The EU FP7 NanoDefine Project

Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial

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NanoDefine Technical Report D3.5

The NanoDefine Consortium
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NanoDefine in a nutshell:

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano, or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

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1 Abbreviations and acronyms

ENM Engineered NanoMaterials

VSSA Volume-Specific Surface Area (in m^2/cm^3)

MSSA Mass-Specific Surface Area (in m^2/g)

BET determination of the overall external and internal MSSA of disperse (e.g. nano-powders) or porous solids by measuring the amount of physically adsorbed gas according to the Brunauer, Emmett and Teller method (definition from ISO 9277:2014-01)

SEM Scanning Electron Microscopy

TEM Transmission Electron Microscopy

EM Electron Microscopy

JRC Joint Research Center of the European Commission

OECD Organization for Economic Cooperation and Development

2 Summary

The VSSA approach has the important advantage over classifying, imaging and counting techniques that it does not involve dispersion protocols. Further, the BET technique as the basis for VSSA determination it is in widespread use, generates low costs and is specified for many commercial materials. Finally, the same equipment allows for a deeper analysis by full isotherm evaluation.

The present deliverable assesses all NanoDefine powders, supplemented by further real-world materials (in total 26 powders), and quantitatively compares the relationship between the median size (by Electron Microscopy – considered as benchmark for the EC nanomaterial definition) vs. the size derived from VSSA.

- Out of 26 materials, VSSA by BET with shape-specific evaluation classified 0 false negatives.
- Out of 26 materials, 23 are correctly classified by VSSA (from BET), and 3 are false positives.
    - The t-plot evaluation of isotherms (contributed by NANoREG) requires isotherm data over an extended pressure range, but resolves the false positives and brings the external VSSA in quantitative accord with EM.

This result was not compromised by the various compositions, strong agglomeration and sizes from 10 nm to 4 µm with typically 50% polydispersity. The VSSA method mitigates the challenges of EM to assess the thickness of platelets, but worked as well on fibbers and particles of irregular shapes. Multimodal substances and mixtures were not tested but are anticipated as not applicable to VSSA.

VSSA is currently applicable only to powders and requires 1 g of material. Stability under vacuum is required and operators must observe maximum admissible temperatures,

With the existing substance data from NanoDefine D1.3 as training set, we derive a screening strategy. If applied to the further data from real-world materials as validation set, this screening does achieve a correct classification, leaving only borderline materials for tier 2 assessment. The decision points are triggered by the D7.3 Materials Classification and by interim measurement results:

- Without prior knowledge on the materials, VSSA (by BET) identifies nano and non-nano materials with appropriate uncertainty margins from the cut-off value.
- Reduction of uncertainty margins is achieved with knowledge of shape and absence of multimodality (e.g. from a single SEM scan).
    - Further reduction of uncertainty margins is anticipated by data on materials with a logical size relationship but was not explored here.
- The t-plot method is not proposed to replace but to supplement BET, in case the material composition raises concerns of false positive classification by microporosity.

With the above strategy, the agreement between the size from VSSA and the size from EM is very good with less than 20% deviation for a wide range of materials. VSSA is not validated for multimodal distributions or mixtures, and is not applicable to suspensions, formulations, articles, consumer products.

VSSA is proposed for monoconstituent substances as Tier 1 screening of both nano- and non-nano materials.

3 Introduction

Volume-Specific Surface Area (VSSA, units of m^2/cm^3) is an ensemble property of powders. VSSA is inverse proportional to the size of the powder particles. An ensemble of monodisperse spheres with 100 nm diameter has a VSSA of 60 m^2/cm^3.

VSSA has the important advantage over classifying and counting techniques (including TEM) that it does not involve dispersion protocols. VSSA is acknowledged in academic literature as an agglomeration-tolerant method with low costs, wide availability, and a wealth of existing data.(Allen 1997) Before publication of the EC nanomaterial definition, VSSA was a candidate criterion for identification of both nano and non-nano materials. VSSA appeared to be a solution especially for particulate materials that only have a size fraction in the nano-scale, or that contain primary nanostructures in highly agglomerated or aggregated forms.(Kreyling et al. 2010)

The EC recommendation for a regulatory definition of nanomaterials (EC, 2011), states that where technically feasible and requested in specific legislation the VSSA can be used to determine compliance with the definition of nanomaterial. A material should be considered as falling under the recommended definition of nanomaterial if the VSSA of the material is greater than 60 m^2/cm^3. However, VSSA was not recommended to classify a material as a non-nanomaterial, because the quantitative relation to the key criterion of the EC nanomaterial definition – the median diameter in number metrics – was unknown due to lack of data.

According to the NanoDefine DoW, following aspects on VSSA are included:
The arrow → designates what the present deliverable reports:

1. Relation of VSSA to the number based particle size average and size distribution on reference and test samples and analytical data from NanoDefine
   → on all NanoDefine powder substances we report BET results from multiple labs (BAM, BASF, NanoDefine producers), and the relation to EM D50 extracted from NanoDefine measurements.
2. Quantitative relation of VSSA to number based particle size distribution for real-world samples
   → a suite of inorganic and organic particles / rods from BASF portfolio, BET and EM;
   → a suite of fillers and pigment from the JRC/Eurocolor round robin, BET and EM.
3. Applicability ranges of the VSSA method as rapid screening tool;
   identification of material properties which lead to false negatives or false positives from VSSA (BAM)
   → false positives of VSSA by BET are linked to powder material characteristics.
   → mitigation of false positives of VSSA by NANoREG isotherm evaluation
   → false negatives of EM are linked to material dimensionality
4. Expanding the VSSA concept to D10 or D1 distribution values to address deviating thresholds chosen in food and cosmetics regulation
   → cancelled, because the European Cosmetics Regulation (legally in force, 2012) and Novel Food Regulation do not use D10 or D1 distribution cutoffs.
5. Close cooperation with the NanoReg project
   → formal agreement via the NANoREG coordinator

→ all NanoDefine materials have been measured (MBN, BASF) according to NANoREG SOP, evaluation by NANoREG is reported here.

4 VSSA determined by BET

The VSSA is obtained by multiplication of mass-specific surface area (MSSA) with the skeletal density ρ. Specifically, MSSA is determined by BET in units of m²/g, and ρ in units of g/cm³.

(Equation 1) $$VSSA = MSSA * \rho$$

The resulting units of VSSA are m²/cm³. It is important to note that the correct density value is not the pour density but must be skeletal density, defined as "the ratio of the mass of discrete pieces of solid material to the sum of the volumes of: the solid material in the pieces and closed (or blind) pores within the pieces" (ASTM D3766).

Volume-Specific Surface Area (VSSA, units of m²/cm³) is an ensemble property of powders. VSSA is inverse proportional to the size of the powder particles. An ensemble of monodisperse spheres with 100 nm diameter has a VSSA of 60 m²/cm³.

4.1 BET method (Excerpt from NanoDefine Technical Report D3.1)

4.1.1 BET for determination of specific surface area - Measuring principle

The Brunauer-Emmett-Teller (BET) theory was derived 1938 to explain the physical adsorption of gas molecules on a solid surface (Brunauer et al., 1938). BET serves as the most often applied technique for the measurement of the specific surfaces of a material – typically porous and powder materials. BET explains multilayer adsorption of gas molecules on a solid and dry material. Nitrogen and argon gas are widely used for measurements. BET is based on three hypotheses:

1.) gas molecules can adsorb physically in multilayers of infinite thickness,
2.) no interaction exist between adsorbed layers, and
3.) the Langmuir theory is applicable for each layer of gas molecules.

The resulting BET equation is applied for fitting experimental gas adsorption isotherms and gives the adsorbed monolayer gas quantity. Knowledge of gas quantity, adsorption cross section of the adsorbing gas and the molar gas volume allows calculation of the specific surface area of the material (Dabrowski, 2001). The method applied here includes the volumetric static measurement of the nitrogen isotherm at 77.3 K with data evaluation according to the BET theory in the relative pressure range between 0.001 and 0.3 according to the international standard ISO 9277:2010. Samples were prepared for adsorption analysis in a degasser, here the samples were heated (up to 250 °C for inorganics, up to 100°C for organics) under vacuum for 30 min or more to remove moisture and other contaminations. At BAM all measurements were performed in a laboratory accredited according to ISO 17025.

