monolayer capacity) is determined by means of the BET equation. With the area covered by each adsorbed gas molecule known, the surface area can be calculated. Before sample measurement starts free space is determined at room temperature and sample temperature (77 K) with Helium 5.6 (Volumetric content more than 99.99 % according to ISO 9277). The saturation pressure of the analysis gas is measured using the P_0 tube where the nitrogen is condensed and the vapour pressure is monitored by a transducer. Samples were prepared for adsorption analysis in a degasser, using flowing inert gas (N_2) passed over a heated (150 °C) sample for 30 min to remove moisture and other contaminations.

The results are reported as (mass) specific surface area S_m in m^2/g. The total measurement uncertainty is typically of the order of +/- 0.5 m^2/g or 1 % of S_m depending on which value is higher.

4.2 Results obtained from BET measurements

All samples of all participants were measured with the same method outlined above.

Table 4-1 BET results for all materials measured by each lab, S_m in m^2/g

	Al-Co-Blue	Pigment Yellow 83 transparent	Pigment Red 101	Pigment Yellow 42	Rutile Sample	Anatase Sample	Pigment Metal 2	Fumed (pyrogenic) SiO2
Itaca	8.1	49.7	9.3	101	14.8	9.7	6.0	190
Clariant	8.0	61	8.0	95.0	15.0	9.0	6.0	215
Lanxess	7.7	60.7	8.1	94.9	14.6	8.9	6.3	215
BASF	8.1	52.5	9.1	85	14.8	9.2	3.6	212
Kronos	7.8	39.7	9.1	89.4	15.4	9.4	5.8	205
Huntsman	7.3	75.9	9.0	74.4	12.9	8.6	3.4	211
Eckart	7.9	55.9	9.1	88.5	15. 7	9.2	3.1	208
Evonik	7.2	74.2	8.7	77.1	15	8.6	3.16	213
Mean	7.8	58.7	8.8	88.2	14.8	9.1	4.7	209
StD	0.3	11.4	0.5	8.6	0.8	0.4	1.4	7.7

The (mass) Specific Surface Area (in m^2/g) and the Volume Specific Surface Area (VSSA, in m^2/m^3) are linked by the density of the material, which was specified by the material manufacturers.

Table 4-2 VSSA values (m^2/cm^3) for all materials measured by each lab

	Al-Co-Blue	Pigment Yellow 83 transparent	Pigment Red 101	Pigment Yellow 42	Rutile Sample	Anatase Sample	Pigment Metal 2	Fumed (pyrogenic) SiO2
Itaca	34.5	72. 6	46.5	375	61.4	37.3	45.6	419
Clariant	34.1	89.0	40.0	352	62.3	34. 6	45.6	473
Lanxess	32.8	88.6	40.7	351	60.6	34.2	48.2	474
BASF	34.5	76.7	45.5	315	61.4	35.3	27.4	466
Kronos	33.2	58.0	45.5	331	63.9	36.1	44.1	450
Huntsman	31.1	110	45.0	275	53.5	33.0	25.8	464
Eckart	33.4	81.6	45.7	328	65.0	35.2	23.7	458
Evonik	30. 7	108	43.5	285	62.35	33.0	24.0	469
Mean	33.04	85.69	43.69	326.38	61.30	34.84	35.55	459.11
StD	1.37	16.60	2.24	31.78	3.23	1.36	10.43	16.88

4.3 A consideration of the VSSA results within the overall inter-laboratory comparison

Three materials gave VSSA results well above the 60 m^2/cm^3 nanomaterial threshold level specified in the EC recommended definition implying that they should be considered as nanomaterials. This is in line with the results from electron microscopy. However the technique would have identified the rutile pigment

K2360 as a nanomaterial which, according to the manufacturer, has a D50n value of 210 nm determined by electron microscopy. The pigment has primary particles coated with oxides of aluminium and silicon which give the pigment a macro-porous shell which results in a higher VSSA value than would be expected in an uncoated material of the same external particle diameter.

It is known that VSSA values alone are not suitable for proving whether the physical particle size distribution in a material should be classified as “nano” or not: porosity and surface roughness, as well as shape, can play an important role which would have be taken into account to avoid incorrect (i.e. false positive) classification. It should also be remembered that, according to the EC definition, VSSA measurements can only be used to identify a nanomaterial and not to classify a material as “non-nanomaterial”. However, this “positive classification” criterion is neither reliable for porous, or partially-porous, structured or surface coated particles, nor for some other cases such as particles with complex shapes.

In fact this method is in general not reliable for either ‘nano’ or ‘non-nano’ particle classification with respect to the EC recommended definition of nanomaterial. If a VSSA greater than 60 m^2/cm^3 is measured, a manufacturer should classify a particulate material as a nanomaterial, and it can be argued that for many simple situations this would be a correct classification. However there are several potential scenarios (especially porous particles or particles with complex shapes) where this positive classification would be shown to be inconsistent with respect to EM measured particle size distributions.

5 Descriptive summary of LD data obtained in the interlaboratory comparison

5.1 Consideration of the results obtained in the interlaboratory comparison

The basic description of the samples renders the Eckart Metal Pigment 2 as well as the BASF Pigment Yellow 42 iron oxide poorly suited to determine their smalllest dimension by LD due to their geometry being platelets or needles respectively. Clariant Pigment Yellow 83 transparent probably has primary particles too small to be measured by the LD method.

In the following sections the D50-values for the volume and number distribution of the various samples will be presented. Obviously erroneous measurements were omitted in case where it would be a hindrance for a clear graphical representation.

5.1.1 D50-values of Al-Co-Blue

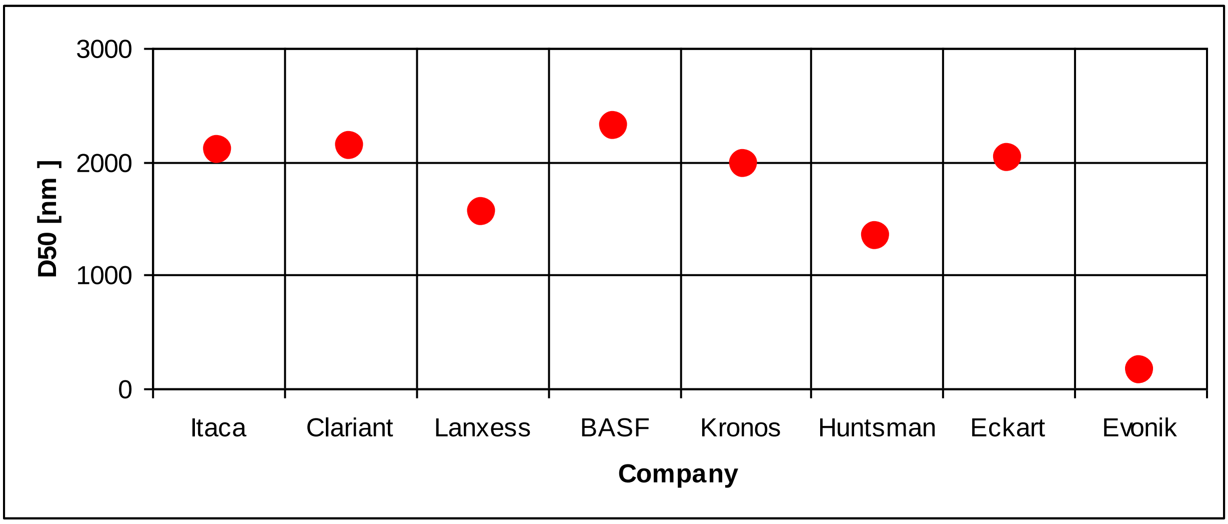


Figure 5-1 Volume-weighted mean particle size D50v of Al-Co-Blue from LD measurements

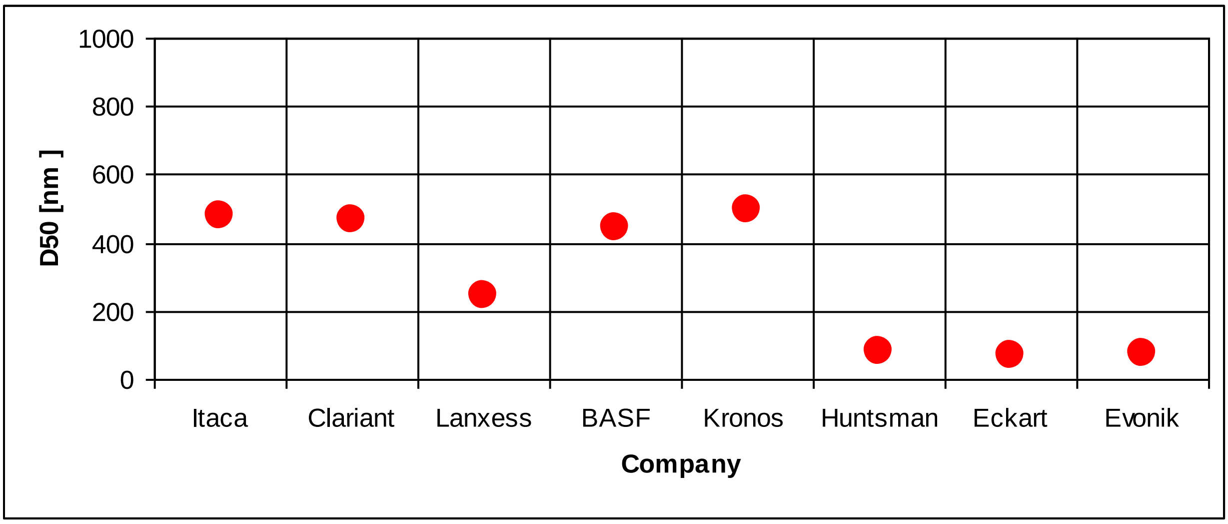


Figure 5-2 Number-weighted mean particle size D50n of Al-Co-Blue from LD measurements

The volume distributions from the different partners (except one) of the Al-Co-blue pigment can be described as being in very rough agreement, though with a notable variability. The much larger disagreement of the results when recalculated to a number distribution shows the effects of the different width of the distribution as well as the difficulties related to the volume to number conversion.

5.1.2 D50-values of Pigment Yellow 42 transparent

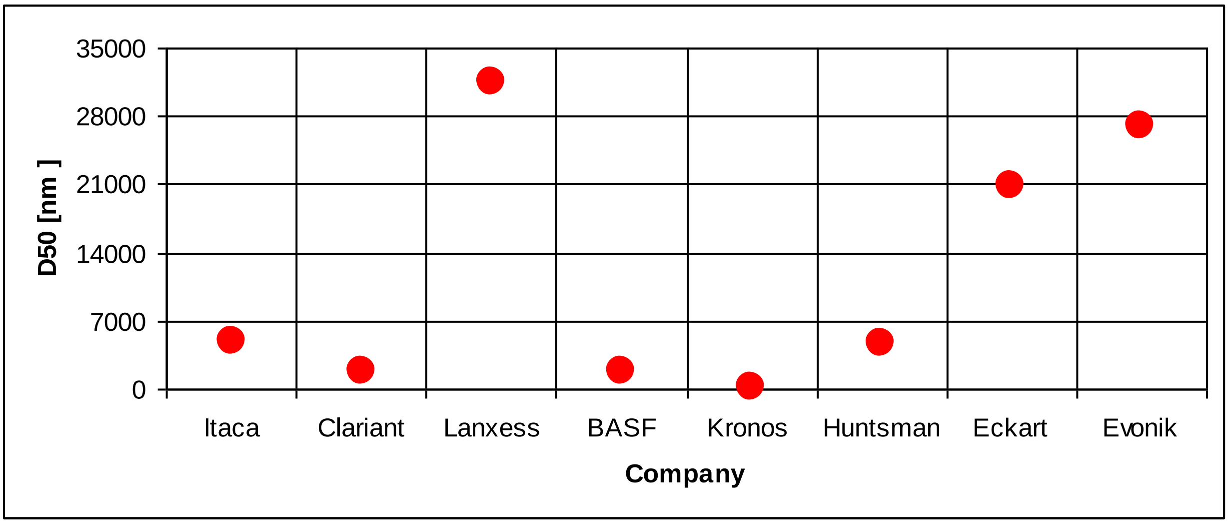


Figure 5-3 Volume-weighted mean particle size D50v of Pigment Yellow 43 transparent from LD measurements

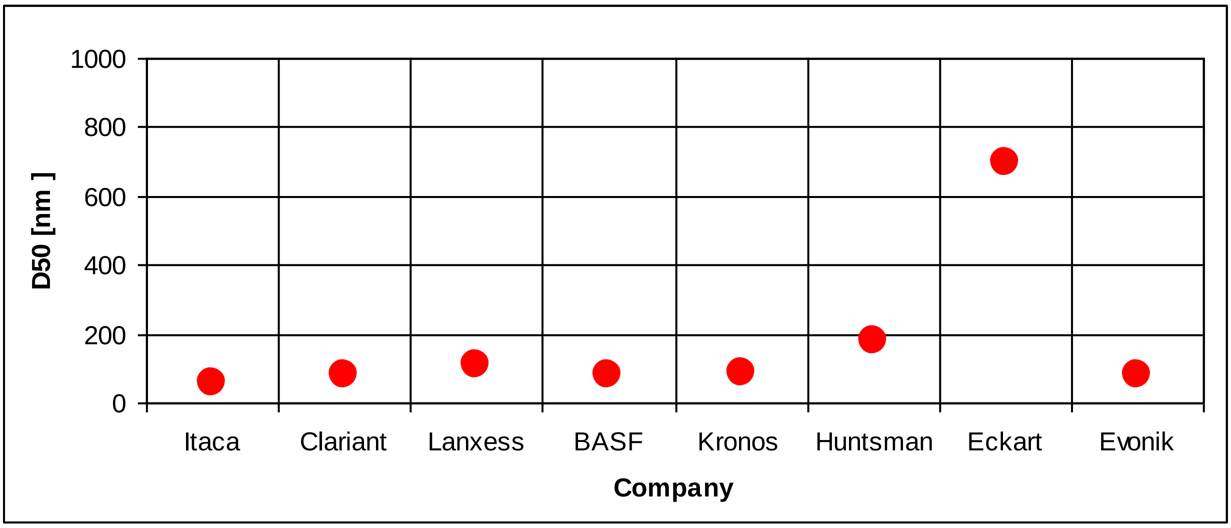


Figure 5-4 Number-weighted mean particle size D50n of Pigment Yellow 42 transparent from LD measurements

The inorganic pigment Yellow 42 is typically described as consisting of needle shaped primary particles with all dimensions smaller than 100 nm. The wide range in reported D50v values may be due to the fact that the instruments are operating at or outside the limit of their ranges and/or dispersion problems. The number distribution will be certainly affected by the low size cut off limit of the instrument.

