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DCS measurements and representative TEM images of sunscreen samples

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Reference: Q122949

Description: DCS measurements and representative TEM images of sunscreen samples

Maker: Various. Please see Section 2 of report for sample listing.

Date of Receipt: All samples received on 14 September 2012 except Cancer Council, Kids sunscreen received on 06 September 2012

Serial Number: M122949

Previous Examination: XRD phase analysis of M122949_002 was conducted previously as described in report RN122439

Date(s) of Test: 05 October 2012 – 16 October 2012

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The meaning and use of specific terms and expressions common to dispersion science and technology used throughout this study are defined as per Special Publication 960-3 of the National Institute of Standards and Technology.^a

Certain trade names and company products are mentioned in the text in order to adequately specify the experimental procedure and equipment used. In no case does such identification imply recommendation or endorsement by the National Measurement Institute, nor does it imply that the products are necessarily the best available for the purpose.

^a V. A. Hackley and C. F. Ferraris, *The Use of Nomenclature in Dispersion Science & Technology*. NIST Recommended Practice Guide, NIST SP 960-3 (2001). Available at www.ceramics.nist.gov/ftproot/PracticeGuides/960-3/SP960-3.pdf (accessed 18 October 2012).

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List of symbols and abbreviated terms used in this report

DCS	differential centrifugal sedimentation
TEM	transmission electron microscopy
x	particle size; Stokes diameter or hydrodynamic diameter
$q_1(x)$	density distribution by intensity
$q_3(x)$	density distribution by volume (or mass)
$q_0(x)$	density distribution by number
$q_1^*(x)$	density distribution by intensity, plotted on a logarithmic axis
$q_3^*(x)$	density distribution by volume (or mass), plotted on a logarithmic axis
$q_0^*(x)$	density distribution by number, plotted on a logarithmic axis
$x_{3,10}$	particle diameter at 10 % of a cumulative distribution by volume (or mass)
$x_{3,50}$	median particle diameter of a cumulative distribution by volume (or mass)
$x_{3,90}$	particle diameter at 90 % of a cumulative distribution by volume (or mass)
$x_{0,10}$	particle diameter at 10 % of a cumulative distribution by number
$x_{0,50}$	median particle diameter of a cumulative distribution by number
$x_{0,90}$	particle diameter at 90 % of a cumulative distribution by number
ISO	International Organization for Standardization
NIST	National Institute of Standards and Technology

1. Scope

This report presents differential centrifugal sedimentation (DCS) measurements and representative transmission electron microscopy (TEM) images of the solid fraction of material extracted from four sunscreen samples to identify nanomaterial^a content that may be present in the samples.

2. Sample list

Table 1: Details of samples received, including lot number, sample reference number, stated ZnO or TiO₂ mass fraction.

Product name and product details as stated on product label	Lot number	Sample reference number ^b	Stated ZnO mass fraction (%)	Stated TiO ₂ mass fraction (%)
Banana Boat, Mineral protect, <i>Very high protection sunscreen. SENSITIVE.</i> SPF 30+ Broad Spectrum UVA/UVB Protection, Naturally sourced zinc goes on clear, Paraben & Fragrance free 4 Hour Water resistant 120g NET	BOO14BB	M122949_001	22	—
Cancer Council, Kids sunscreen. <i>Very high protection for delicate skin.</i> Fragrance free. SPF 30+ UVA UVB Broad spectrum 4 HOURS Water Resistant 110 mL	BOO89	M122949_002	—	2.5
Soleo organics, all natural SUNSCREEN <i>for all skin types. NO chemical UV absorbers, NO titanium dioxide, NO synthetic preservatives. Biodegradable! For adults and children of all ages 30+ SPF 3HR UV-A UV-B 80g</i>	B36218	M122949_006	22.3 ^c	—
Miessence, reflect OUTDOOR BALM BFA registered product 100g Made in Australia	12O343	M122949_007	Not specified	—

In addition to the client's samples, TEM imaging of NIST Reference Material 8013^d (nominally 60 nm Au particles) was performed to establish the calibration of the length scales of the TEM images. This calibration is reflected in the scale bars shown in the TEM images presented in this report.

^a As defined by ISO 80004-1:2010(E) ¹ nanomaterial is material with any external dimension in the nanoscale (2.1) or having internal structure or surface structure in the nanoscale.