4.1.2 Performance – general remarks

According to NanoDefine Technical Report D3.1, VSSA (by BET) is the only technique apart from SEM and TEM to cover the entire size range from 1 nm to 10 µm diameter, with limitations of SEM and TEM to reach the lower and upper limits, respectively.
The BET method is widely used and accepted in Industry, academia and (governmental and regulatory) research institutes. For example, the National Institute of Standards and Technology (NIST, US) and BAM provide a practical guide for its application which is available without charge from NIST (Klobes et al., 2006). BET is standardized (ISO 9277:2014-01). Certified reference materials are currently available from BAM in Germany, JRC-IRMM in Belgium, NIST in the USA, and APPIE in Japan. The ISO 9277:2014-01 standard lists 19 certified reference materials with BET surfaces from 0.104 ± 0.012 m²/g to 550 ± 5 m²/g, thus covering well the relevant range from non-nano to nano materials. BET can be applied easily. The BET theory is based on expansive assumptions (see above), but the results obtained by BET can be made SI-traceable (Hackley, 2013). Round-Robin tests for the development of reference materials for BET as performed by BAM proved good accuracy of the BET method. An inter-laboratory study to evaluate "real-world" precision and bias of specific surface area measurements was reported on a powdered TiO2 material containing sub-30 nm primary crystallites (NIST RM 1898). Based on results from 19 laboratories, overall performance was good. Estimates of precision ranged from 0.10 to 3.96 % and measurement bias was generally within ±5 % of the certified sur-

face area value of the material. Between-laboratory variability accounted for 91 % of the total variance and is likely explained by gravimetric errors.(Hackley and Stefaniak 2013)

Type of samples	dry solid
Particle property measured	Surface area
Type of quantity	surface per mass ratio as integral value over all particles in a test sample: Mass-specific Surface Area (MSSA)
size range	all size ranges from 1 nm to 10 µm
Main advantages with regard to NanoDefine	
• BET is a standardised methodology (ISO 9277:2014-01) • BET does not require dispersion; operates on as-produced agglomerated and aggregated powders. • BET values are widely specified for commercial particulate materials. • Widespread availability; the total cost of ownership is low • Although measurement times are hours for some samples, the technician time for a complete measurement and evaluation is about 0.5 h, resulting in low costs per measurement • Certified reference materials are available for a wide range of specific surfaces up to 1300 m^2/g and down to 0.1 m^2/g, thus covering wide ranges on both sides of the VSSA cutoffs.	
Main disadvantages with regard to NanoDefine	
• Particles and non-particulate porous materials cannot be distinguished • Materials must not release volatile compounds. • Measurement times can be in the range of hours and increase with increasing surface area • As yet, the quantitative relationship between VSSA and EM D50 has not been assessed.	

4.2 He-pycnometry

The density of the samples is determined by He-pycnometry according to DIN 66137-2:2004. This method measures the volume of a sample by placing it in a chamber of known volume, which is connected via a valve to a second chamber of known volume. Before starting the measurement, the whole system is flushed with He gas to remove remaining air in both chambers, which are subsequently sealed off by closing the valves. The He pressure in the sample chamber is increased by adding He gas until a certain constant value is reached, while the second chamber stays at ambient pressure. When opening the valve between the two chambers, both pressures equilibrate and from the pressure change in both chambers and the known chamber volumes, the sample volume can be calculated. The density is then obtained by relating the measured sample volume to its mass and is commonly referred to as the skeletal density.

4.3 VSSA (by BET) results on NanoDefine test materials

NanoDefine Technical Report D1.3 reports values of MSSA as measured by BET at partner BAM in compliance with DIN ISO 9277:2010 (Multi-Point-BET mit N_2) as well as at material producers and of skeletal density (measured by He-pycnometry at partner BAM). Additionally, BET measurements were repeated by partner BASF. The resulting values and standard deviations (n=3: each one result from BAM, from BASF and from the supplier) are reported in **Fehler! Verweisquelle konnte nicht gefunden werden.**, including the conversion to VSSA by (Equation 1). The relative standard deviation (RelStDev) is below 10% for most materials, below 20% for all materials.

For IRMM-385 Kaolin the table reports the values measured by BASF, and two replicates measured by BAM. These agree within 2.5% relative standard deviation. An outlying value from the supplier was excluded. IRMM-381 BaSO4 (fine grade) has a relative standard deviation approaching 20%. Although this may be linked to its low MSSA, another low-surface-material (IRMM-384 CaCO3) has below 3% relative standard deviation. Replicate measurements at MBN identify different evacuation times as source of the 15% standard deviation for the microporous BAM11- Zeolite.

Material	**MSSA (BET) (n=3)**	**MSSA (BET) StDev**	**skeletal density**	**VSSA (BET)**	**VSSA (BET) StDev**	**VSSA (BET) RelStDev**
	m^2/g	**m^2/g**	**g/cm^3**	**m^2/cm^3**	**m^2/cm^3**	**%**
IRMM-380 – organic pigment (transparent)	67.7	4.7	1.5	100.4	6.9	7
IRMM-386 – organic Pigment (opaque)	17.5	0.9	1.5	26.2	1.3	5
IRMM-381 - BaSO4 (fine grade)	2.5	0.5	4.4	11.1	2.0	18
IRMM-387 – BaSO4 (ultrafine grade)	36.9	0.4	4.0	148.0	1.6	1
IRMM-382 - MWCNT	252.7	16.9	1.8	454.8	30.5	7
IRMM-383 – Nano Steel	9.7	1.0	7.8	75.3	7.5	10
IRMM-384 – CaCO3 (fine grade)	5.8	0.1	2.7	15.5	0.4	2
IRMM-385 - Kaolin	16.0	0.4	2.61	41.8	1.0	2
IRMM-388 – coated TiO2	14.8	0.4	4.0	58.9	1.4	2
BAM11 – Zeolite powder	370.5	54.5	2.1	766.9	112.8	15
IRMM-389 – basic methacrylate co-polymer	1.3	0.1	1.1	1.5	0.1	8

Table 1: VSSA (by BET) results on NanoDefine test materials

4.4 VSSA (by BET) results on fillers and pigment from JRC/Eurocolor round robin

In a pilot round robin, BET and VSSA were reported on a series of fillers and pigments from eight labs throughout Europe.(Gilliland et al., 2014) The results (Table 2) were reproducible within a relative standard deviation (RelStDev) of less than 20%, an exception being again the material of lowest MSSA (Cu/Zn pigment metal 2). TEM with manual or semi-automated or automated (only fumed SiO2) image evaluation of the smallest external diameter was performed as benchmark. Due to the complex shapes, the authors noted a considerable ambiguity in TEM evaluation with respect to the selection of the smallest external dimension. They express concerns that TEM may have not measure the smallest dimension.

Material	MSSA (BET) (n=8)	MSSA (BET) StDev	skeletal density	VSSA (BET)	VSSA (BET) StDev	VSSA (BET) RelStDev
	m^2/g	m^2/g	g/cm^3	m^2/cm^3	m^2/cm^3	%
Fumed SiO_2	209	7.7	2.2	459	16.9	4
FeOOH Pigment Yellow 42	88.2	8.6	3.7	326	31	10
TiO_2 Rutile	14.8	0.8	4.1	61	3.2	5
Cu/Zn Pigment metal 2	4.7	1.4	7.7	36	10	28
Fe_2O_3 Pigment Red 101	8.8	0.5	5.0	44	2.2	5
$CoAl_2O_4$ Al-Co-Blue	7.8	0.3	4.2	33	1.4	4
TiO_2 Anatase	9.1	0.4	3.8	35	1.4	4
Azo Pigment Yellow 83 transparent	58.7	11.4	1.5	86	17	20

Table 2: VSSA (by BET) results on inorganic and organic fillers and pigments from the JRC/Eurocolor round robin (JRC 2014), both below and above the cutoff

4.5 VSSA (by BET) results on further real-world materials

Pigments, fillers, anticaking agents are clearly particulate, and product performance is linked to their relatively well-defined morphology. There is however no technical relevance of size in number metrics. Datasheets typically specify size in volume metrics or specific surface area. For the present evaluation, the BET values and skeletal density values were used as provided in online datasheets, to determine the VSSA (by BET) of a suite of materials representative for the BASF pigments portfolio, comprising both organic, inorganic, and metal-organic complex materials (all powders). Results are summarized in **Fehler! Verweisquelle konnte nicht gefunden werden.**. TEM with semi-automated image evaluation of the smallest external diameter of constituent particles inside aggregates was performed as benchmark.