5.1.3 D50-values of Fumed Silica

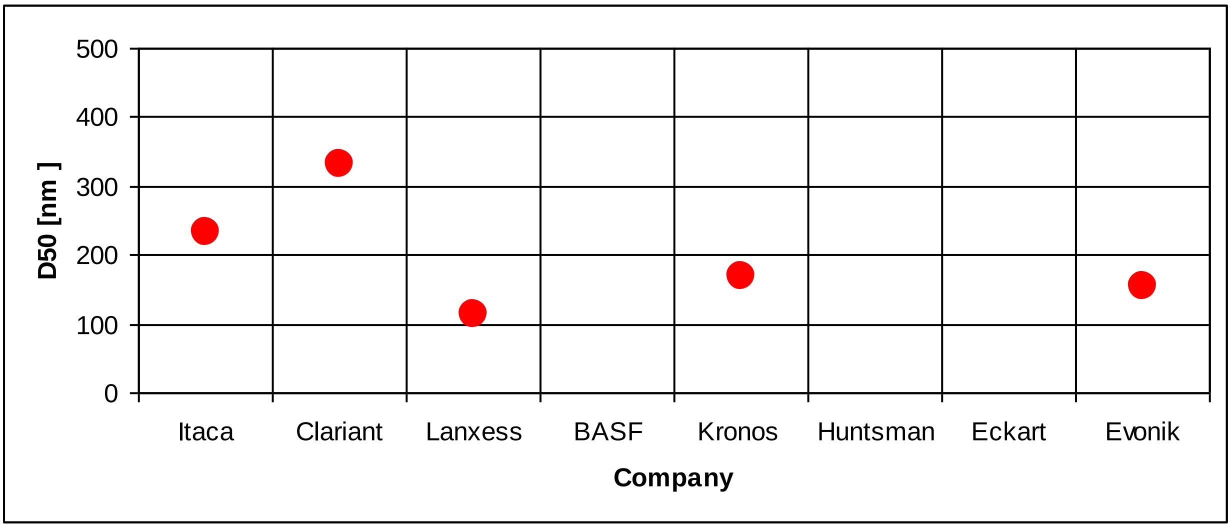


Figure 5-5 Volume-weighted mean particle size D50v of Fumed Silica from LD measurements

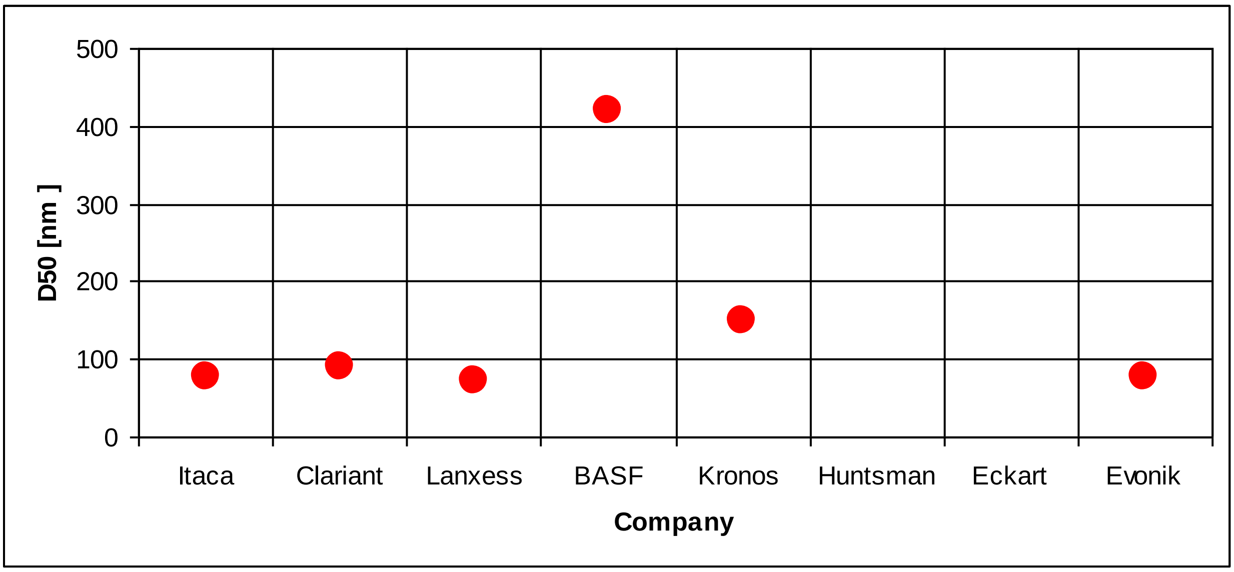


Figure 5-6 Number-weighted mean particle size D50n of Fumed Silica from LD measurements

The fumed pyrogenic silicon dioxide sample is considered to have a mean primary particle size of 12 nm and is highly agglomerated/aggregated. The D50v results from laser diffraction show a broad range of results. LD cannot determine primary particle size and it appears that the instruments were operating outside their practical size range even at the scale of the aggregate/agglomerate size, and additionally achieving a good dispersion has been problematic. This is further illustrated by the Huntsman results which are not shown as the D50 values were off scale for both volume and number distribution values.

5.1.4 D50-values of Anatase Pigment

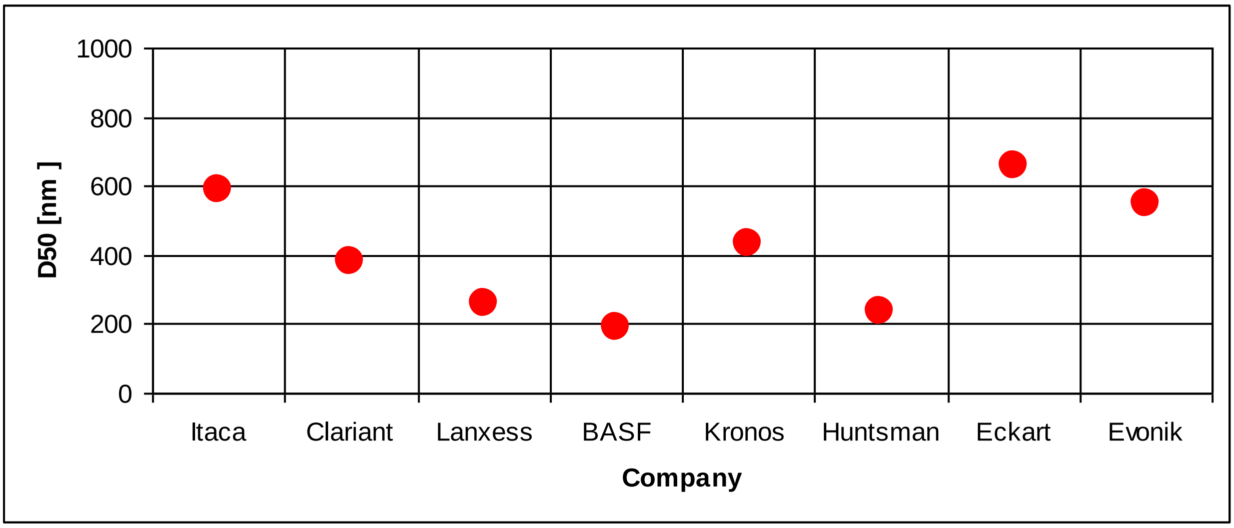


Figure 5-7 Volume-weighted mean particle size D50v of Anatase Pigment from LD measurements

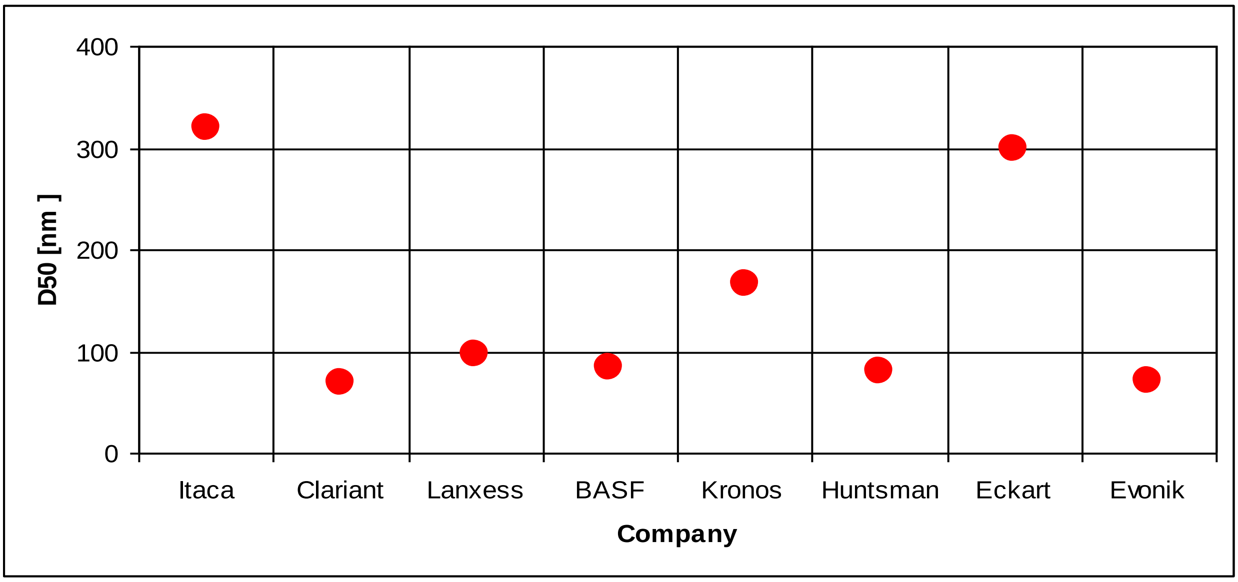


Figure 5-8 Number-weighted mean particle size D50n of Anatase Pigment from LD measurements

The sample is considered to have a mean primary particle size of 130 nm based on electron microscopy (data supplied by the manufacturer). The discrepancies in the volume distribution data between the different participants are bigger than expected especially when compared with those of the rutile sample cited in the Figures 5-9 and 5-10. Assuming that similar dispersion could be achieved for the two titanium dioxide samples the increased variability seen here might indicate that LD instrumentation is operating outside its optimum range. The D50n results are very variable with several participants reporting a value less than 100 nm.

5.1.5 D50-values of Rutile Sample

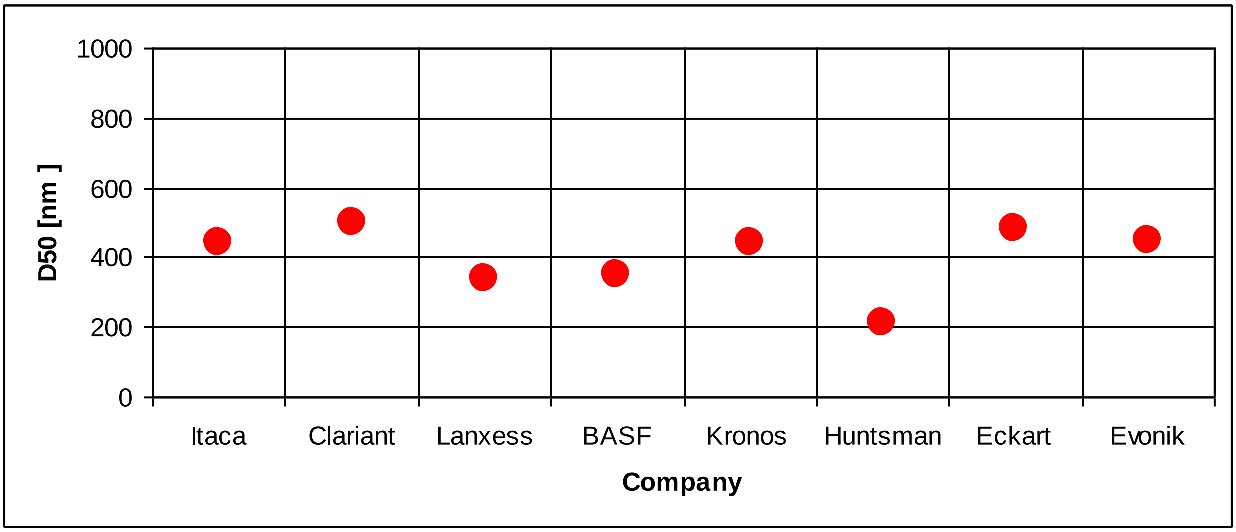


Figure 5-9 Volume-weighted mean particle size D50v of Rutile Sample from LD measurements

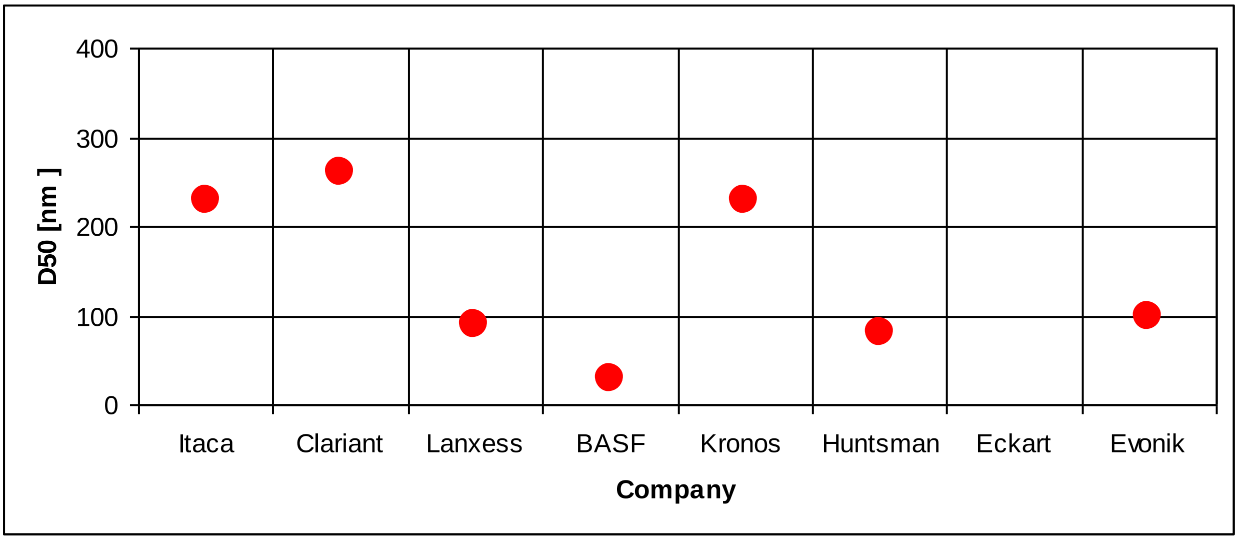


Figure 5-10 Number-weighted mean particle size D50n of rutile sample from LD measurements

The sample is considered to have a mean primary particle size of 210 nm based on electron microscopy (data supplied by the manufacturer). The results for the rutile pigment show a rough correlation concerning the volume distribution data, though considering the instruments were not operating at their lower size limits, the variation between partners is still surprising if it is assumed full dispersion was achieved. The results concerning the number distribution again show that LD is not reliable for material classification against the recommended EC definition even for relatively large and roughly spherical particles.