NOTE 1 This generic term is inclusive of nano-object (2.5) and nanostructured material (2.7).

NOTE 2 See also engineered nanomaterial (2.8), manufactured nanomaterial (2.9) and incidental nanomaterial (2.10).

^b Internal NMI code given to sample.

^c The value of 22.3 % given on the packaging does not specify if it is a mass fraction.

^d RM 8013 has a reference diameter (mean equivalent spherical diameter of the Au particles) measured by TEM of (56.0 ± 0.5) nm.

3. Summary of results

Table 2: Summary of findings for the four samples measured. The liquid phase (aqueous or organic) in which the particles remained during washing is listed, as well as other relevant information.

Sample reference number	Particles remained in organic or aqueous phase after washing	Nanomaterials present yes/no	Other comments
M122439_001	aqueous	yes	Both nano and non-nanomaterial observed in TEM
M122439_002	organic	yes	—
M122439_006	aqueous	yes	—
M122439_007	organic	yes	Both nano and non-nanomaterial observed in TEM

There are currently no methods available for determining quantitatively the fraction of particles with dimensions in the nanoscale (i.e., below 100 nm) in complex formulations such as sunscreens.²

4. Experimental details

To produce sample material suitable for analysis, it was necessary to use a washing procedure to separate the particles from the formulation.³ In performing the washing procedure, the aggregation/agglomeration state of the particles in the formulation may be changed. Also, particles may be incompletely separated from the formulation, or different particle sub-populations may be present in different liquid phases, i.e., the aqueous or organic phases. Thus the size distributions presented in this report are not necessarily based on the entire particle content of the samples and therefore cannot be used to determine the proportion of particles with sizes below a particular value, such as 100 nm, that may be present in the sunscreen formulation.

4.1. Formulation removal by washing

The washing procedure was adapted from that described by Contando *et al.*³ The procedure used was as follows:

1. The tube of sunscreen was shaken.
2. 1 g of sunscreen was weighed into a small (50 mL) beaker.

3. 20 mL of ultrapure water (MilliQ, 18 M Ω cm, filtered through final filter of 0.22 μ m) was added to the beaker, and the mixture was ultrasonicated (Misonix S4000, 12.7 mm diameter horn, 32 W, 20 s).
4. 20 mL of methanol (Sigma Aldrich, Chromasolv for HPLC \geq 99.9 %, Lot# SZBB111SV) was added to the beaker, and the mixture was again ultrasonicated using the same settings.
5. The sample was then poured into a clean separating funnel. 10 mL of hexane (Sigma Aldrich, Chromasolv for HPLC \geq 97.0 %, Lot# SZBC085CV) was added and the separating funnel was stoppered and shaken vigorously for \sim 30 s.
6. The sample was then allowed to settle overnight.
7. After settling, the sample formed into a number of different layers.
8. The lowest layer of the formulation (aqueous layer) was carefully drained and retained for measurements.

Samples M122949_001, and M122949_006 contained particles in the aqueous phase (as indicated by the turbidity of the phase and by DCS measurements).

Samples M122949_002, and M122949_007 did not disperse well at step 3 of the above procedure, and after settling, the aqueous phase appeared clear, suggesting that no particles were present. The interface between the different liquid phases in these samples was not sharply delineated and highly indistinct. Since for these samples the particles appeared to be present in the organic (hexane) phase, a different washing process was applied following the procedure outlined below:

1. The tube of sunscreen was shaken.
2. 1 g of sunscreen was weighed into a small (50 mL) beaker.
3. 20 mL of hexane (Sigma Aldrich, Chromasolv for HPLC \geq 97.0 %, Lot# SZBC085CV) was added to the beaker, and the mixture was ultrasonicated (Misonix S4000, 12.7 mm diameter horn, 32 W, 20 s).
4. The sample was then poured into a clean separating funnel. 20 mL of ultrapure water (MilliQ, 18 M Ω cm, filtered through final filter of 0.22 μ m) was added and the separating funnel was stoppered and shaken vigorously for \sim 30 s.
5. The sample was then allowed to settle overnight.
6. After settling, the sample formed into a number of different layers.
7. The top layer of the formulation (organic layer) was retained for measurements.