Material	MSSA (BET) (n=1)	skeletal density	VSSA (BET)
	m^2/g	g/cm^3	m^2/cm^3
Pigment Yellow 42 (transparent)	83	3.9	323.7
Pigment Red 101	93	4.5	418.5
Pigment Yellow 139	25	1.7	42.5
Pigment Red 254 (opaque)	15	1.63	24.5
Pigment Red 254 (transparent)	94	1.63	153.2
Pigment Blue 15:4	64	1.61	103.0
Pigment Orange 73	23	1.3	29.9

Table 3: VSSA (by BET) results on further real-world materials, both below and above the cutoff.

Within the real-world application of the EC nanomaterial definition, one has to screen an even wider diversity of materials than usually considered in nanomaterial definition impact assessments, such as by BiPRO (2013). Since no criteria other than size are used, and since no upper size limit is given by

the EC nanomaterial definition, essentially all granular matter that has "particle boundaries" needs to be screened against the EC nanomaterial definition criteria. We can designate these as "non-engineered particulates", if their product performance does not depend on properties in the particulate state of matter, because that is only an intermediate state useful for storage or shipping, whereas the product performance is after melting, dissolution, reaction or other loss of particulate structure. Examples include mortars, organics, solidified waxes, polymer granulates, salts.
Due to the non-engineered shape of these materials, very complex and very inhomogeneous structures are common, this making an image evaluation certainly cumbersome, to some extent arbitrary, if not impossible. Large average size is common and limits electron-transparency, making them inaccessible to TEM. Very often, already the dispersion step before TEM analysis fails because the materials react or dissolve. SEM without sample preparation will still deliver an image, at the price of increased image evaluation complexity.
Also BET values are typically not specified for non-engineered particulates, but at least they can be determined under conditions discussed below in section 6.1.1. Also very complex structures thus become accessible to a classification by the EC nanomaterial definition.

4.6 Quantitative relation of VSSA (by BET) to EM D50

A quantitative relation between VSSA and EM can be provided only for engineered particulate materials (both nano and non-nano), where the EM evaluation is unambiguous and allows identification of the remaining discrepancies.

4.6.1 VSSA cut-offs adapted to the dimensionality as introduced by JRC-report #2 (2014).

The JRC report #2 introduces VSSA cutoffs that are adapted to the shape of the material.(Roebben et al., 2014) With D = number of small dimensions, the shape-specific VSSA cutoff is given by:

Equation 2 $$\text{VSSA cutoff} = \mathbf{60}\frac{\boldsymbol{m^2}}{\boldsymbol{cm^3}} * \frac{\boldsymbol{D}}{\mathbf{3}}$$

This concept was recognized by JRC (Roebben et al., 2014) as a powerful tool to provide an average value of the smallest dimension in the sense of the EC nanomaterial definition. NanoDefine Deliverable 3.1 recognizes that all dispersion-based techniques fail for shapes other than roughly spherical, whereas few techniques (EM, SAXS, XRD, BET) provide access to the smallest dimension of primary particles, and of these, only EM and BET cover the entire size range from 1 nm to 10 µm.(NanoDefine D3.1, section 6)

We use the same concept to derive a quantitative relation between the VSSA (determined by BET) and and the median diameter in number metrics (determined by EM, in short: D50 (EM)):

Equation 3 $$\text{relative VSSA (in \% of VSSA cutoff)} = \mathbf{60}\frac{\boldsymbol{m^2}}{\boldsymbol{cm^3}} * \frac{\boldsymbol{D}}{\mathbf{3}} * \frac{\mathbf{1}}{\boldsymbol{VSSA}} \times \mathbf{100}$$

Equation 4 $$\text{relative size (in \% of D50 cutoff)} = \frac{\boldsymbol{D50}}{\mathbf{100}\boldsymbol{nm}} \times \mathbf{100}$$

In words, Equation 3 extracts from a specific surface area measurement the diameter of the smallest dimension, and compares it to the 100 nm cutoff. Equation 4 does the same for the median diameter in number metrics. Section 6.1.1 discusses the material properties that are implicitly required to perform this quantitative evaluation, and 6.1.2 discusses the pragmatic application as a screening tool.

In a strict sense, the shape-dependent cutoffs assume that the contribution to surface area is negligible from the surfaces that delimitate the large dimensions (the edges of platelets, the ends of rods). Here we employed a pragmatic approach and used the aspect ratio of 3:1 to differentiate whether a given material is best approximated as consisting of particles, rods, or platelets. Specifically, the number of small dimensions and VSSA cutoffs are

- Particle (aspect ratio <3:1) D=3 → nano, if VSSA > 60 m²/cm³
- Rod (aspect ratio >3:1:1) D=2 → nano, if VSSA > 40 m²/cm³
- Platelet (aspect ratio >3:3:1) D=1 → nano, if VSSA > 20 m²/cm³

The resulting quantitative comparison is summarized in Table 4. The color code highlights agreements and discrepancies, with results "nano" in green, results "non-nano" in red, and results close to the cutoff in white.
As can be immediately seen, cases of discrepancy between the "nano"/"non-nano" classification by VSSA from BET and D50 from EM are rare, and even in the most naïve evaluation (simply using all 26 lines of available data, without quality filter), the average discrepancy is only 21%. Adding to this surprisingly good agreement, even the worst case has less than a factor of 2 discrepancy in the decisive size criterion.

4.6.2 Discrepancy by porosity: VSSA (by BET) classifies some materials false positive

Visually, the discrepancies between D50 (EM) and VSSA (BET) are lines where different color codes collide. This is the case for:

- IRMM-388 – coated TiO2
- BAM11 – Zeolite powder
- TiO2 Rutile (from JRC/Eurocolor round robin, possibly the same material as IRMM-388).

These materials are classified as "nano" by VSSA, whereas EM shows a D50 above 100 nm. For Zeolites, quite obviously the mesoporous nature (inner porosity) adds to the BET surface area, but should not be considered since the EC nanomaterial definition does not include internally nanostructured and porous materials. Similarly, the surface coating (coating porosity) of the TiO2 is known to contribute to the specific surface.
These are cases of false positives, where VSSA classification would mark materials for nano-specific regulation. To resolve the discrepancy, one can either exclude mesopores from the adsorption isotherm evaluation (see section 5 , t-plot methodology), or one performs EM evaluation to prove that by the D50 criterion the material is actually non-nano.

4.6.3 Discrepancy by shape: VSSA (by BET) implements the EC definition stricter than EM

Discrepancies cumulate whenever EM image evaluation contributes significantly to uncertainty. The JRC/Eurocolor report (Gilliland et al., 2014) comments: "identification and detection errors [in EM] … 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."

This is the case for:	(NanoDefine D1.3), (Gilliland et al., 2014)
• IRMM-383 – Nano Steel	platelets: SEM not measurable
• IRMM-385 – Kaolin	platelets: SEM false negative - measured lateral dimension
• Cu/Zn Pigment metal 2	platelets: SEM false negative, does not detect thickness
• Fe2O3 Pigment Red 101	complex shape: TEM difficulty to assign minimal dimension
• CoAl2O4 Al-Co-Blue	not dispersable: TEM cannot distinguish constituent particles
• Pigment Orange 73	platelets: TEM false negative, does not detect thickness

For all these materials, VSSA delivers an equivalent diameter value, whereas EM completely failed, or EM reported larger diameters that are considered false negatives.

Obviously, platelets are a class of materials that tend to be false negatives in EM (unless evaluated manually, with great care and sample dispersion skills), whereas they can be assessed easily by VSSA, in accord with the JRC report #2. (Gilliland et al., 2014) A simple and single EM image, even SEM without dispersion, is enough to inform the user of the approximately platelet shape. An appropriate screening strategy for known and unknown shape is presented in section **Fehler! Verweisquelle konnte nicht gefunden werden.**.