5.1.6 D50-values of Pigment Red 101

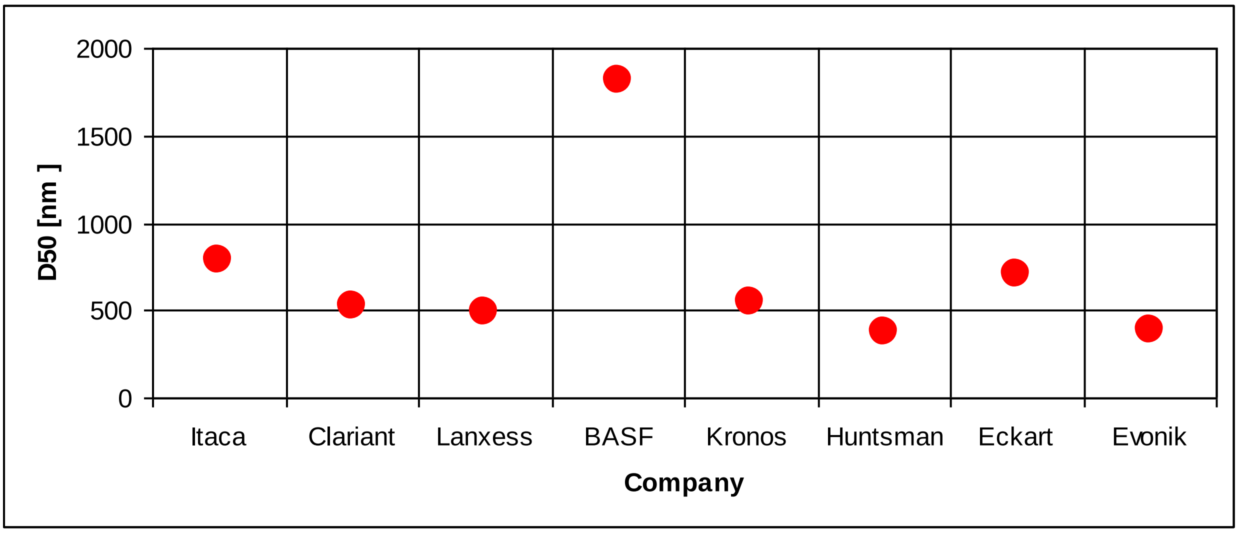


Figure 5-11 Volume-weighted mean particle size D50v of Pigment Red 101 from LD measurements

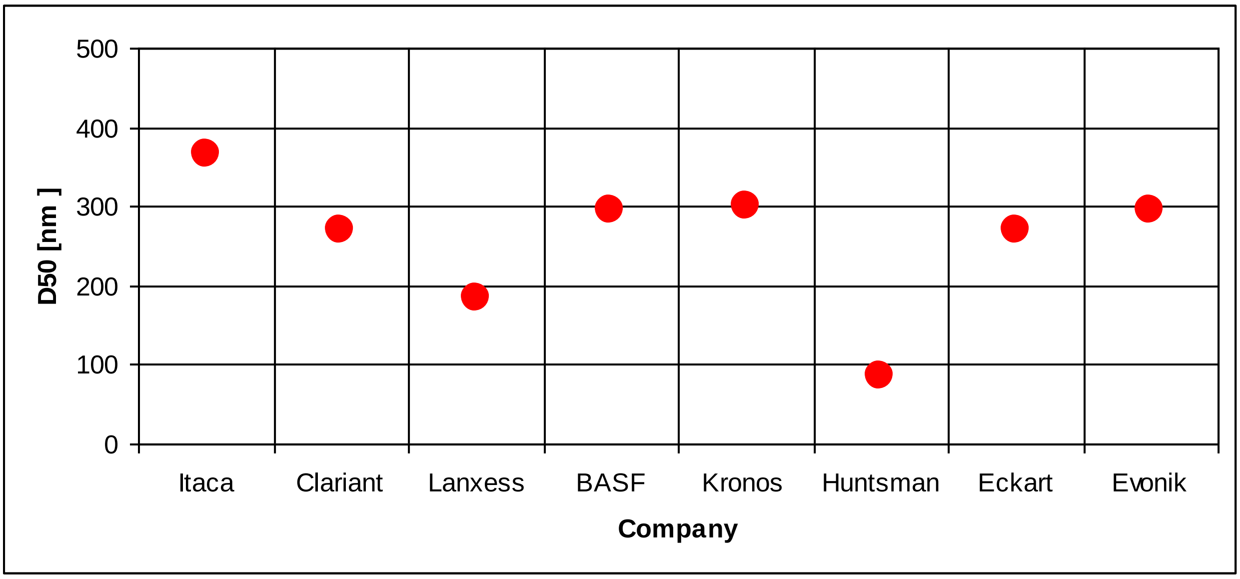


Figure 5-12 Number-weighted mean particle size D50n of Pigment Red 101 from LD measurements

Pigment Red 101 is considered to have a mean primary particle size of 250 nm based on electron microscopy (data supplied by the manufacturer). In this case a rough correlation of D50v (though with a factor of 2 between lowest and highest values) between all the laboratories except one is observed, the choice of optical constants used being especially important (see section 5.2) in this case. The instruments were not operating close to the limit of detection and though the average of the D50n values correlates quite well with the mean size specified by the manufacturer, there is a variation by a factor of 4 between partners.

5.1.7 D50-values of Pigment Metal 2

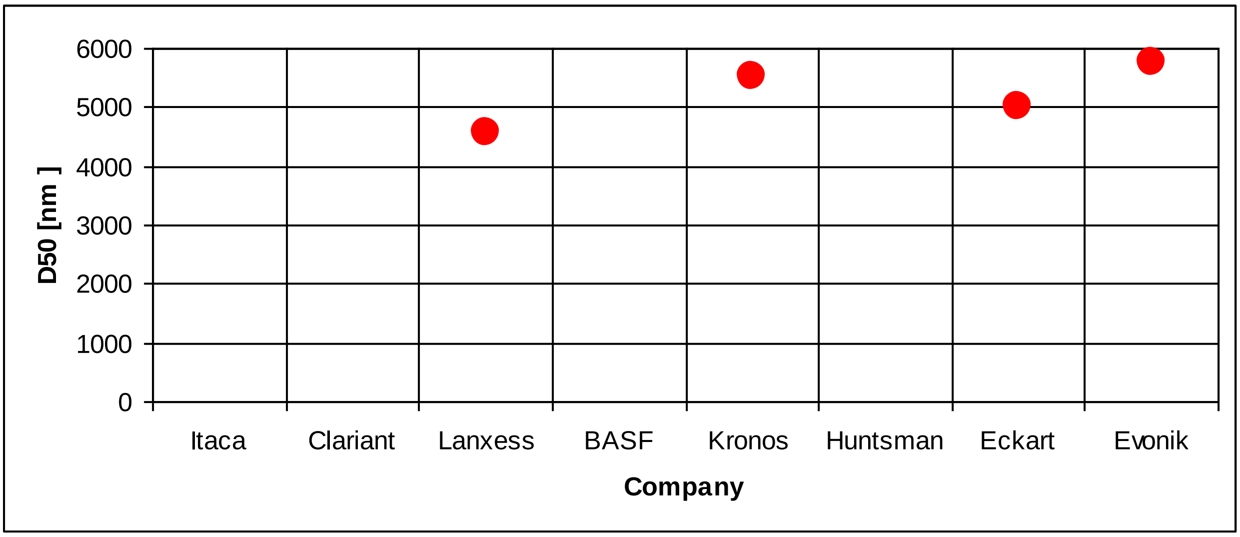


Figure 5-13 Volume-weighted mean particle size D50v of Pigment Metal 2 from LD measurements

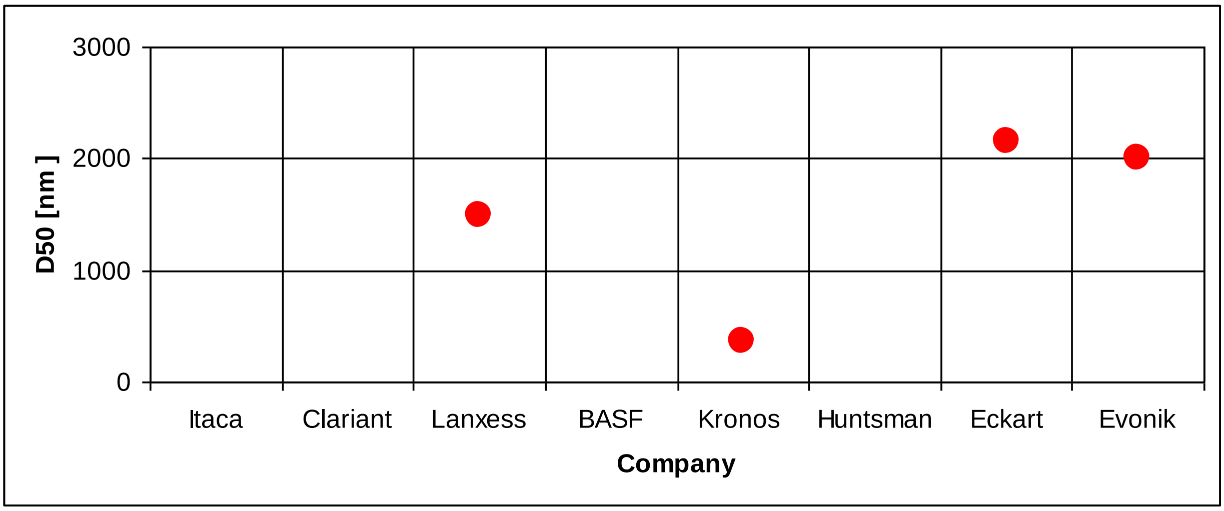


Figure 5-14 Number-weighted mean particle size D50n of Pigment Metal 2 from LD measurements

Electron microscopy studies of this material show it to be platelet in form with the major axis being around 4000 nm. Due to the complexity of the material the thickness of the platelets has not been accurately determined and so no verified value can be quoted at this time.

The Pigment Metal 2, being platelet in form, is poorly adapted to measurements based on laser diffraction and most other optical instrumental techniques. The result of the volume distribution seems to correlate approximately with the length of the particle. Several of the partners experienced sample preparation problems with this unusually shaped product and were unable to report meaningful results.

5.1.8 D50-values of Pigment Yellow 83 transparent

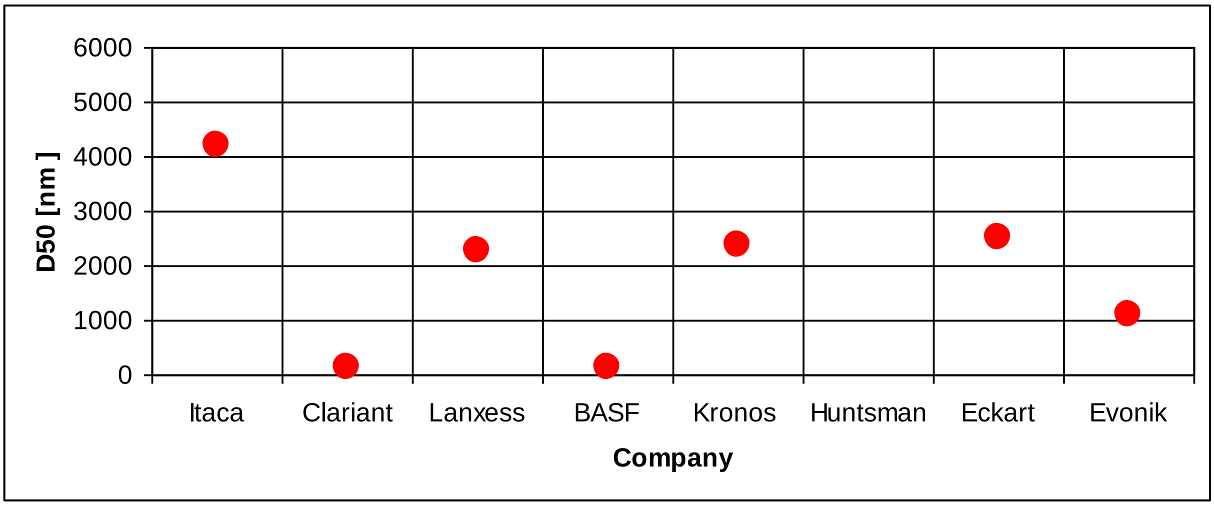


Figure 5-15 Volume-weighted mean particle size D50v of Pigment Yellow 83 transparent from LD measurements

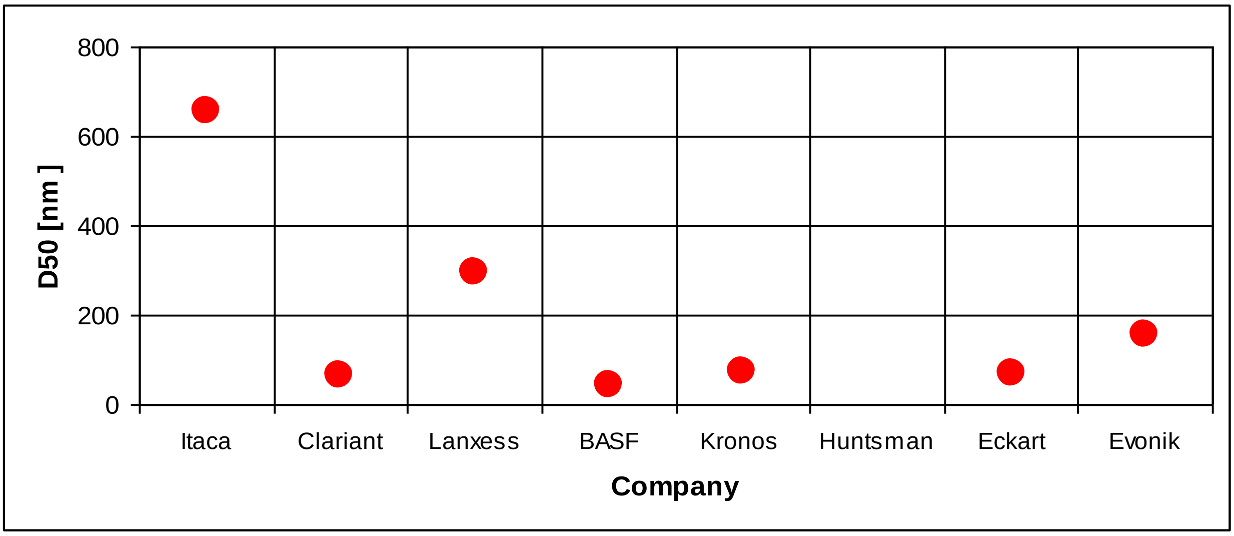


Figure 5-16 Number-weighted mean particle size D50n of Pigment Yellow 83 transparent from LD measurements

The sample is considered to have a mean primary particle size of 47 nm based on electron microscopy. The size of the particles renders this a borderline case for measurement by laser diffraction. Pigment Yellow 83 transparent is an organic pigment and therefore not an especially good scatterer of light, increasing the difficulties in this case.

The large observed differences both in D50v and D50n are the result of the instruments operating at the limit of their size ranges, possible difficulties with dispersion, and unreliable conversion from volume to number distributions.

Table 5-1 LD derived D50v and D50n values (in nm)

Material	Type of weighing	D50 values (in nm) per participant							
		Itaca	**Clariant**	**Lanxess**	**BASF**	**Kronos**	**Huntsman**	**Eckart**	**Evonik**
Al-Co-Blue	Vol	2101	2140	1552	2320	1970	135	2030	153
	Num	481	470	247	445	500	80	70	78
Pigment Yellow 42	Vol	4880	1920	31590	1920	180	4660	20970	27000
	Num	60	80	110	80	90	180	700	80
Fumed Silica	Vol	234	330	114	198100	170	66517	59100	153
	Num	78	90	72	420	150	10577	10000000	78
Anatase Pigment	Vol	590	380	262	193	433	237	660	549
	Num	319	70	97	85	167	80	300	71
Rutile Pigment	Vol	440	500	340	350	440	210	480	450
	Num	230	260	90	30	230	80	1000000	100
Pigment Red 101	Vol	787	530	489	1820	550	377	710	393
	Num	366	270	185	296	300	87	270	295
Pigment Metal 2	Vol	100000	100000	4572	100000	5520	100000	5000	5746
	Num	100000	100000	1500	100000	370	100000	2150	2000
Pigment Yellow 83	Vol	4198	130	2263	148	2387	1000000	2520	1101
	Num	658	65	297	44	73	10000	70	156

5.2 Complex index of refraction as a source of error in LD

Instruments based on laser light scattering and diffraction as well as centrifuges with a detection unit based on such optical effects require the input of the complex index of refraction to obtain correct results.