4.2. DCS

DCS measurements were conducted on a disc centrifuge (CPS Instruments USA, model UHR24 000, operating software CPSV95). The instrument was internally calibrated

immediately before each measurement with a polyvinyl chloride calibration standard (CPS Instruments, mean diameter, 0.263 μm , Lot# 123) dispersed in propan-2-ol (Labscan HPLC grade Lot# 10080381), or water (MilliQ, 18 M Ω cm, filtered through final filter of 0.22 μm). This reference material is quoted by the supplier as being traceable to the realisation of the definition of the SI metre at NIST, USA.

The disc centrifuge was loaded with two different types of density gradient solution. For the samples suspended in the aqueous phase (M122949_001 and M122949_006), 17 mL of an 8-240 mg g^{-1} density gradient solution of sucrose (Sigma, 99.5% Lot# 029k0010) in ultrapure water (MilliQ, 18 M Ω cm, filtered through final filter of 0.22 μm) was used. For the samples suspended in the organic phase (M122949_002 and M122949_007), 17 mL of an 8-240 mg g^{-1} density gradient solution of 1.8 oil (Halocarbon, Lot# 8C09162) in propan-2-ol (Labscan HPLC grade Lot# 10080381) was used. Subsequently, 0.5 mL of dodecane (Sigma, Reagent Plus, $\geq 99\%$, Lot# MKBB8417) was added as a gradient evaporation barrier during measurement. The samples were measured with the disc centrifuge operating at a rotation frequency of 21 500 min^{-1} to access the particle size range below 600 nm. After reaching the requisite rotation frequency, the system was allowed to equilibrate for 1 h before measurement.

The samples were bath-ultrasonicated for ~ 1 min before measurement in DCS. A 0.1 mL aliquot of each sample was used per measurement. Triplicate measurements were performed on selected samples to verify run repeatability.

The physical properties used in the analysis of the measured data, i.e., for input into the Stokes equation and for conversion of the measured extinction signal to the volume- and number weighted particle size distributions, using Mie theory, are given in Table 3.

Table 3: Physical properties of the particle material and gradient fluid used for the determination of the particle size distribution by DCS.

	ZnO	TiO ₂	Gradient fluid ^e (aqueous)	Gradient fluid ^f (organic)
Refractive index	2.23 ^g	2.59 ^h	1.072	1.38 ⁱ
Absorption coefficient ^j	0.010 ^g	0.0015 ^h	—	—
Density (g cm ⁻³) ^k	5.6	4.2 ^l	1.361	0.97
Dynamic viscosity (mPa s)	—	—	1.2	1.86 ^m

4.3. TEM

Calibration samples consisting of NIST Reference Material 8013 (aqueous suspension of gold nanoparticles, nominal diameter 60 nm) was prepared by diluting the reference material with ultrapure water (MilliQ, 18 MΩ cm, filtered through final filter of 0.22 μm) in the ratio 1 part to 9. A 5 μL aliquot of the diluted Au suspension was placed on the reflective side of a carbon-coated grid (Agar Scientific, UK).

The samples were prepared by bath-ultrasonicated the samples for ~1 min and dropping a 5 μL aliquot of the particle-containing liquid phase (either water or hexane) on the reflective side of a carbon-coated grid (Agar Scientific, UK). The TEM samples were allowed to dry overnight in a clean, dust-free environment.

All TEM images were recorded on a Morada CCD camera, attached to a Philips CM12 TEM operating at an accelerating voltage of 120 keV. The samples were positioned at eucentric height and imaged during two sessions that took place within a week. All images were acquired at a nominal magnification value of 66 000 ×. This magnification of the microscope/camera system was calibrated with NIST Reference Material 8013 (gold nanoparticles, nominal diameter 60 nm) which has a reference value of the mean particle diameter as determined by TEM of (56.0 ± 0.5) nm. This diameter is traceable to the SI metre

^e These values represent an approximation of the density gradient fluid parameters that are likely to exist at the location of the detector in the DCS. For the purposes of these measurements, the density gradient fluid is assumed to contain 180 mg g⁻¹ sucrose in water at the detector location. Values derived from information at: <http://homepages.gac.edu/~cellab/chpts/chpt3/table3-2.html> accessed on 26 November 2012 and from the CPS Disc Centrifuge Operating Manual (2007), CPS Instruments Inc., Florida, USA.

^f These values represent an approximation of the density gradient fluid parameters that are likely to exist at the location of the detector in the DCS. For the purposes of these measurements, the density gradient fluid is assumed to contain 180 mg g⁻¹ Halocarbon 1.8 oil in propan-2-ol at the detector location.