Further, many materials resist standard dispersion protocols. These cannot be assessed by EM, and if subjected to semi-automatic evaluation, EM will produce false negatives.

Material	D	VSSA (BET) m²/cm³	VSSA (BET) % of cutoff	D50 (EM) % of cutoff	VSSA OK?	Comments
IRMM-380 – org. P. (tr)	2	100	40	40	OK	
IRMM-386 – org. P. (op)	2	26	153	157	OK	acc. to NanoDefine D1.3 "particle", but elongated in EM. Evaluated with D=2.
IRMM-381 - BaSO4 (f)	2	11	360	214	OK	acc. to NanoDefine D1.3 "rhombohedral", but elongated in EM. Evaluated with D=2
IRMM-387 – BaSO4 (uf)	3	148	41	35	OK	
IRMM-382 - MWCNT	2	455	9	10	OK	
IRMM-383 – Nano Steel	1	75	27		OK	platelets: EM false negative / not measurable
IRMM-384 – CaCO3 (f)	2	15	258	158	OK	
IRMM-385 – Kaolin	1	42	48	128	OK	platelets: SEM measured lateral dimension
IRMM-388 – coatd TiO2	3	59	102	213	false positive	surface coating porosity
BAM11 – Zeolite powder	3	767	8	133	false positive	internal pores
IRMM-389 – basic methacrylate copolymer	3	1	4 084	2 000	OK	
Fumed SiO2	3	459	13	12	OK	
FeOOH P. Yellow 42	2	326	12	20	OK	
TiO2 Rutile	3	61	98	210	false positive	surface coating porosity
Cu/Zn P. metal 2	1	36	56		OK	platelets: EM false negative / not measurable
Fe2O3 P. Red 101	3	44	136	249	OK	complex shape: TEM hard to assign small'st dimens.
CoAl2O4 Al-Co-Blue	3	33	182	527	OK	not dispersable: TEM cannot assign particles
TiO2 Anatase	3	35	171	130	OK	
Azo P. Yellow 83 tr	3	86	70	47	OK	
Pigment Yellow 42	2	324	12	10	OK	
Pigment Red 101	2	419	10	9	OK	
Pigment Yellow 139	3	43	141	150	OK	
Pigment Red 254 (op)	3	24	245	233	OK	
Pigment Red 254 (tr)	3	153	39	36	OK	
Pigment Blue 15:4	2	103	39	30	OK	
Pigment Orange 73	1	30	67		OK	platelets: EM false negative / not measurable

Table 4: Quantitative relation between VSSA (by BET) and D50 in number metrics by EM. D is the number of small dimensions (D=1 for platelets, D=2 for rods/fibbers, D=3 for all other shapes). The % of cutoff are calculated by **Equation 3** and **Equation 4**, and refer for both VSSA and EM D50 to the smallest dimension being 100 nm diameter. The color code highlights agreements and discrepancies, with results "nano" in green, results "non-nano" in red, and results close to the cutoff in white.

4.6.4 Material classes with close agreement between VSSA (by BET) and D50 (by EM)

Out of 26 materials, 23 are correctly classified by VSSA.

If we remove the cases with known issues in the benchmark EM evaluation (platelet shape and indispersible materials), and with known issues in VSSA evaluation (porous materials), the remaining list follows. For the NanoDefine materials, the EM evaluation from D1.3, despite their limited statistics, provide orientating values of the polydispersity of these materials, indicated as relative standard deviation in brackets (EM StD divided by the EM median).

- IRMM-380 – organic pigment (transparent) (polydispersity: 29%)
- IRMM-386 – organic Pigment (opaque) (polydispersity: 55%)
- IRMM-381 - BaSO4 (fine grade) (polydispersity: 56%)
- IRMM-387 – BaSO4 (ultrafine grade) (polydispersity: 57%)
- IRMM-382 – MWCNT (polydispersity: 19%)
- IRMM-384 – CaCO3 (fine grade) (polydispersity: 52%)
- IRMM-389 – basic methacrylate copolymer (polydispersity: 70%)
- Fumed SiO2
- FeOOH Pigment Yellow 42
- TiO2 Anatase
- Azo Pigment Yellow 83 transparent
- Pigment Yellow 42
- Pigment Red 101
- Pigment Yellow 139
- Pigment Red 254 (opaque)
- Pigment Red 254 (transparent)
- Pigment Blue 15:4

The above set of materials – regardless of various compositions, polydispersity, irregular shapes, strong agglomeration – features an average deviation between VSSA (by BET) and D50 (by EM) of 11%, with a maximum deviation of 21%.

4.6.5 Quantitative relation: absence of false negatives despite polydispersity

Out of 26 materials, VSSA with shape-specific evaluation classified 0 false negatives (none).

This result was not compromised by the various compositions, irregular shapes, around 50% polydispersity, and strong agglomeration.

It should be kept in mind that theoretical considerations on calculated VSSA values of polydisperse or multimodal materials can lead to false negatives (Roebben et al., 2014). Specifically, a normal distribution with 50% polydispersity was shown to induce less than 10% mismatch between the VSSA cutoff and the median size cutoff (Figure 1 in Roebben et al., 2014). Our experimental data in section 4.6.4 and Table 4 show that these assumptions coincide with the typical polydispersity of real-world particulate materials, and confirm that the mismatch is indeed limited. For a lognormal shape of the distribution the discrepancy is maybe larger, but the decisive parameter is the relative polydispersity. A theoretical test case further below (section 6.1.3) explores the boundary conditions for the use of VSSA as screening tool.

Strong disagreement between surface-based and number-based classification may arise with multimodal materials which can, depending on their particle size distribution, display a very low VSSA even if they are a nanomaterial, i.e. having a median diameter below 100 nm. One example is the case of sea sand in Roebben et al., 2014. To include the case of a mixture, one can theoretically model a bimodal mixture of 20 nm particles and 840 nm particles, each with 110% polydispersity, which will have a VSSA of only 5.9 m^2/cm^3 if 0.19% in mass metrics (corresponding to 97.7% in number metrics) of this mixture are below 100 nm diameter. As no multimodal material is included in this report, the influence of multimodality and mixtures on the VSSA-based classification remains completely open. Consequently, for a reliable classification of a material as non-nano by VSSA, it needs to be proven non-multimodal by another experimental method.

5 Cooperation with the NANoREG project

5.1 Extract from the scope of the NANoREG DoW (WP2A)

"To use the criteria VSSA > 60 m^2/cm^3 to measure the particle size, we have to assume that the particles are mono-modal, spherical and non-porous. This implies to measure the external surface area of the particles, without taking into account the internal porosity of these particles. Up to now the only existing proposal is to define the VSSA by combining the BET surface area and the density of the powder. This way is misleading because the BET surface area takes into account the internal porosity of the particles instead of their external surface. This will then define false positive nano-materials. It means that a lot of powder will be falsely considered as "nano". "

Complementary to and beyond NanoDefine activities on VSSA, in NANoREG a more in-depth analysis of these isotherms is foreseen in order to develop a tool to discriminate between the external particle surface and the internal or coating porosity in line with the VSSA criteria.

5.1.1 Terms of cooperation (Annex I).

To secure that all intellectual property rights are respected, formal agreements have been made with the NANoREG coordinators at the Dutch Ministry of Infrastructure and the Environment.

- NANoREG will provide the preferred parameters for optimal raw data quality and will point to suitable instrumentation to measure nitrogen adsorption isotherms (done, Envicat, Nov 2014)
- NanoDefine will determine nitrogen adsorption isotherms on their series of samples and provide the isotherms files to NANoREG (done, BASF, Jan 2015)
- There is even no exchange of materials, only of data. The materials will be labelled with their unique IRMM-3xx and BAM-xxx codes and their chemical identification.(done, BASF, Jan 2015)
- NANoREG will then proceed to the analyses of these isotherms and provide the results of this analysis back to NanoDefine. (done, Envicat, Feb 2015)
- It is envisaged that the VSSA (by BET) and VSSA (by isotherm, NANoREG evaluation) are integrated into the NanoDefiner decision tree that points to valid methodology for specific material properties. (WP7)

Ministry of Infrastructure and the Environment

> Return address P.O. Box 1, 3720 BA Bilthoven, The Netherlands

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Attn Mr. A LeCloux
General Manager

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Our reference
NANoREG 2014/023

Date 24 December 2014
Subject Collaboration on VSSA

Dear Mr LeCloux,

Collaboration between NANoREG project and the NANoDefine project on the development and evaluation of methods for measuring the Volume Specific Surface Area (VSSA) of nanomaterials, will be beneficial to the NANoDefine project as well as to the NANoREG project.