The complex index of refraction $\tilde{n}(\lambda)$ $\tilde{n}(\lambda)$ is a constant defined to describe the refraction $n(\lambda)n(\lambda)$ and absorption $\kappa(\lambda)$ $\kappa(\lambda)$ of a substance at a given wavelength λ. Together they are necessary to describe the scattering and absorption cross sections of particles:

$$\tilde{n}(\lambda) \quad = \quad n(\lambda) + i\,\kappa(\lambda)$$

As indicated, both the refraction and the absorption of the substance depend on the wavelength. This dependence is naturally more pronounced in the case of coloured pigments. The measurement of this material parameter is not trivial for substances with elevated indices of refractions, such as pigments, as few methods are available covering the range of the indices of refraction encountered here.

The simultaneous use of different light sources as practiced by several instruments naturally aggravates this problem. The use of different wavelengths is mandatory to increase the size range particularly to smaller particles and for an increased accuracy.

As valid values for index of refraction are not available for all cases needed, this has led to the practice of using "fixed values" for every substance, which is sometimes even recommended by the manufacturers. This is acceptable for a size range of more than about 5 µm, where the scattering of particles can be described without knowledge of the index of refraction, but will lead to wrong results in lower size ranges. This can be seen in the example shown below which compares the size distributions obtained using either typical standard values or more realistic physical constants.

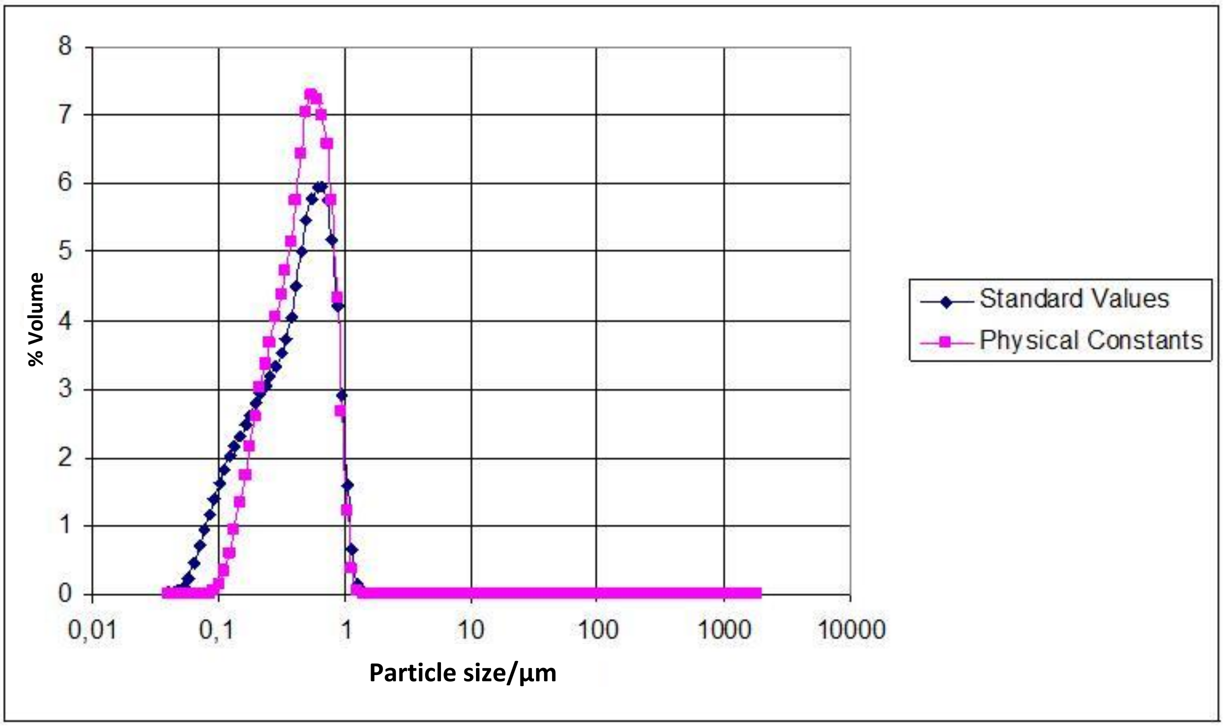


Figure 5-17 Influence of incorrect optical material parameters on measured volume-weighted particle size distributions

The above measurement on the Pigment Red 101 sample was done with the same Coulter LS 13320 Laser Diffractometer used in the comparison exercise. The values for index of refraction used are as shown in Figure 5.18. It should be especially noted that the resulting variation in the size distribution is particularly noticeable in the small particle size range below 100 nm.

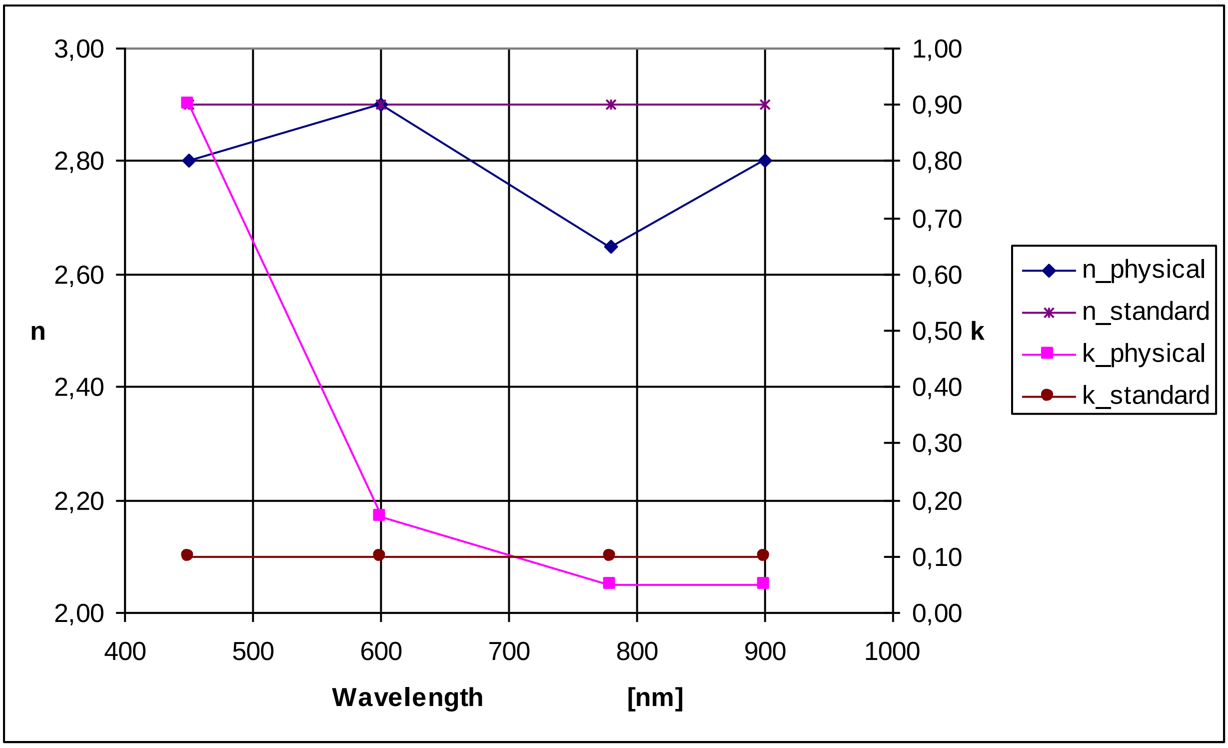


Figure 5-18 The values for index of refraction (fixed values vs measured material parameters) used in previous Figure 5.17

As the variation of the refraction (real component) was relatively small, the differences in the results were mainly attributable to variations in absorption (imaginary component) at the shorter wavelengths.

If no trustworthy values for the complex refractive index can be found in the literature and measurement is not possible, the only way forward is a two-step approach:

1) Using known values of similar compounds, estimate the real and imaginary parts of the index of refraction for the wavelength in question.
2) Check the effect of the varying the input values within the uncertainties (if known) on the results of the measurements of the instrument used.

5.3 Summary of the LD-results

The results of the measurements made by laser diffraction show that:

1) Reproducible results are not obtained between laboratories even when identical dispersion protocols and calculation parameters are used.
2) A good dispersion has to be assured to get reproducible results. Each sample type generally requires a specifically developed dispersion protocol and knowledge and experience of the material characteristics can help to achieve this faster.
3) A shortcoming of LD is its applicability only to particles with a well-defined, usually spherical, geometry.

4) Depending on the material the complex index of refraction must be known for all wavelengths employed.
5) LD has a large dynamic range in terms of particle size and thus can provide information about the particle size distribution even for materials with a broad size distribution. Although LD gives information about the size of larger particles it cannot distinguish large primary particles from agglomerates/aggregates nor can it give information about constituent particles within aggregates and/or agglomerates.
6) LD has a limited sensitivity in the nanometre range, especially below 50 nm, depending on the index of refraction.
7) The size resolution achievable with LD may not be sufficiently high as to allow its reliable use with narrow size distribution materials.
8) As with all methods used in this study, the transformation of the volume to number distribution must be viewed with caution (see chapter 8). It is especially important to ensure that the sample particle size is within the useful working range of the instrument.

In conclusion, Laser Diffraction is a widely used technique in the pigments industry that has great value for product quality control and for the determination of particle characteristics related to product specifications. However, in view of the above-mentioned limitations, and its high dependence on sometimes poorly determined optical parameters, we conclude that it has limited practical value in the classification of pigments or fillers with respect to the EC definition of nanomaterials, and can only be used as a complementary method to TEM analysis.

6 Descriptive summary of DLS data obtained by partners

6.1 Consideration of the results obtained in the interlaboratory comparison

In this study only four participants had access to DLS instruments and in some cases it was not possible to measure all 8 pigment types being examined in the study.

Furthermore, since the final objective is to assess the suitability of this method for the determination of a number size distribution the data considered will be the Z-average and the number size distribution values of D10n, D50n, and D90n.

To allow easier comparison, the previously shown manufacturer-supplied data has been reproduced here (Table 6-1) to show the particulate sizes and agglomeration/aggregation states which are expected from electron-microscopic evaluation, and a summary of the D(50)n results of the measurements made by dynamic light scattering have been tabulated alongside this (Table 6-2). Considering the D50n values it can be seen (Table 6-2 and Table 6-5) that the different laboratories obtained relatively consistent values for Pigment Red 101, Rutile Sample, Pigment Yellow 42 and fumed (pyrogenic) silicon dioxide while the other materials had a larger scatter. This is possibly an indication that all laboratories managed to achieve more or less equivalent states of dispersion for these four samples, though it should be noted that the D10n and/or D90n values showed more scatter for these samples.

Table 6-1 Manufacture supplied data on particle size and agglomeration/aggregation state

Material	Dimensions of primary particles	Agglomeration state in solid
Al-Co-Blue	Not specified	Not specified
Pigment Yellow 83 Transparent	>90% below 100 nm (D50n≈ 50 nm)	Aggregation/agglomeration
Pigment Red 101	Mean 270 nm (Range 3 nm-1 µm)	Aggregation/agglomeration
Pigment Yellow 42	<100 nm all dimensions	Aggregation/agglomeration
Rutile Sample	D50n≈ 210 nm	Aggregation/agglomeration
Anatase Sample	D50n≈ 130 nm	Aggregation/agglomeration
Pigment Metal 2	Platelet width≈4000 nm	Aggregation/agglomeration
Fumed(pyrogenic) SiO_2	12 nm	Aggregation/agglomeration

Table 6-2 Comparison of D50n values obtained by DLS in different laboratories

Material under Test	D50n min (nm)	D50n max (nm)	Average D50n (nm)*	Is Nano by DLS?	Expected Nano-status from EM
Al-Co-Blue	211	383	263 [4]	NO	NO
Pigment Yellow 83 Transparent	38	103	70 [3]	YES	YES
Pigment Red 101	201	259	229 [3]	NO	NO
Pigment Yellow 42	54	56	55 [3]	YES	YES
Rutile Sample	201	227	221 [4]	NO	NO
Anatase Sample	146	261	196 [4]	NO	NO
Pigment Metal 2			446 [1]	NO	unknown
Fumed(pyrogenic) SiO_2	101	110	106 [4]	NO	YES

*The number of accepted values used to calculate the average is indicted in brackets [ ]

From the results, the following specific points can be noted:

1) Z-average values, being scattered light intensity-weighted, generally overestimate the size, but in any case are not directly useful for implementation of the definition.
2) A wide variability of the results was observed between laboratories for several samples - this effect is probably due in large part to variation in sample preparation rather than instrument errors, together with further errors introduced when calculating number size distributions from intensity data for polydisperse samples.
3) An examination of the graphical representation of the number size distributions shows in many cases that the simple numerical values such as D50n, although apparently consistent with the manufacturer-supplied size values, may come from data which has been distorted during the mathematical conversion from the scattered light intensity–weighted distribution to number distribution.
4) It is probable that better-developed, sample-specific, protocols for complete dispersion, avoiding flocculation, that ensured the stability of the measured dispersions would lead to more reproducible results.
5) To have the correct basis for the calculation of number distributions the DLS technique requires that the complex index of refraction be known for all wavelengths employed. This requirement is not always easy to satisfy for all materials but in many cases a more detailed optical characterisation of samples could improve the situation.
6) One of the strengths of DLS over methods such as CLS and LD is that it is technically capable of measuring particle sizes from below 1 nm to several micrometres. Unfortunately this advantage may not be realised in many cases since there is a very large variation of scattering power with

particle size. This effect, when combined with its limited size resolution, means that the presence of large numbers of smaller particles may be masked by even a relatively low proportion of larger particles. This problem may be overcome if DLS can be used with pre-fractionated samples produced by chromatographic (AF4) or centrifugal separation methods. This approach to using DLS has much promise but due to its limited application in the pigment industry was not included in this study.

It should be remembered that any data obtained from DLS (also LD and CLS) is derived from particulate entities and not necessarily the constituent particles of aggregates or agglomerates. Furthermore the values obtained depend on a series of assumptions about the material characteristics (optical properties, density and shape) which for many materials are not valid.

Overall, the DLS technique when applied to the measurement of number size distributions of polydisperse particulate materials, is unlikely to give false positive results but may often produce false negatives.