BASF (partner in both the NANoREG project and the NANoDefine project) and Envicat (partner in the NANoREG project) have expressed their willingness to come to such a collaboration and have defined the scope of this collaboration in the annex to this letter.

Both organisations agree that the confidentiality and IPR aspects of this collaboration will be governed by the relevant provisions of the NANoREG Consortium Agreement that previously has been signed by both organisations.

I kindly ask you to confirm this agreement by signing this letter and return it to me.

Yours sincerely,

ing. A.H.J. Dijkstra
Project Manager NANoREG

Organisation: Envicat
Name: André Lecloux
Function: General Manager

Signature

Date 6 January 2015

Ministry of Infrastructure and the Environment
Project Office NANoREG

NANOREG

5.1.2 Evaluation Method: t-plot isotherm analysis

Via an in depth analysis of nitrogen adsorption isotherms and mercury penetration techniques, it was demonstrated that it is possible to separate external and internal surface area of particles. (Lecloux, 1981). This possibility was demonstrated on a series of silica spherical particles in the nano range (Lecloux et al, 1988). This method is using in particular the t-plot method as proposed by (de Boer et al, 1965) and modified by Lecloux (1981 and 1988). This approach of the adsorbed amount as a function of relative pressure is compared graphically with a normalized reference isotherm obtained on a non-porous sample. This reference isotherm is chosen according to the value of the C_{BET} constant of the isotherm under test, this constant being representative of the adsorption energy. This plot is able to identify the presence of a microporosity and can provide information on the total and external surface area of the particles. The Dubinin diagram is also used to characterize the microporosity when it is present.

This method is easily applicable to the dry powders without any treatment, but it requires longer evacuation time with more data points at low pressure than a routine BET measurement. The aim is to extend the application and validity of this method for non-spherical and/or poly-disperse particles.

5.2 Results of NANoREG on NanoDefine materials

Nitrogen adsorption isotherms were measured by two NanoDefine laboratories (BASF and MBN) and the raw data supplied to NANoREG, where the evaluation was performed.

5.2.1 Exemplary material: IRMM 388 coated TiO2 (non-nano) and NM103 coated TiO2 (nano)

The t-plot is shown here after, indicating the presence of small micropores (confirmed by the Dubinin plot) of about 0.9 nm and responsible for about half the surface area (S_μ). The size of these micropores is what could be expected from the coating process. St represents the total surface area which is similar to the BET value. The "external" surface is obtained by subtracting S_μ from S_t. Additional very low pressure isotherms were just supplied by MBN, but not yet evaluated.

All the surface area values are expressed in m²/g.

S_{BET} BAM	S_{BET} BASF	S_t	S_μ	S_w
14.4	15.1	15.5	7.7	7.8

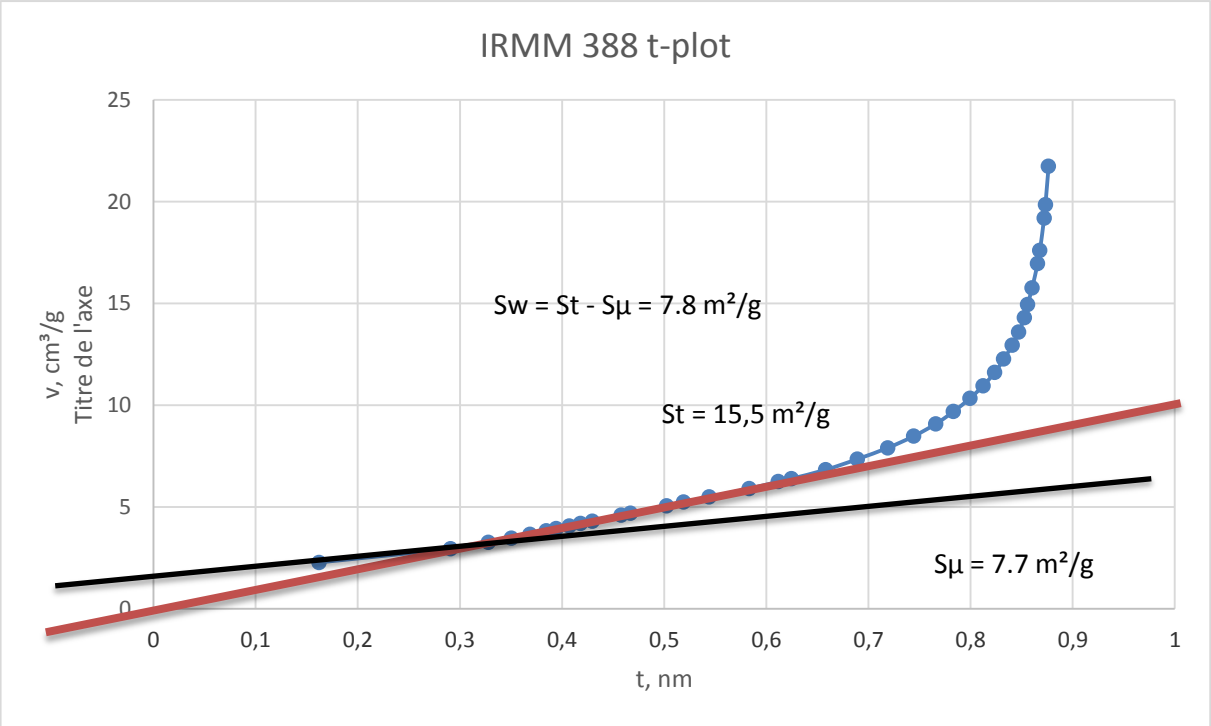

Figure 1 t-plot evaluation of IRMM 388 isotherm (courtesy of NANoREG)

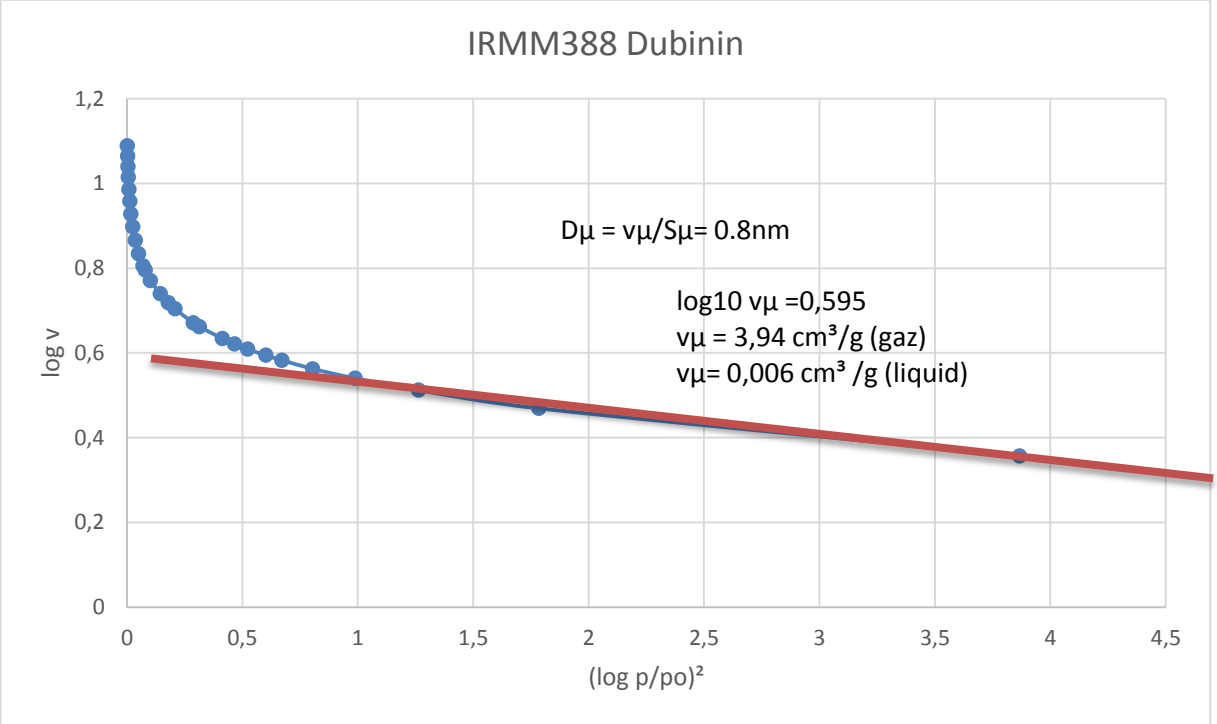

Figure 2 Microporosity contributions to pore volume of IRMM 388 (courtesy of NANoREG)

In the frame of the NanoReg project another coated sample of titanium dioxide (NM103 from the JRC repository of nanomaterials) was also considered. The nitrogen adsorption isotherm on this sample was obtained in two different laboratories with an excellent reproducibility. The resulting t-plot is showing the presence of a microporosity confirmed by the Dubinin plot with a pore size of about 0.5 nm. This is a very similar behavior to the IRMM 388 sample even if NM 103 is nano while IRMM 388 is not.