Table 6-3 Tabulated values of Z-average diameters from DLS analysis

Measuring Partner	Evonik	JRC-IHCP	Kronos
	Z-Average (nm)	Z-Average (nm)	Z-Average (nm)
Al-Co-Blue	572	435	380
Pigment Yellow 83 Transparent	238	233	242
Pigment Red 101	398	338	366
Pigment Yellow 42	----	105	114
Rutile Sample	350	278	315
Anatase Sample	408	260	285
Pigment Metal 2	-----	----	446
Fumed (pyrogenic) SiO_2	210	140	302

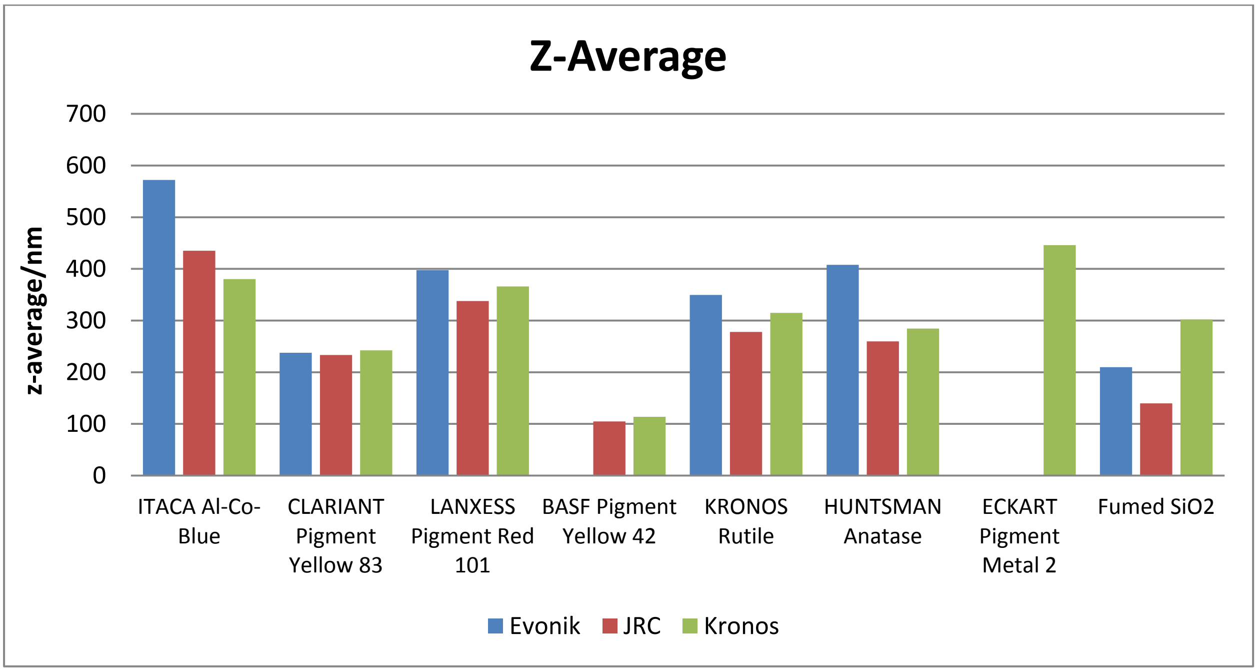


Figure 6-1 Graphical representation of the Z-average (nm) from DLS analysis

Table 6-4 Tabulated values of D10n from DLS analysis

Measuring Partner	Evonik	Kronos	JRC-IHCP	ITACA
	D10n (nm)	D10n (nm)	D10n (nm)	D10n (nm)
Al-Co-Blue	170	126	136	233
Pigment Yellow 83 Transparent	46	72	29	
Pigment Red 101	184	134	162	
Pigment Yellow 42		40	40	42
Rutile Sample	196	138	150	173
Anatase Sample	199	95	115	156
Pigment Metal 2		51		
Fumed(pyrogenic) SiO_2	95	81	75	70

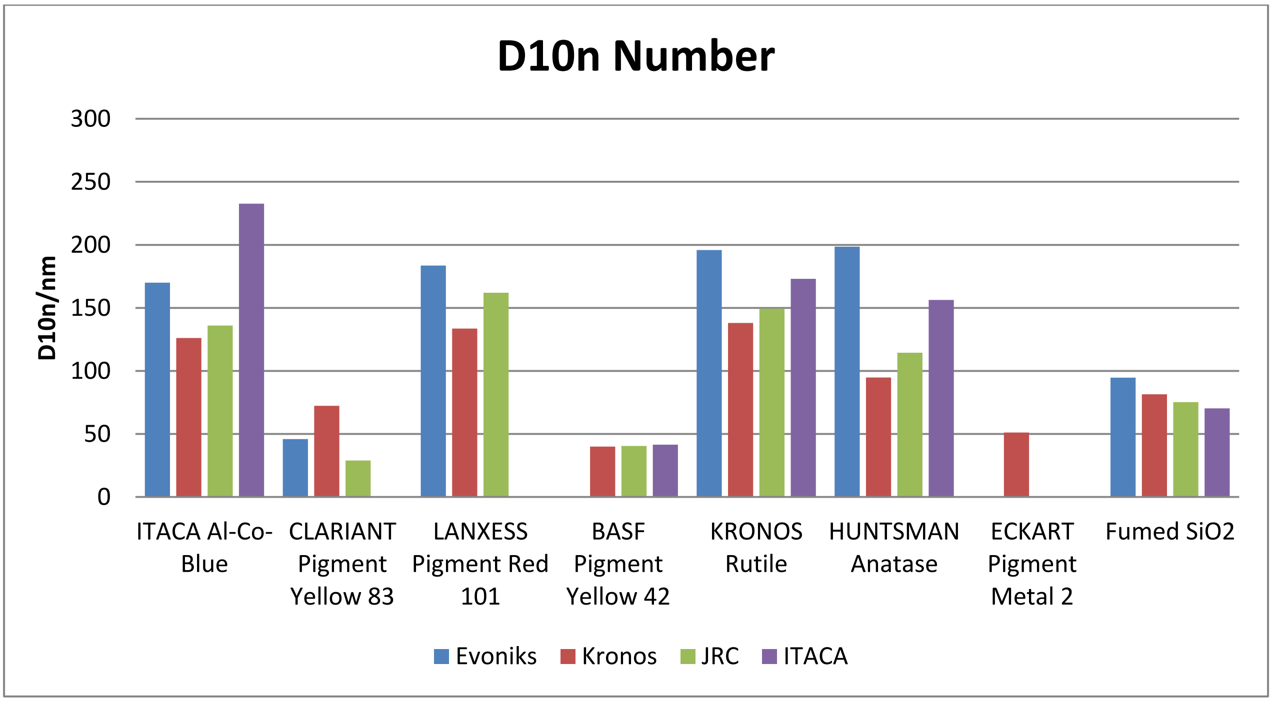


Figure 6-2 Graphical representation of D10n from DLS analysis

Table 6-5. Tabulated values of D50n from DLS analysis

Measuring Partner	Evonik	Kronos	JRC-IHCP	ITACA
	D50n/nm	D50n/nm	D50n/nm	D50n/nm
Al-Co-Blue	261	198	211	383
Pigment Yellow 83 Transparent	70	103	38	
Pigment Red 101	227	201	259	
Pigment Yellow 42		54	54	56
Rutile Sample	227	201	221	235
Anatase Sample	261	154	146	224
Pigment Metal 2		446		
Fumed(pyrogenic) SiO2	109	110	101	105

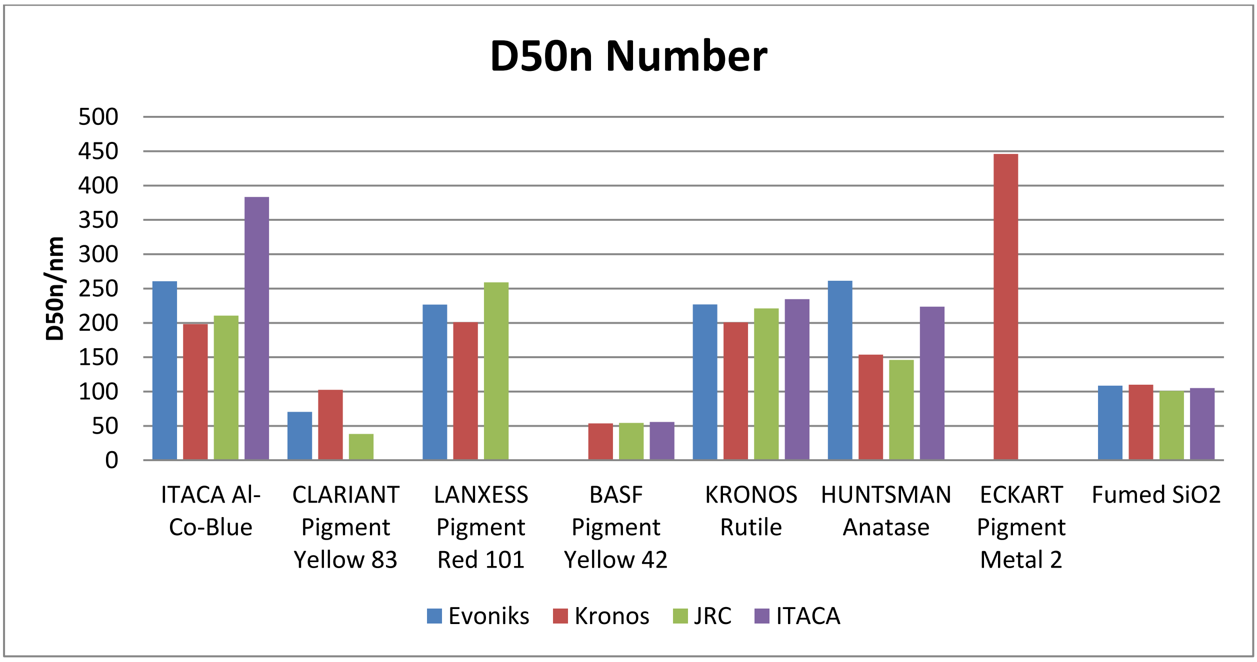


Figure 6-3 Graphical representation of D50n from DLS analysis

Table 6-6 Tabulated values of D90n from DLS analysis

Measuring Partner	Evonik	Kronos	JRC-IHCP	ITACA
	D90n/nm	D90n/nm	D90n/nm	D90n/nm
Al-Co-Blue	524	472	381	670
Pigment Yellow 83 Transparent	140	58	190	
Pigment Red 101	391	454	397	
Pigment Yellow 42		80	79	89
Rutile Sample	339	340	369	345
Anatase Sample	392	215		374
Pigment Metal 2		716		
Fumed(pyrogenic) SiO2	187	149	202	250

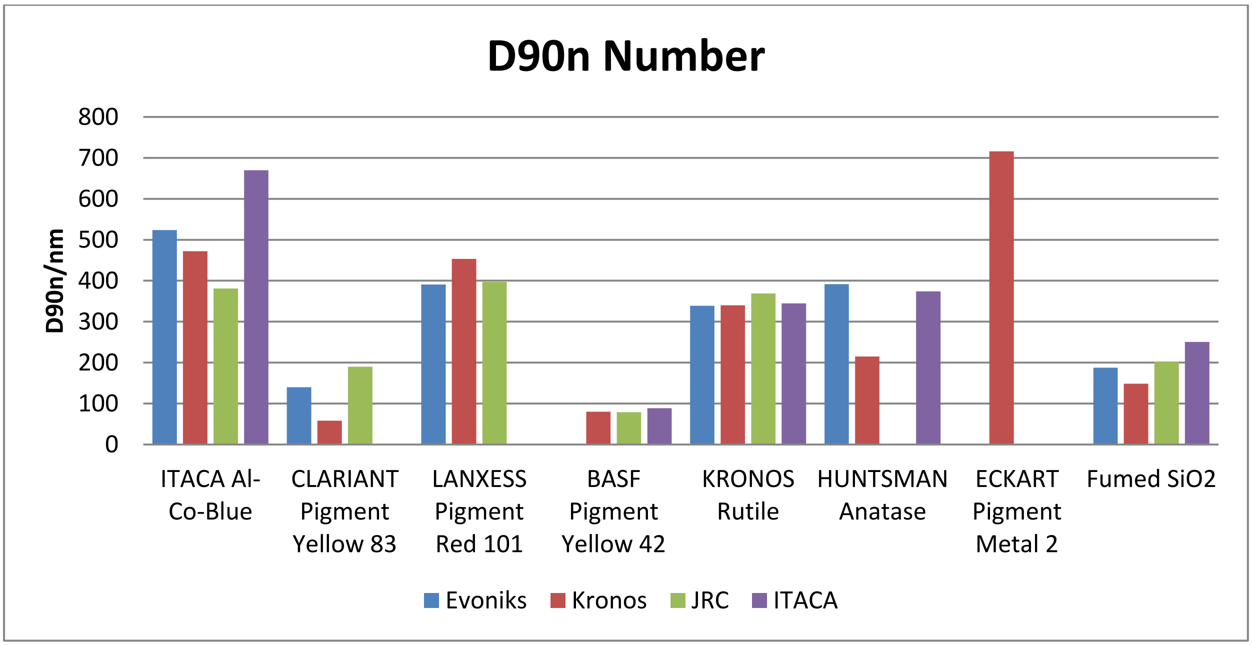


Figure 6-4 Graphical representation of D90n from DLS analysis

7 Descriptive summary of CLS data obtained by partners

7.1 Consideration of the CLS results obtained in the inter-laboratory comparison.

In the present study 6 participants had access to CLS instruments, and measurements were made on all 8 materials. It should be noted that one material type, Pigment Metal 2, proved to be problematic and results were reported by only two participating laboratories.

In this part of the study the aim was firstly to assess the suitability of the CLS method for the determination of a number size distribution and secondly to assess the reproducibility and reliability of the method when applied to nominally identical samples prepared by a standard procedure. To evaluate this in a simplified manner, which might be relevant to the final application of classifying nanomaterials, a comparison of the numerical output from the various instruments was made rather than undertaking a detailed consideration of the particle size distribution curves themselves.

Table 7.1 below has been compiled showing the D50 values expressed in volume and number obtained by the 6 laboratories participating in this part of the study.

Table 7-1 Measured values of D50v and D50n

Material	D50v (nm)					D50n (nm)					
Al-Co-Blue	635	462	320	1019	559	202	434	20	215	35	133
Pig. Yellow 83 Transparent	109	30000	40		109	39	29474	30		44	45
Pigment Red 101	360	342	310	378	366	76	185	10	249	43	52
Pigment Yellow 42	49	3019		84	95	36	2367	-	55	45	30
Rutile Sample	329	447	290	291	332	245	378	210	219	28	297
Anatase Sample	314	356	250	296	390	183	325	160	223	289	108
Pigment Metal 2	378				555	125				46	
Fumed(pyrogenic) SiO2	71	92	60	427	76	55	73	60	83	66	43

Technical note: The CLS instrument used by one laboratory does not offer the option of calculating D50v values but does allow D50n. For this reason only 5 results of D50v have been quoted while 6 results of D50n could be reported. (Under scored numbers considered to be outliers).The final shaded column indicates the D50n results which have no corresponding D50v values.

After eliminating the clear outliers the tabulated D50v data values still show quite a considerable variation for most of the materials. There is a rough agreement between labs for some samples which indicates that for some sample types a common method of sample preparation and common analysis parameters might lead to comparable D50v results.

When these size distributions are then mathematically converted from mass/volume base to number base, a much higher degree of disagreement is observed between the various results, as reflected in the scattered D50n values. This is attributable, in large part, to the fact that small errors occurring in the low size range of the weight/volume distribution are magnified in the mathematical conversion to number base. The origin of the errors may, in part, be attributed to instrumental effects such as background noise, variations in sensitivity at the lower size range and the minimum size of particle which is able to sediment during the analysis process. The last of these parameters is, for any one type of particle, dependent on a series of different instrument parameters and in particular the gradient density, rotational speed/centrifugal force used by the instrument and the minimum size cut-off imposed by the total sedimentation time used for each sample. In the study no detailed specification was made for the maximum/minimum particle sizes which had to be reached. A consequence of this was that the D50 values obtained in the different laboratories could be derived from particle size distribution data sets with different maximum/minimum size cut-off values. This error would, in most cases, not be expected to greatly influence the D50v values but could certainly have a much greater influence on the D50n values.