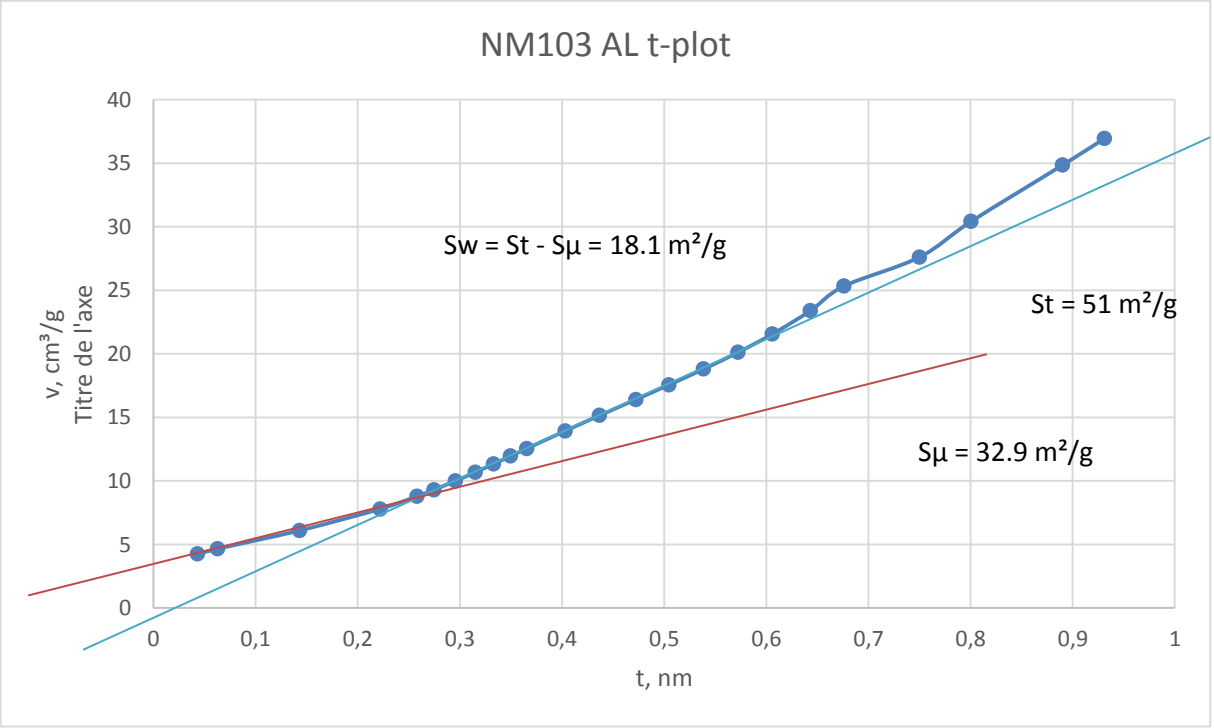

Figure 3 t-plot evaluation of NM103 isotherm (courtesy of NANoREG)

5.2.2 Exemplary material: BAM 11 Zeolite

The t-plot is shown here after, indicating that the major part of the surface area is due the presence of a very significant microporosity which due to the internal structure of the zeolite. The "external" surface is directly measured from the high pressure part of the t-plot (red line); the number of experimental points in the very low pressure region of the BASF isotherm is not sufficient to accurately assess the microporosity; this explains also the low value of the S_{BET} from BASF compared to the S_{BET} from BAM. A preliminary S_t fit through the origin and the data point at lowest pressure provides a value of S_t = 425 m²/g, which actually coincides with the S_{BET} evaluation at BAM. The microporosity contribution is estimated from the difference between S_t and S_w. Additional very low pressure isotherms were just supplied by MBN, to enable a consistent evaluation.

All the surface area values are expressed in m²/g.

S_{BET} BAM	S_{BET} BASF	S_t	S_μ	S_w
425	316	425	395.8	29.2

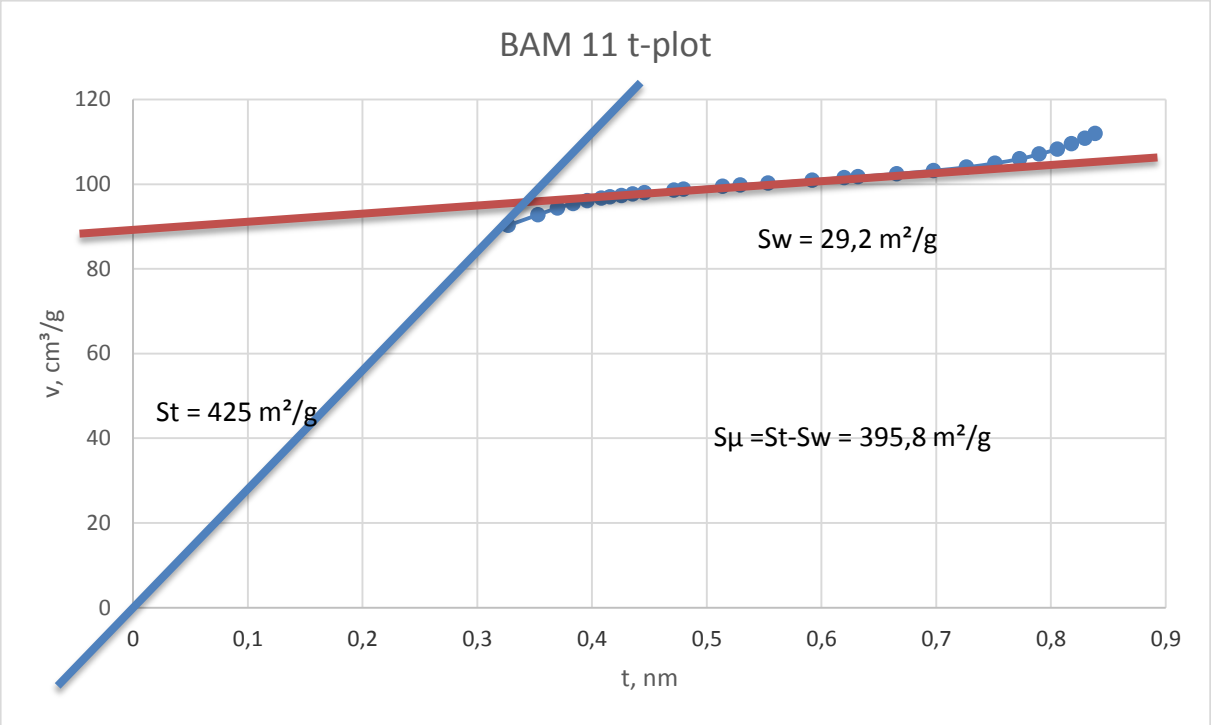

Figure 4 t-plot evaluation of BAM 11 isotherm (courtesy of NANoREG)

5.2.3 Summary on t-plot results on all NanoDefine materials

Microporosity is found to be zero (S_μ=0; S_t = S_w) for the majority of the NanoDefine materials. This is in line with the expectations based on the chemical composition and synthesis. For these materials, the standard BET evaluation (S_t) is fully sufficient.