From the rather variable D50n values averages were calculated for each material (excluding the outlier values) and the resulting nano-status was compared (Table 7-2) with that expected by the manufacturer based on their EM evaluation. For 7 cases the results were consistent, though in the case of Pigment Red 101 the status was borderline. The result for Pigment Metal 2 is not conclusive given the platelet shape of this material.

Table 7-2 Particle classification based on average CLS measured values of D50n

Material	D50n min (nm)	D50n max (nm)	Average of D50n (nm)	Is Nano by average CLS?	Expected status (by manufacturer) based on EM
Al-Co-Blue	**20**	**434**	**173**	**NO**	**NO**
Pigment Yellow 83 Transparent	**30**	**45**	**40**	**YES**	**YES**
Pigment Red 101	**10**	**249**	**103**	**NO**	**NO**
Pigment Yellow 42	**30**	**55**	**42**	**YES**	**YES**
Rutile Sample	**28**	**378**	**230**	**NO**	**NO**
Anatase Sample	**108**	**325**	**215**	**NO**	**NO**
Pigment Metal 2	**46**	**125**	**86**	**YES**	**not known**
Fumed(pyrogenic) SiO2	**43**	**83**	**63**	**YES**	**YES**

Note: The data are reported and averages were calculated excluding the 2 outlier results indicated in Table 7-1

If, instead of considering the average measured values in the study, the individual classifications coming from each laboratory are considered (Table 7-3) it can be seen that the situation is not in fact so clear-cut, with only two materials (anatase and pyrogenic SiO_2) being unanimously classified according to the manufacturer classification.

This evaluation is extremely simplistic in nature but does serve to show that, although CLS derived number distributions from multiple laboratories can produce superficially correct classification results, the fact that only 2 of 8 materials were correctly assigned by all laboratories implies that there is a significant possibility of the method giving false positive or false negative values.

However, given that the method can deal, at least in principle, with polydisperse samples, with appropriate development the technique may have promise for use in classification of some very specific sample types (spherical, non-agglomerated, non-aggregated, uniform known density).

Table 7-3 Particle classification based on individual laboratory derived values of D50n

Material	No. of lab results		Classification by majority	Expected classification
	D50n<100nm	1000nm >D50n>100nm	Nano	Nano
Al-Co-Blue	2	4	No	No
Pigment Yellow 83	4	1	Yes	Yes
Pigment Red 101	4	2	Yes	No
Pigment Yellow 42	4	1	Yes	Yes
Rutile K2360	1	4	No	No
Anatase Sample	0	6	No	No
Pigment Metal 2	1	1	---	not known
Fumed(pyrogenic) SiO2	6	0	Yes	Yes

7.1.1 Comparison of D50n values from DLS and CLS analysis

One of the main motivations for using the CLS method is that, compared to the faster and technically easier light scattering techniques (LD and DLS), this method could be less subject to the biasing towards larger particle size. It is therefore interesting to compare the results of D50n data obtained by the DLS and the CLS methods - see Table 7-4 below.

Although in some cases, as expected, the size values for CLS are smaller than DLS, the level of scatter of the number based results is much higher for CLS.

Table 7-4 Comparison of the D50n values (in nm) obtained by DLS and CLS in partner laboratories

Material	DLS				CLS					
AlCo-Blue	261	198	211	383	202	434	20	215	35	133
Pigment Yellow 83	70	103	38		39	29474	30		44	45
Pigment Red 101	227	201	259		76	185	10	249	43	52
Pigment Yellow 42		54	54	56	36	2367		55	45	30
Rutile K2360	227	201	221	235	245	378	210	219	28	297
Anatase Sample	261	154	146	224	183	325	160	223	289	108
Pigment Metal 2		446			125	-	-	-	46	
Fumed(pyrogenic) SiO2	109	110	101	105	55	73	60	83	66	43

7.1.2 Additional considerations of CLS analysis

The previously reported results relating to the CLS are clearly quite disappointing under the circumstances of the particular test conditions used. The high variation is presumably a combination of the increased sensitivity of CLS to smaller particles, the types of particles under examination, and the high dependency of the results on dispersion state. It is worth noting, however, that a more extensive experience of using the method with materials which are non-aggregated and properly dispersed have shown that the CLS technique can, in general, give more accurate and reproducible results than DLS for particle sizes above a few tens of nanometres. This statement is valid provided the data is evaluated as a distribution based on weight/volume. Conversion to number basis can result in much higher variability and an increased chance of serious errors. Experience with highly monodisperse materials with known densities has shown that CLS data can approach the accuracy and size resolution normally achievable only with statistical analysis of many particles in TEM images. The use of CLS in this particular application suffers, like DLS, from the distortions which can occur during the step of converting weight/volume data in a number based distribution. The fact remains that the CLS method has two other disadvantages which are its inability to distinguish aggregates from primary particles and more importantly the current generation of instruments cannot operate at sufficiently high speeds as to guarantee sedimentation of the smallest particle size, 1 nm, which is fundamental to the EC nanomaterial definition. For these reasons the CLS technique, although potentially valuable for use in a multi-techniques strategy is not able to resolve the difficulties of reliably assessing nanomaterial status as specified in the EC recommended definition.

8 Transformation of mass/volume distributions to number distributions – cautionary notes

The particle sizing instruments based on light scattering such as laser diffraction, dynamic light scattering or centrifugal sedimentation (with optical detection) produce particle size distributions based on mass/volume (CLS and LD) or intensity (DLS) rather than particle number. This happens because the physical or optical phenomena on which each instrument bases its measurement are most strongly linked to either the mass or volume of the particles rather than the number of particles. To make such data relevant to the EC definition it is necessary to mathematically convert the standard type of distribution into an equivalent number size distribution. This conversion is based on a number of assumptions, and becomes increasingly prone to error as the quantity and size of sub-100 nm particles decreases. To highlight the importance of this, it is necessary to consider the steps required to convert a mass/volume distribution into a number distribution.

For the simplest case of spherical particles the transformation of a mass/volume distribution to a number distribution is achieved arithmetically by dividing each value of the measured distribution by the third power of the geometric mean of the size band in question. The need to divide by the third power of an experimentally determined value means that errors which may be minor in the original mass-volume distribution become greatly magnified in the resulting number size distribution. The origin of the effect is illustrated in Figure 8-1 which shows the calculated particle number concentrations in solutions containing different sizes of monodispersed Ag nanoparticles at constant mass concentration.

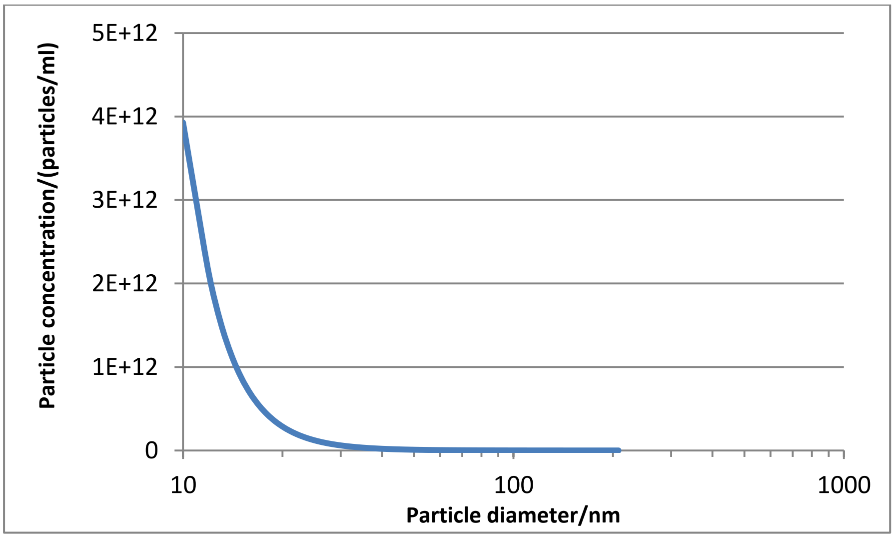
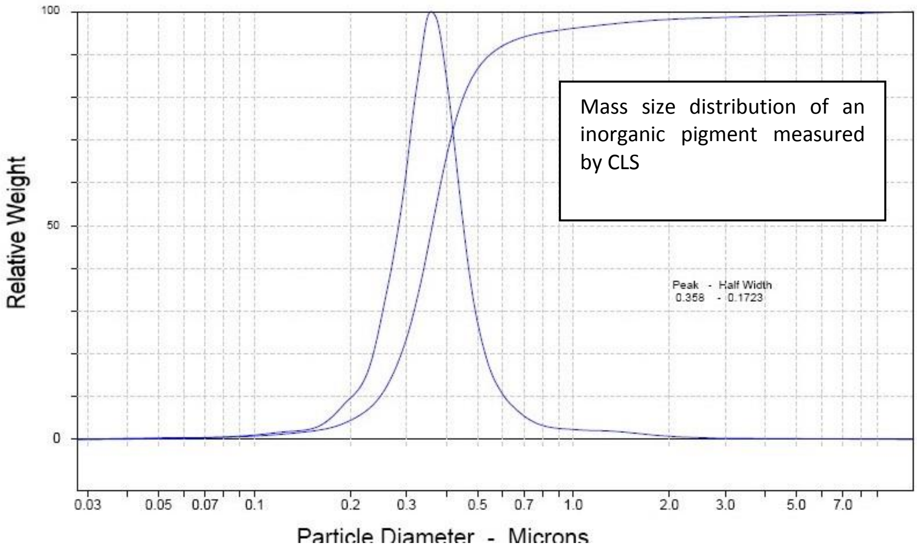


Figure 8-1 Variation of silver nano-particle number concentration with size at constant mass concentration (0.02mg/ml)

As can be seen from Figure 8-1 the particle number concentration at constant mass begins to increase very rapidly below a size of around 20-25 nm. The consequence of this effect is that, as particle size decreases, inaccuracies in the experimentally determined weight-size distribution are disproportionately magnified by the mathematical conversion to the desired number-size distribution.

With a few notable exceptions such as NTA and TEM the accuracy and dynamic response of most types of particle sizing instruments is tailored towards deriving mass or volume distributions. This may be incompatible with the accuracy and sensitivity required to produce data which can reliably be used as the basis for the highly non-linear conversion to a number size distribution.

To illustrate the strong influence that the conversion steps can have on eventual number size distributions derived from mass-size distribution this chapter will present a simplified simulation based on a model mass-particle size distribution. To increase the relevance of this simulation to the work conducted in this study, the starting model distribution has been based on a real-world example from this study which is shown in Figure 8-2



Mass size distribution of an inorganic pigment measured by CLS

Figure 8-2 CLS derived particle size distribution of an inorganic pigment expressed on a mass basis

An examination of the CLS derived weight/size distribution of the pigment material shows it to be composed of mainly particles in the range 150 - 700 nm with only a small fraction of material in the sub-100 nm range. From the CLS data analysis software the particular experimental example was found to have D50m and D50n values of 320 nm and 60 nm respectively. The example chosen as the basis of this discussion is considered representative of many samples as the form of the particle weight-size distribution exhibit the Gaussian type form which is commonly found in nanomaterials whose manufacture employs milling methods.

The basic model size distribution (Figure 8-3) has been made from the sum of three simple log-normal functions (f(x1), f(x2), f(x3)) which have been adjusted to produce a data set of mass and size which matches as closely as possible that of the main peak in the experimentally determined example. In all cases the simulations consider the size range from 10 nm to 5000 nm although for clarity the graphics will show the range of interest from 10 nm to 1000 nm.

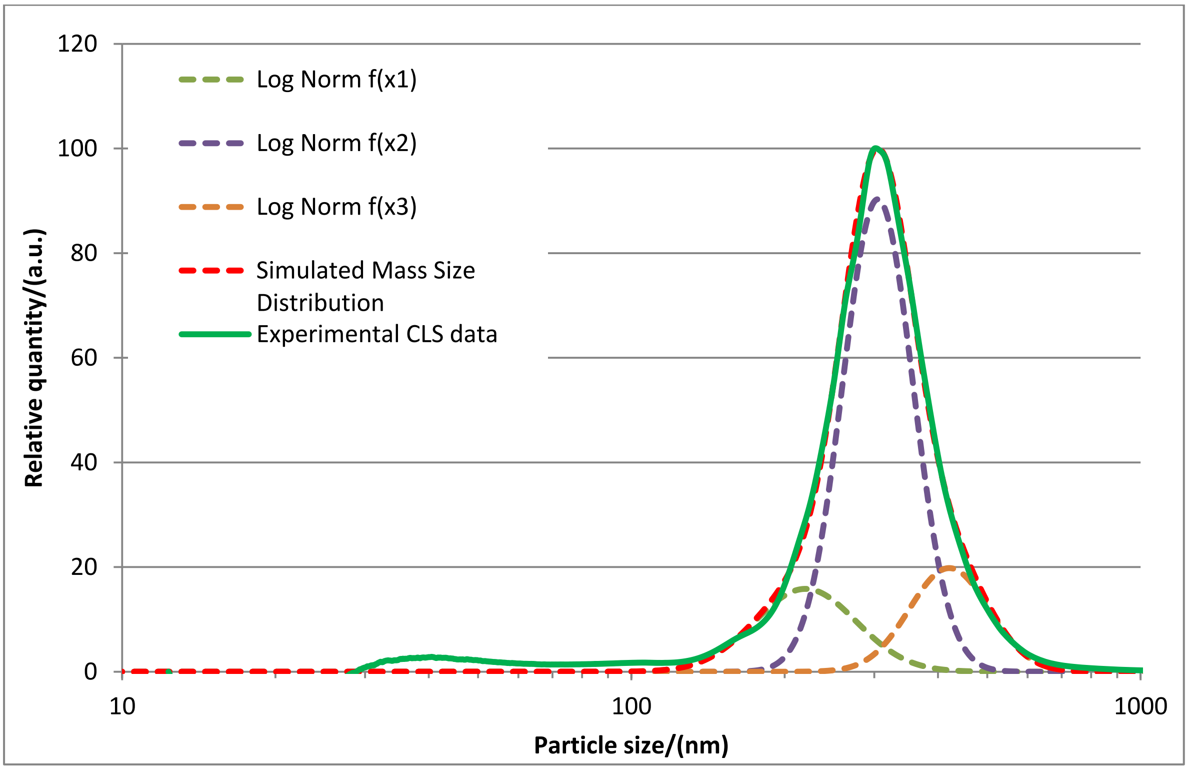


Figure 8-3 Comparison of experimental and model particle size distribution expressed on a mass basis

For the purposes of simulations a further mass function (f(x4)), initially set to zero, can be added to the composite to simulate the effect of adding material in the size range around and below 100 nm. A lower size cut-off of 10 nm has been imposed in the basic simulation as this corresponds to the lower useable size range of medium density particles (metal oxide) when analysed by typical disc centrifuges. It should be noted that the principles being illustrated in this section, although based on data from a disc centrifuge, are equally applicable for other analysis methods which rely on transformation of mass to number distribution.

From this simplified model it has been possible to examine the effect on calculated number size distribution which results from making small systematic variations to the starting weight size distribution. As the starting point for the evaluation the basic model particle size distribution has been used to calculate the cumulative particle size distributions expressed in both weight and number as shown in Figure 8-4. From these curves, the values of D50m and D50n were then determined to be 321 nm and 242 nm respectively. The amount of material <100 nm is 0.05wt%.

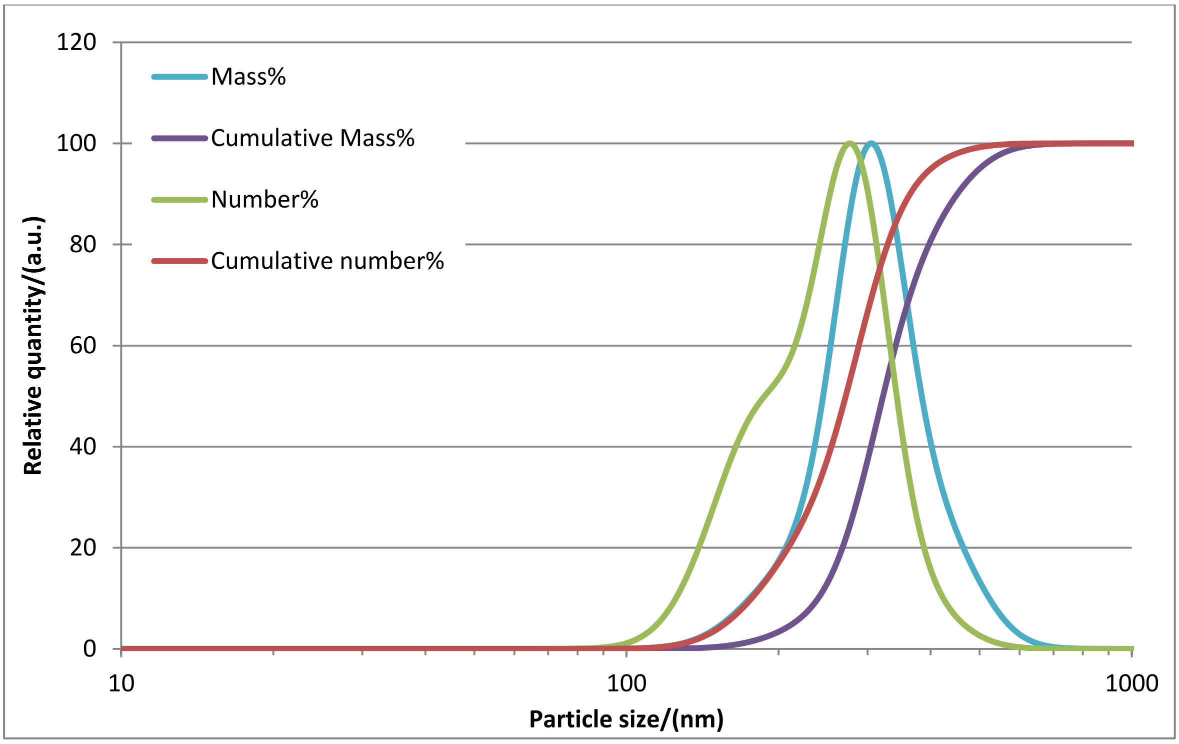


Figure 8-4 Model particle size distribution showing cumulative mass and number distributions: D50m=321nm and D50n=271nm

To illustrate the extent to which this number size distribution can be influenced by the inclusion of a small quantity in mass of sub-100 nm material the example shown in Figure 8-5 (enlarged in Figure 8-6) has been prepared. This example has been derived by taking the initial distribution shown in Figure 8-4 and adding gradually increasing quantities of a second population of particulate material (peak maximum around 50 nm) until the calculated D50n value decreased to 100 nm. In this way it was possible to estimate the relative quantity of sub-100 nm material necessary to change the classification of the initially non-nano starting material to nano under the terms of the nanomaterials definition.

In this case, increasing the initial 0.05wt% of sub-100 nm material to a total of 0.44wt% was found to be sufficient to reduce the D50n value from 271 nm to below the critical 100 nm transition value. In contrast to this the equivalent D50m value underwent only a minimal change from 321 nm to 320 nm.

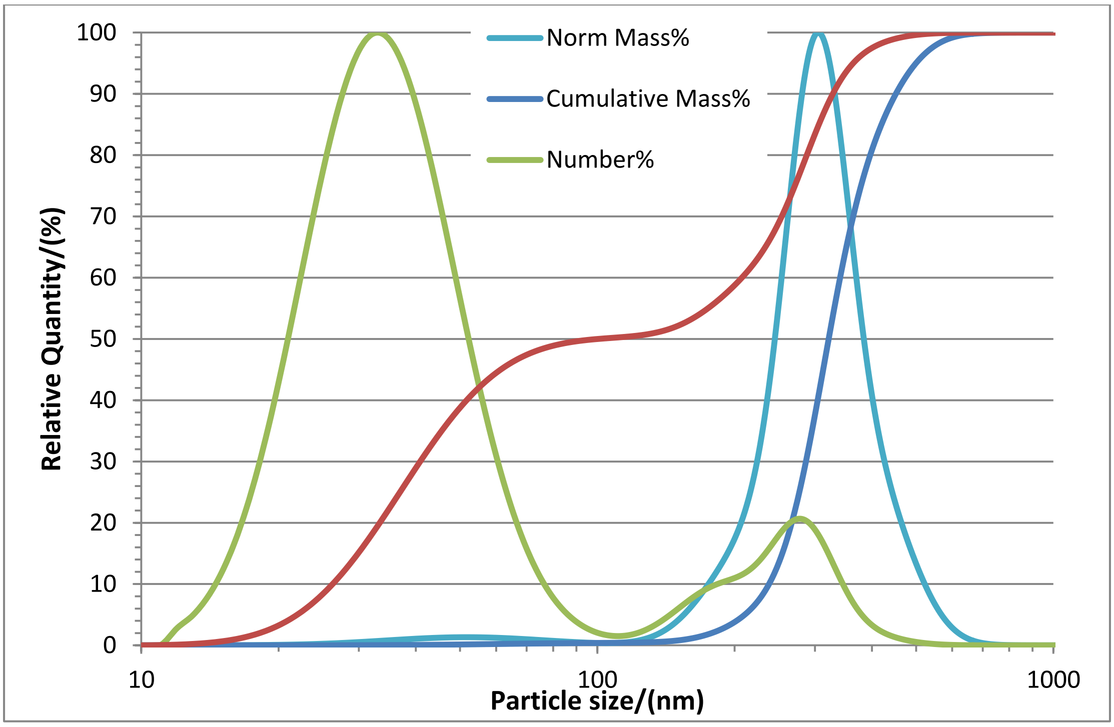


Figure 8-5 Simulated particle size distributions after inclusion of 0.44% mass of particulate materials with peak maximum of 50 nm and FWHM of 50 nm: D50m=320 nm and D50n=100 nm

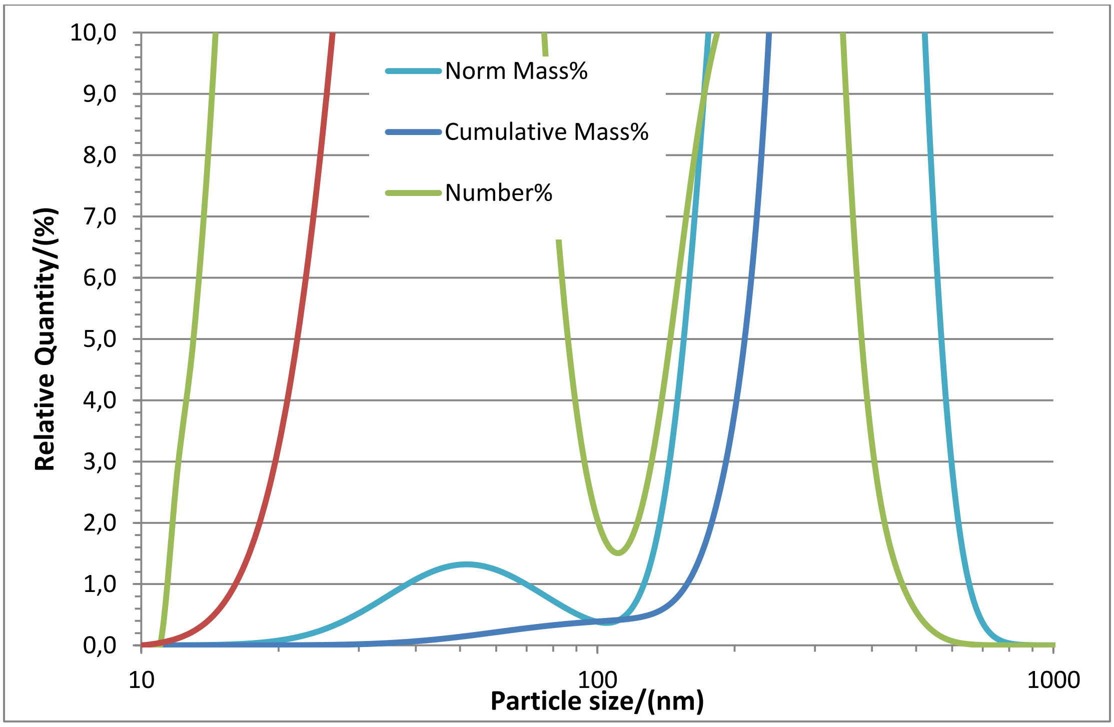


Figure 8-6 Enlargement of Figure 8-5 Simulated particle size distributions after inclusion of 0.44% mass of particulate materials with mean size centred 50 nm

From this simulated example it can clearly be seen how a small variation in the sub-100 nm portion of the initial mass distribution can have a very strong influence (positive or negative) on the number size distribution and therefore on the evaluation of the nanomaterial status of a sample. In practice, such small differences between the real and the measured mass size distributions could result from a variety of experimental factors such as instrument sensitivity, dynamic range, signal-to-noise ratios and choice of effective measured size range (instrument or operator dependant)-in particular the value of any lower cut-off. While it would be possible to undertake numerous other simulations to evaluate the relevance of these other factors, this is beyond the scope of this discussion which has the simpler aim of merely drawing attention to the importance that the accuracy of any the mass-size distribution will have on the validity of any derived number-size distribution.

9 Summary and conclusions

The JRC-IHCP and Eurocolour have carried out a program of work to study a number of methods of measuring particle size distributions which have been suggested as possibly useful for assessing the status of particulate materials against the EC Recommendation for a definition of nanomaterial. The study has examined the use of five methods applied to a range of eight widely different but industrially relevant powder pigments. The materials used were chosen so as to exhibit a wide diversity of physico-chemical properties which would serve to test the general effectiveness, suitability and reliability of the measurement techniques. In particular the samples consisted of pigments with different primary particle sizes, shapes and levels of aggregation/ agglomeration, as well as both inorganic and organic substances, and both coated and uncoated pigments.

During the course of the study it was very clearly seen that sample preparation was a key part of the measurement process since each of the three primary techniques under assessment (LD, DLS and CLS) requires the generation of a stable dispersion of particles. Effectively there are three requirements to achieving this:

- wetting: the solvent must contact the surface of the particle and air must be displaced
- disintegration: agglomerates need to be separated by the input of external energy
- stabilisation: establishing a repulsive force between the particles to avoid re-agglomeration and maintain the dispersed state.

The appropriate energy input is substance dependant and needs to be optimised both in terms of the power input and treatment time. The aim is to break up agglomerates into constituent particles but avoiding excessive input that might introduce artefacts. For certain materials the destruction of even primary/constituent particles may be possible with some dispersion methods. However, this is not likely with ultrasonic probes which were generally considered as acceptable for the present study, although it is important to note that some materials can show partial re-agglomeration with excessive ultrasonic energy input. To minimise this aspect of particle behaviour it is recommended that particle size measurement be made on dispersions across a range of sonication energies so as to determine the energy necessary to achieve minimum stable particle size measurement values i.e. that neither an additional input of energy nor more time leads to a significant change in the particle size detected. Because there is no guarantee at this point that the majority of agglomerates have been broken up, and pre-existing aggregates will still be present, it is necessary to verify the effectiveness of the dispersion. This may be done by comparing the minimum stable particle size measurement to the size of the constituent particles expected from a suitable EM micrograph.

Additives, used to achieve dispersion and stability, need to relate to the surface chemistry of the sample, in particular whether the substance is hydrophilic or hydrophobic. Filtration of samples to remove very large particles which might dominate the measured results was tried but was not successful in many cases due to blocking of the filters if the dispersed sample was not sufficiently diluted or the particles were too large.

In this study, the original intent was to adopt a single, identical dispersion protocol for all 8 different materials but in practice this could not be achieved due to the widely varying physico-chemical properties of the test materials. The materials manufacturers provided the knowledge necessary to permit the adoption of only two main dispersion protocols. It should be noted that some partners had to further adapt these basic protocols to make them compatible with their own specific instruments and techniques, and that the variability of the results between laboratories suggest that the dispersion protocols did not always produce identical dispersions. Should materials analysis be entrusted to independent test laboratories who do not have detailed knowledge of individual samples it may be expected that significant effort would be needed to optimise dispersion protocols and ascertain whether they achieved full dispersion into stable suspensions of constituent particles.

9.1 Techniques - General Remarks

LD, DLS and CLS have some characteristics in common which affect the applicability of these techniques for implementation of the recommended EC definition of nanomaterial:

- they measure volume/mass distributions which must then be converted to a number distribution using mathematical algorithms – this can result in potentially large errors as discussed in more detail in chapter 8;
- the evaluation of particle size commonly assumes the presence of spherical particles – deviation from spherical geometry can render results invalid;
- none of these techniques can determine the size of constituent particles within aggregates or agglomerates, the presence of which will render classification as non-nanomaterial unreliable;
- LD and DLS cannot be relied upon to produce valid results for polydisperse samples with any significant fraction of particles in the "nano range" (1 nm to 100 nm);
- CLS and LD are limited in sensitivity at the lower sizes in the "nano range" so that the presence of any significant fraction of particles outside their range of practical sensitivity will render results invalid;
- dispersions must often be (highly) diluted to avoid errors due to multiple light scattering or streaming events.

9.1.1 Laser Diffraction

LD is a quick and easy method to apply since LD instruments give a particle size distribution from one single measurement. Knowledge of refractive indices and absorption coefficients is critical. The lower cut off level for size measurement is more restricted than for the other techniques being in the range 10 nm to 50 nm depending on the particular LD instrument used and the samples being analysed. The sensitivity to particles in the range below 100 nm depends on the index of refraction of the substance in question but is relatively low since the technique is optimised for particles of 500 nm and above. Care has to be taken if the calculation of the number size distribution includes a significant proportion of particles below the measurement range as this can lead to completely erroneous results. This problem was confirmed by one partner where deliberate dosing of a sample with nanoparticles did not alter the measured distribution confirming the lack of sensitivity of this technique in the nanosize range.

The results from the LD studies indicated a high variability, and for seven of the eight samples types examined using this technique false positive or false negative results versus the manufacturer defined nanomaterial status were reported by at least one of the laboratories.

While laser diffraction can possibly produce valid results for well dispersed and spherical primary particles if the primary particle size distribution can be shown to be entirely within the operating limits of the instrument in question, its various drawbacks mean that it certainly cannot be used as a technique for classification of samples against the EC definition if there is any significant nanomaterial (< 100 nm) content.

9.1.2 Dynamic Light Scattering

On a theoretical basis DLS reports a light-scattering intensity weighted average value that is strongly biased in the presence of (even a small fraction of) large particles. To produce output as a particle size distribution it is necessary to fit a sum of functions corresponding to monodisperse nanoparticles of the same properties to the measured scattering intensity. This can introduce high errors for polydisperse samples. Another factor of relevance is particle shape since in the case of non-spherical particles, the Brownian motion of the particles is a combination of both translational and rotational diffusion. Both types of diffusion cannot be distinguished by the traditional DLS technology and as a result the Stokes-Einstein relationship is in essence not valid. As for LD, the results are therefore influenced by the algorithm and fitting parameters used. They are further complicated by the required conversion to a number weighted result to allow comparison to the definition. In many ways the technique has similar problems to LD but it has the advantage that the measurement range extends to the smallest particles of the nano size range, increasing its applicability for this particular task.