Non-zero microporosity was anticipated and confirmed for

- IRMM 382 (MWCNT) – attributed to the inner volume of the tubes
- IRMM 388 (coated TiO_2) – attributed to the alumina coating
- BAM 11 (Zeolite) – attributed to the inner structure of zeolite

For IRMM 388 (coated TiO2) and BAM 11 (Zeolite), the t-plot method resolves the discrepancy between surface metrics and EM size determination, as evidenced by the close match of the % of cutoff by VSSA (by S_w) and D50 (by EM) in Tables 4 and 5. For IRMM 382 (MWCNT), this match is slightly worsened against the good match with VSSA (by BET), although still plausible.

Non-zero microporosity was also found for IRMM 380 (Pigment Yellow 86). However, the VSSA (by S_w) % of cutoff derived from the t-plot evaluations in contradiction to the EM size, whereas the VSSA (by BET) closely matches the % of cutoff from EM size.

	Total surface	Micro-porosity	External surface	VSSA (S_w) % of cutoff.
	S_t in m²/g	S_μ in m²/g	S_w in m²/g	
IRMM-380 – organic pigment (transp.)	73.9	52.2	21.7	124.2
IRMM-381 - BaSO4 (fine grade)	2.3	0	2.3	
IRMM 382 – MWCNT	262	108	154	14.4
IRMM 383– Nano Steel	9.1	0	9.1	
IRMM 384– CaCO3 (fine grade)	6.2	0	6.2	
IRMM 385 – Kaolin	15.5	0	15.5	
IRMM 386– organic Pigment (opaque)	17.4	0	17.4	
IRMM 387– BaSO4 (ultrafine grade)	37.8	0	37.8	
IRMM 388 – coated TiO2	15.5	7.7	7.8	192.8
IRMM 389– b. methacrylate copolymer	1.5	0	1.5	
BAM 11– Zeolite powder	425	395.8	29.2	99.3

Table 5: Summary of NANoREG results on NanoDefine materials. The "% of cutoff" is calculated by **Equation 3**. See Table 4 for the color coded EM size comparisons.

5.3 Discussion of the t-plot results

The value of the external surface Sw of the particles can be obtained from the t-plot, if the fitting ranges for S_t (blue lines, through the origin) and S_μ (red lines) are well specified. This is the duty of NANoREG WP2. In any case to detect the presence of microporosity inside the particles, it is very important to have a sufficient number of experimental points in the low pressure region of the isotherm, because the micropore filling is always occurring at very low pressure. This makes the degassing and data acquisition more time-consuming, so that t-plot will not replace BET as screening methodology.

The t-plot results confirm the standard BET evaluation for all materials that were not anticipated to be porous, except for one material: The evaluation of the isotherm is in contradiction to the logical relationship by chemical composition and synthesis for IRMM 380 (Pigment Yellow 86). This is from the same synthesis route as the larger analogue IRMM 386, and the additional processing is known to reduce the primary particle size (to make it applicable as "transparent" pigment), but is not expected to induce microporosity. Compared to the TEM evaluation, the t-plot evaluation (not the BET evaluation) for IRMM 380 results in a false negative classification, because the constituent particles within agglomerates are not recognized (or considered as porous material) by the t-plot evaluation. However, adjustments of the evaluation procedure depend on the fuzzy transition between aggregates (in scope of the EC nanomaterial definition) and internally porous materials (out of scope).

The agreement between the measured surface and the surface derived from EM is very good if the particle shape is taken into account. Such an agreement provides confidence in both approaches: It confirms that standard BET is successful with no false negatives for screening purposes. It further confirms that the t-plot evaluation of the adsorption isotherm is a successful correction to resolve false positives that arise from compositions that can be anticipated to induce microporosity of nm or sub-nm dimension. The t-plot should not be applied for any material, because one false negative occurred when the BET result was over-corrected.

6 Applicability ranges of the VSSA method as rapid screening tool

6.1.1 Applicability range

The experimental data from Table 4 is plotted in Figure 5 to show that the VSSA classification is contained in a range of less than a factor 2 deviation around the EM D50 as benchmark. No outliers were removed, all materials are plotted. This plot relies on the VSSA with shape-specific evaluation.

From the list of materials in section 4.6.4 with good correlation between EM D50 and VSSA, we conclude that in the terminology of the NanoDefine Materials Classification Scheme, the VSSA screening is applicable for the following checkboxes (possibly more):

- Monoconstituent substance
  - Inorganic
  - Carbon-based
  - Organic, particulate
- Nanoscaled dimension (called here “number of small dimensions”)
  - 1 or 2 or 3
- Shape
  - Sphere
  - Elongated
  - Flat
  - Unknown (with proper uncertainty ranges)
- Presence of different sized particles (with baseline EM to exclude multimodality)
  - Unknown (polydispersity OK, multimodality not)
  - Important presence of susnanoparticles (polydispersity OK, multimodality not)
- Trade form
  - Powder (suspensions may suffer drying artifacts)
- Dispersability (any!)
  - In aqueous media
  - In material-specific media
  - Can be aerosolized
  - None (this option is missing in NanoDefine Materials Classification System)
- Stability of particles during testing (none critical, except vacuum and thermal)
  - Vacuum (mild vacuum is applied)
  - Heating (off-gassing is often performed at elevated temperatures (200°C), but all equipment allows longer off-gassing at room-temperature if required to ensure sample stability)
- Specific properties (none critical except porosity)
  - Particle surface porosity (false positives from Alumina-on-TiO2, not for generic coatings, such as organic functionalizations)

6.1.2 Screening strategy

With the existing data from NanoDefine as training set, we derive the following screening strategy. If applied to the further data from real-world materials as validation set, this screening does achieve a correct classification, leaving only borderline materials for tier 2 assessment:

1. Measure skeletal density and BET (outgassing conditions within thermal stability range)
2. If VSSA is more than a factor x10 below cutoff (VSSA < 6 m^2/cm^3), classify as non-nano.
   - Reason: uncertainty factor of x3 arises from the possible reduction of the shape-adjusted VSSA cutoff. Another uncertainty factor x2 from the experimentally determined range of VSSA mismatch against EM D50 (Figure 1). Combined uncertainty is x6, hence, x10 is considered as conservative, but subject to discussion of the exact screening cutoff value.
3. If VSSA > 6 m^2/cm^3, identify the shape from a simple SEM image. Re-evaluate with pragmatic as-

pect ratio criteria as proposed here to select the appropriate shape-specific cutoff as proposed by JRC report #2 (Roebben et al., 2014):

- Particle (aspect ratio <3:1) D=3 → nano, if VSSA > 60 m²/cm³
- Rod (aspect ratio >3:1:1) D=2 → nano, if VSSA > 40 m²/cm³
- Platelet (aspect ratio >3:3:1) D=1 → nano, if VSSA > 20 m²/cm³

4. Quit VSSA screening and escalate to Tier 2 methods (EM or other)
   - If the simple SEM image shows multimodality
   - If the VSSA value is within an uncertainty range x2 around the shape-corrected cut-off (Figure 5)
5. Options to remove false positives from inner or coating porosity
   - Acquire and evaluate adsorption isotherms by NANoREG method (requiring long evacuation, dense data points at low pressures). Re-evaluate with outer VSSA.
   - NanoDefine Tier 2 methods.
6. Options to reduce the x2 uncertainty range around the cut-off:
   - "baseline" EM evaluation of closely related materials with a logical relationship of processing conditions and size distribution (Gilliland et al, 2014)

Of note, the need to know the shape for step 3 will typically require a simple SEM scan, but it does not require dispersion of the material, and it does not require statistical evaluation of EM images. These are the two most time-consuming steps and sources of EM uncertainty, as agreed by both JRC report #1, NanoDefine Technical Report D3.1 and by industry. Considering additionally that BET is already known and publicly available for many materials, the above screening is a tremendous reduction of the technical hurdles to implement the EC nanomaterial definition.