On the other hand, DLS is performed on unstirred samples and those of high density may be prone to sedimentation and may be also susceptible to flocculation which might lead to erroneous results. In practice for the samples tested, based on the mean D50n values averaged across all the laboratories, DLS gave what was considered by the manufacturers to be a correct classification versus the definition for all the samples tested with the exception of the fumed (pyrogenic) silica sample. This sample is highly aggregated and agglomerated and gave a borderline result with DLS of D50n = 106 nm while the manufacturer considers the constituent particles to have a size of around 12 nm. For some samples CLS indicated a possibly significant content of particles in the nano size range which could perhaps also be interpreted from the corresponding electron micrographs. The number distribution values reported from the DLS did not support this, a fact attributable to the problems DLS has in dealing with polydisperse samples.

Considering all the results reported a number of false negative results were measured but no false positives. In general the reported results indicated a larger particle size than determined by EM with considerable distortion of the particle size distribution due to the required algorithmic conversions. Given its limitations, DLS cannot be recommended as a suitable technique for classification of any of the examined sample types with respect to the EC definition. However, the results suggest, in agreement with what is known about DLS, that a positive classification as “nanomaterial” is quite likely to be correct. For most materials produced by the pigment industry it is unlikely that DLS can be relied on for classification of samples as “non-nanomaterial” without supporting evidence .

9.1.3 Centrifugal Liquid Sedimentation

CLS has the theoretical advantage over LD and DLS in that there is some separation on a size basis by the application of centrifugal force prior to concentration detection by light or X-ray scattering. This means that problems associated with the measured signal from large particles dominating the signal from the smallest particles are reduced but the risk of errors from the volume to number conversion is still inherent to the technique.

Despite the theoretical advantage of a method using particle size separations, the results achieved in this comparison exercise were disappointing. For samples that were clearly nanomaterials as determined from EM data the lowest measured D50n values were slightly closer to the EM result than with DLS. However, overall, it could be observed that there was significant variation between the laboratories with both false positives and false negatives determined against the definition (based on the nanomaterial status specified by the manufacturers). It is likely that this is due to a combination of problems with dispersion, different instruments and operating protocols, and the high uncertainties associated with the conversion steps from, firstly, light absorption to material mass and then from mass to particle number.

9.1.4 Volume Specific Surface Area (VSSA)

VSSA is identified as a proxy measurement method within the definition (2), but only for positive identification of nanomaterials, against a threshold value of 60 m^2/cm^3. Measurement by BET has the advantage over LD, DLS and CLS (and EM) in that it does not involve dispersion protocols. However, VSSA is only directly applicable to the measurement of smooth, non-aggregated particles. The presence of surface treatments or porosity can alter the relationship between VSSA and particle size, as can particle shape and polydispersity.

Measurement of a surface coated titanium dioxide sample in this study by BET/VSSA showed the errors related to the presence of a porous coating. However, for dry powdered materials which are approximately spherical, uncoated and non-porous this is an effective technique for positive classification of materials as being nanoparticulate in nature.

9.1.5 Electron Microscopy (EM)

With the exception of some modern 3D imaging systems, both SEM and TEM give a two-dimensional projection of the three-dimensional particle ensemble of interest. When attempting to apply EM methods to the determination of particle size distribution one of the main challenges is to reliably extract information about a 3-dimensional system starting from a two-dimensional projection which can vary greatly in complexity based on the properties of the sample material. EM based methods have the advantage over all the previously discussed methods that they can provide information about the size and shape of individual particles and as well being able to distinguish (especially high resolution TEM) constituent particles within larger aggregates/agglomerates. The major disadvantage of EM methods is their limited availability in industry, the high purchase and running costs and slow throughput.

Given the significant limitations of the other size determination methods examined in this study, TEM has to be considered as the most reliable for classification of the pigments studied. However, in addition to this being an expensive and time consuming technique, it must again be stressed that TEM also has significant inherent difficulties in terms of preparing a representative sample of well dispersed particles, image interpretation, and determination of appropriate size parameters. With respect to the EC definition, it can be expected that a significant lab-to-lab variability of TEM determined median values of minimum constituent particle size will be observed for many industrial particulates. In order to reduce one of the sources of this variability, guidance and clarification regarding some aspects of, and terms used in, the EC definition is required.

9.2 Practical application of the EC nanomaterial definition with existing non-EM technology

In the course of this study a number of the more commonly available particle sizing instrumental methods (LS, DLS, CLS and BET), have been applied to the challenging task of determining the particle size distributions of industrial pigment materials. The study was conducted to evaluate what can reasonably be achieved using the facilities and knowledge available in the research and development or quality control laboratories within industry.

This study has shown the extent to which particle size measurements conducted with current technology and preparative procedures considered basic but realistic can produce results which are poorly reproducible and divergent from the results which had been expected from the extensive prior knowledge available to the manufacturers of the materials. Overall it was not possible to show that a simple unambiguous classification according to the EC definition could be made with the types of readily available technology and generic sample preparation procedures used in this study.

In considering this conclusion it is very important to note that this study has concentrated only on those methods which are commonly available in the pigment Industry. No attempt was made to compensate for the intrinsic limitations of these methods by use of alternative complimentary techniques.

Examples of moderately priced, commercially available measurement techniques which could compliment the methods examined in this study are Particle Tracking analysis (PTA) [10] or Asymmetrical Flow Field-Flow Fractionation (AF4) [11]. The additional information which could be gained from these methods could be valuable in screening tests and provide a cost-effective way of reducing but not eliminating the need for EM analysis. At this time electron microscopy remains the only method which can distinguish between primary/constituent particles and aggregates/agglomerates, as well as being able to determine appropriate size parameters for non-spherical particles. It therefore remains necessary for the application of the EC recommended definition.

9.3 The need for a pragmatic/practical approach to the task of materials classification

As mentioned above, the typical instrumentation used in this study, with a simple dispersion protocol, cannot be considered as being able to reliably classify pigments as nanomaterials or non-nanomaterials against the recommended EC definition. There are other techniques available (PTA, AF4, X-ray diffraction, small-angle X-ray scattering, atomic force microscopy, etc.) that can give some information about particle sizes, but which are not fully capable of addressing rigorously the various aspects of the EC Recommendation on the definition [3]. Even TEM, the most suitable technique ‘in principle’, presents major experimental and inherent difficulties which could lead to unreliable results for many samples. Thus it cannot be seen at this stage as a general solution to the problem of materials classification. Additionally, it is expensive and time consuming, and therefore not viewed by industry as a desirable method for routine sample analysis.

An additional source of uncertainty may be found in the EC definition itself. There are several aspects that require clarification, such as what constitutes an aggregate and what level of “fusion” of constituent particles is required before an aggregate becomes a single particle. This is more than just a problem of nomenclature. Some production processes include high temperature fusion or partial fusion of individual particles, and the resulting entities may have all dimensions above 100 nm and almost no likelihood of disintegrating into smaller particles in normal application environments, yet they may appear as complex aggregated entities when examined with TEM. In such cases it will be the TEM operator to decide subjectively what to take as the “constituent particles” in the size distribution analysis.

Another uncertainty arises in the choice of size parameter to determine for those constituent particles. The definition states “one or more dimensions” in the size range 1 nm to 100 nm. What is meant may be clear for spherical or rectangular parallelepiped shaped particles, but needs clarification for the more common situation of complex and non-uniform shapes.

Together with the known difficulties associated with measuring number size distributions of polydisperse materials using ensemble methods, these aspects rule out “in principle” the sole use of the large majority of particle sizing methods available on the market, making high resolution TEM analysis necessary for the implementation of the definition.

This is a situation that should be addressed firstly through the provision of a number of clarifications with respect to the definition, with possibly some adjustment of the wording and criteria of the definition itself, as part of the current revision process [12],[13].

Secondly, given that classification will be a requirement before significant further analytical development can take place, guidelines are needed on how to move ahead, at least using a provisional and pragmatic approach, with the problem of how to classify the broad range of industrial particulate materials already on the market, not only manufactured by the pigments industry, in a way that is acceptable to all stakeholders at the present time. This approach should not preclude the development of improved methodologies which is considered as essential to achieve reliable classification of nanomaterials.

As part of such an approach, we suggest that in some cases product families can be defined in which it is possible to unequivocally determine which specimen has a higher mean particle size than the other. In these cases it may be sufficient to characterise one “lead” member of the family by thorough methods, including EM, and do the relational grouping by other means. This might include simple logical considerations (based on synthesis parameters such as longer growth times, etc.) or, in the case of identical particle production methods where other parameters are changed, comparison of product characteristics that are shown to be related to final particle size. This would clearly require convincing case-by-case justification, but may allow a fast and cost-efficient screening of large existing portfolios of pigment materials.

The product family idea is presented in Fig. 9-1. In the case where a rigorous EM analysis classifies the smallest of the product family as being non-nano then logically the rest of the group can be similarly classified without the need for further EM analysis. The same is of course true for the reverse situation – if the member with the highest median particle size is a nanomaterial, the rest of the family members are also nanomaterials.

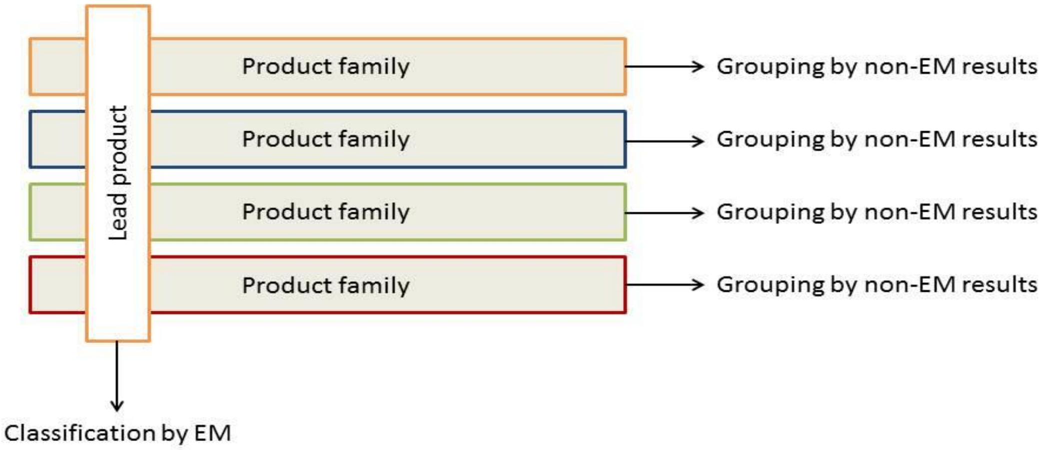


Figure 9-1 Possible grouping approach to screen large existing product portfolios

10 References

1. European Parliament. Strasbourg 2009. European Parliament resolution of 24 April 2009 on regulatory aspects of nanomaterials (2008/2208(INI)). P6_TA(2009)0328 Nanomaterials. Available at http://www.europarl.europa.eu/sides/getDoc.do?type=TA&reference=P6-TA-2009-0328&format=XML&language=EN.

2. European Union. Luxembourg 2011: Commission Recommendation of 18 October 2011 on the definition of nanomaterial. Publications Office of the European Union, Official Journal of the European Union (20.10.2011): L 275/38.

3. Linsinger T, Roebben G, Gilliland D, Calzolai L, Rossi F, Gibson N, Klein C. Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial". JRC Reference Reports, EUR 25404 , 2012. DOI: 10.2787/63995.

4. Van Doren EAF, De Temmerman P-J RH, Daoud Francisco MA, Mast J. Determination of the volume-specific surface area by using transmission electron tomography for characterization and definition of nanomaterials. Journal of Nanobiotechnology 2011. 9:17.

5. Wohlleben W. Validity range of centrifuges for the regulation of nanomaterials: from classification to as-tested coronas. J. Nanoparticle Res. 2012. 14:1300.

6. Braun A, Couteau O, Franks K, Kestens V, Roebben G, Lamberty A, Linsinger TPJ. Validation of dynamic light scattering and centrifugal liquid sedimentation methods for nanoparticle characterisation. Advanced Powder Technology 2010. 22:766-770.

7. ISO 13318-1:2001 Determination of particle size distribution by centrifugal liquid sedimentation methods - Part 1: General principles and guidelines, International Organization for Standardization, Geneva.

8. ISO 13318-2:2007 Determination of particle size distribution by centrifugal liquid sedimentation methods - Part 2: Photocentrifuge method, International Organization for Standardization, Geneva.

9. ISO 13318-3:2004 Determination of particle size distribution by centrifugal liquid sedimentation methods[9] Part 3: Centrifugal X-ray method, International Organization for Standardization, Geneva[10].

10. ASTM E2834 - 12 (2012) Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA), Active Standard ASTM E2834 Developed by Subcommittee: E56.02|Book of Standards Volume: 14.02, DOI: 10.1520/E2834-12.

11. Karl-Gustav Wahlund, Flow field-flow fractionation: Critical overview. Journal of Chromatography A, 1287 (2013) 97– 112.

12. H. Rauscher, G. Roebben, V. Amenta, A. Boix Sanfeliu, L. Calzolai, H. Emons, C. Gaillard, N. Gibson, T. Linsinger, A.Mech, L. Quiros Pesudo, K. Rasmussen, J. Riego Sintes, B. Sokull-Kluttgen, H. Stamm: Towards a review of the EC Recommendation for a definition of the term "nanomaterial" Part 1: Compilation of information concerning the experience with the definition, JRC Scientific and Policy Report, EUR 26567 EN, Eds H. Rauscher, G. Roebben, 2014.
13. G. Roebben, H. Rauscher, V. Amenta, K. Aschberger, A. Boix Sanfeliu, L.Calzolai, H. Emons, C.Gaillard, N.Gibson, U.Holzwarth, R. Koeber, T. Linsinger, K.Rasmussen, B.Sokull-Klüttgen, Hermann Stamm: Towards a review of the EC Recommendation for a definition of the term "nanomaterial" Part 2: Assessment of collected information concerning the experience with the definition. JRC Scientific and Policy Report, EUR 26744, Eds G. Roebben, H Rauscher, 2014.

European Commission
EUR 26916 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: **Basic comparison of particle size distribution measurements of pigments and fillers using commonly available industrial methods**

Author(s): Juan Bautista Vicent Pena, Klaus Kund, Uwe Hempelmann, Wendel Wohlleben, Thomas Koch, Alison Burke, Greg McNult, Angelika Hartl-Gunselmann, Stefan Knobl, Martin Reisinger, Douglas Gilliland, Neil Gibson, Birgit Sokull-Klüttgen, Hermann Stamm and Heike Liewald

Editor(s): Douglas Gilliland, Neil Gibson and Uwe Hempelmann

Luxembourg: Publications Office of the European Union

2014 – 90 – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series –ISSN 1831-9424 (online)

ISBN 978-92-79-43974-2 (PDF)

doi:10.2788/21024

LB-NA-26916-EN-N

JRC Mission

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Serving society
Stimulating innovation
Supporting legislation

doi:10.2788/21024

ISBN 978-92-79-43974-2

Publications Office