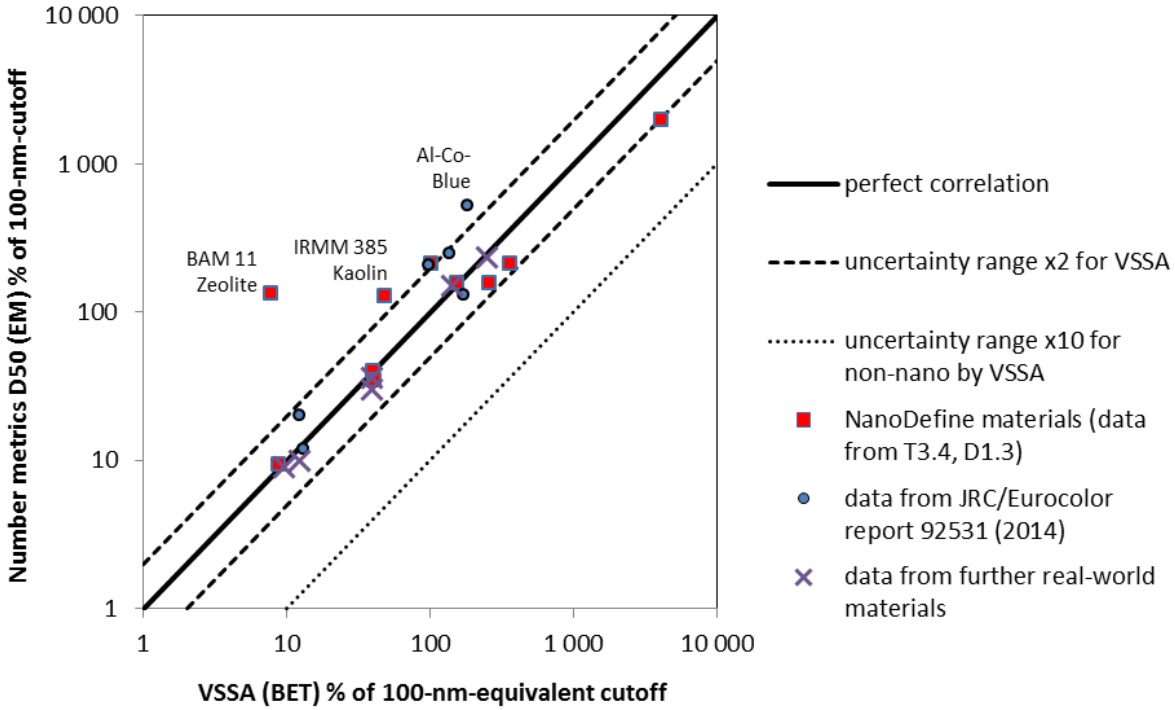

Figure 5 Applicability of VSSA as screening tool.

6.1.3 Can agglomeration, aggregation and polydispersity limit the applicability or motivate different screening cutoff values?

Agglomeration is not a criterion of the D7.3 Materials Classification. The available SEM data of the NanoDefine materials suggests that the majority of them are heavily agglomerated and many aggregagated (indispersably) in their powder trade form. Contrary to concerns in JRC report #2, p. 47, agglom-

eration appears not to be critical: VSSA (by BET) on the 26 materials of heavily agglomerated ENM powders covered in this report shows excellent agreement with EM D50. This finding is rationalized by the fact that the N_2 cross-sectional area of 0.16 nm² is far smaller than agglomerate interstitial spaces even for the smallest ENM, and can thus access all surfaces within agglomerates.

JRC report #2, p. 48 then addresses aggregates. One definition of aggregates is that (in contrast to agglomerates) part of the surface area of the primary particles is lost due to the necks where particles are partially sintered (fused). This effect obviously challenges an assessment of the primary particle size by VSSA. However, if only one tenth of the surface remains (corresponding to the x10 uncertainty range used for the 6 m²/cm³ VSSA screening cutoff), one might consider that the particulate nature is lost by such a near-complete sintering and has transformed into a bulk solid with internal nanostructures. **Aggregates with near-complete sintering represent a "risk" of having false negatives by the 6 m²/cm³ VSSA screening cutoff.**

Finally, polydispersity can reduce the VSSA below the proposed screening cutoff even for monomodal nanomaterial substances, if only the polydispersity is large enough. A lognormal distribution with modal value of 38 nm and a D50 median of 98 nm in number metrics (0.19% below 100 nm in mass metrics) results in a VSSA of 5.9 m^2/cm^3, hence a false negative. However, such a distribution has a polydispersity of 258%, which is five times more than the real-world polydispersities measured on the materials provided for NanoDefine (see NanoDefine Technical Report 1.3 and section 4.6.4). **Materials with excessive polydispersity (related to mixtures) thus represent another "risk" of having false negatives by the 6 m²/cm³ VSSA screening cutoff.**

Polydispersity of real-world materials, both nano and non-nano, had only limited impact on the correlation against EM. So far, the polydispersity of the NanoDefine materials has been evaluated by relatively simple SEM and TEM evaluation. Optimized TEM methodology may revise the polydispersity values, thus modifying our interpretation on its influence on the VSSA. However, as the selection of the NanoDefine materials was done in a way to cover a broad range of particulate substances used for industrial applications and in consumer products, these "real-life" materials are probably rather polydisperse indeed and consequently – as no false negatives were reported – the 10 fold extended uncertainty range is probably large enough to classify polydisperse materials correctly.

In summary, the user should know the associated "risk" of a specific VSSA screening cutoff value and should consider the above implications[2]. Altough the 26 real-world materials reported here necessitate no lower than 10 m^2/cm^3 as screening cutoff, some lower value will be chosen for a general guidance, in order to accommodate the limited representation of the particulate world by the test materials. A screening cutoff at 6 m^2/cm^3 is our proposal to be discussed further.

7 Conclusions

According to NanoDefine Technical Report D3.1, VSSA (by BET) is the only technique apart from SEM to cover the entire size range from 1 nm to 10 µm diameter. Further, it is among the very few techniques to assess smallest dimensions without dispersion. The BET technique is standardized, in widespread use, generates low costs and is specified for many commercial materials.

Agglomeration is not critical: VSSA (by BET) on the 26 materials of heavily agglomerated ENM powders covered in this report shows excellent agreement with EM D50. This finding is rationalized by the fact that the N_2 cross-sectional area of 0.16 nm² is far smaller than agglomerate interstitial spaces even for the smallest ENM, and can thus access all surfaces within agglomerates.

In line with the 10% contribution of polydispersity to the discrepancy between VSSA and EM D50, calculated by theoretical size distributions in JRC report #2, we find that the agreement is good for real-world particulate materials with polydispersity around 50% in their size distribution. This is a minor fac-

[2] An additional technical boundary condition: The two lowest-surface Certified Reference Materials for BET measurements have VSSA values of 0.4 m²/cm³ (alumina BCR-169) and 3.5 m²/cm³ (tungsten BCR 175), both from IRMM. The VSSA cutoff might want to stay well above these lower extremes of the measurement range of available BET instruments.

tor against the shape factors.

In the range 6 < VSSA < 60 m^2/cm^3 the JRC concept of shape-related cut-off values was successful to achieve excellent agreement between VSSA and EM D50. This concept requires, however, the knowledge of shape and absence of multimodality (e.g. from a single SEM scan) as "baseline EM" to validate VSSA.

The VSSA method mitigates the challenges of EM to correctly assess the minimal external dimension (thickness) of platelets and the even bigger challenge of counting methods to assess the minimal external dimension of platelets and fibers (potentially false negatives by counting and EM methods).

False positives occurred with VSSA (by BET) for materials with inner or coating porosity. VSSA (by isotherm t-plot evaluation) is effective to resolve these artefacts. Newly acquired adsorption isotherms can cover both BET and t-plot pressure ranges.

False negatives did not occur beyond a factor of 2 disagreement vs. EM D50. Considering the possibility that the shape might be unknown, the data suggest an uncertainty margin of 10. Within the diverse set of 26 materials investigated here, there are no contradiction to the median diameter in number metrics (D50 EM) if we classify all materials with VSSA < 6 m^2/cm^3 as non-nano without further assessment, even if shapes are unknown. As multimodal materials or mixtures are not covered in this report, they remain a possibility for false negatives.

Within these boundaries, VSSA (by BET) is well suited to screen both for nano *and* non-nano materials in monoconstituent powders, and is developed into a detailed screening strategy with decision points triggered by the D7.3 materials classification and by interim measurement results.

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