^g Value for ZnO at 30 °C and a wavelength of 405 nm. Taken from: <http://www.luxpop.com/> accessed on 23 November 2012.

^h Value for rutile TiO₂ at 27 °C and a wavelength of 405 nm. Taken from: <http://www.luxpop.com/> accessed on 23 November 2012.

ⁱ This value was measured with a digital refractometer (Schmidt + Haensch, DSRλ) at a wavelength of 405 nm and a temperature of 30 °C.

^j Estimated value.

^k Taken from the CRC Handbook, 90th edition.

^l Density, rutile.

^m This value was measured at a temperature of 30 °C with a vibro viscometer (AND, SV10).

as realised at NIST. Calibration images were recorded at both sessions and combined for the final analysis of the scale factor.

Image J softwareⁿ v1.45s was used for quantitative analysis of the TEM images of the reference material to obtain calibration information. Each image was thresholded to isolate the particles from the background. The resulting binary images were critically assessed, and any particles that overlapped, had ill-defined boundaries due to insufficient contrast or were only partially contained within an image were excluded from analysis. The area, measured in (pixel)², of the remaining particles was measured, and this value was used to determine the equivalent diameter, measured in pixels, of a spherical particle with the same projected cross-sectional area (equivalent spherical particle diameter). A total of 25 images and 588 particles were analysed. The resulting distribution of measured equivalent spherical particle diameters has a mean value of (48.3 ± 3.1) pixels. This value, in conjunction with the NIST reference value of the particle diameter of (56.0 ± 0.5) nm was used to determine a scale factor of (1.159 ± 0.012) nm (pixel)⁻¹ for the conversion of lengths measured in pixels to lengths measured in nanometres. This scale factor was applied to generate the calibrated scale bars on the representative TEM images.

5. Results

5.1. DCS

The measured time-dependent extinction is converted to an intensity-based density distribution $q_1(x)$ using the Stokes equation. Mie theory is then used to convert the measured $q_1(x)$ into the density distribution by volume (or mass), $q_3(x)$. This conversion involves modelling the light scattering from a particle with given geometry and optical properties. Since small particles scatter less light than large particles (scatter intensity is roughly proportional to the square of the particle volume), this conversion tends to enhance the smaller-diameter end of $q_3(x)$ compared to the measured $q_1(x)$. Inaccuracies in the values used for the optical properties of the particles will create errors in the calculated scatter intensities which will translate to inaccurate weightings and distortions of $q_3(x)$.

When converting a density distribution by volume (or mass), $q_3(x)$, to a density distribution by number, $q_0(x)$, the data is inversely scaled by the equivalent volume of a sphere, which leads to an even greater enhancement of lower sizes. Since smaller particles scatter much less light, the signal-to-noise ratio at the smaller size-end is lower, and the influence of the background signal is more pronounced. The density distributions by intensity,

ⁿ <http://rsbweb.nih.gov/ij/>

mass and number, plotted on a logarithmic axis, ($q_1^*(x)$, $q_3^*(x)$ and $q_0^*(x)$, respectively) are given below.

The $q_1^*(x)$ data illustrates that all samples contain a detectable fraction of primary particles or aggregates/agglomerates with a Stokes diameter below 100 nm. Samples M122040_002 and M122040_007 show non-zero intensity at the maximum accessible diameter (~530 nm), indicating that the size distributions extend to higher diameters.

The $q_3^*(x)$ distributions are calculated by applying Mie theory to the measured $q_1^*(x)$ data. This results in a shift of the distributions to lower sizes (as explained earlier).

The $q_0^*(x)$ distributions by number are calculated by scaling the $q_3^*(x)$ data by the mass of an equivalent sphere with diameter x . As explained above, this strongly emphasises the lower end of the size distribution where the signal-to-noise ratio is low. The $q_0^*(x)$ data indicate that for the measured fraction of extracted material and in the size range below 450 nm, nearly all particles (>90 %) are in the nanoscale. As stated above, this result does not necessarily represent the true proportion of particles with sizes below 100 nm relative to the entire particle population present in the sunscreen formulation. This is due to the incomplete coverage of the particle size range by the DCS measurements, due to the possibility of incomplete extraction of the particulate content from the formulation during sample preparation, and due to the potential transformation of particulate material by the extraction process.

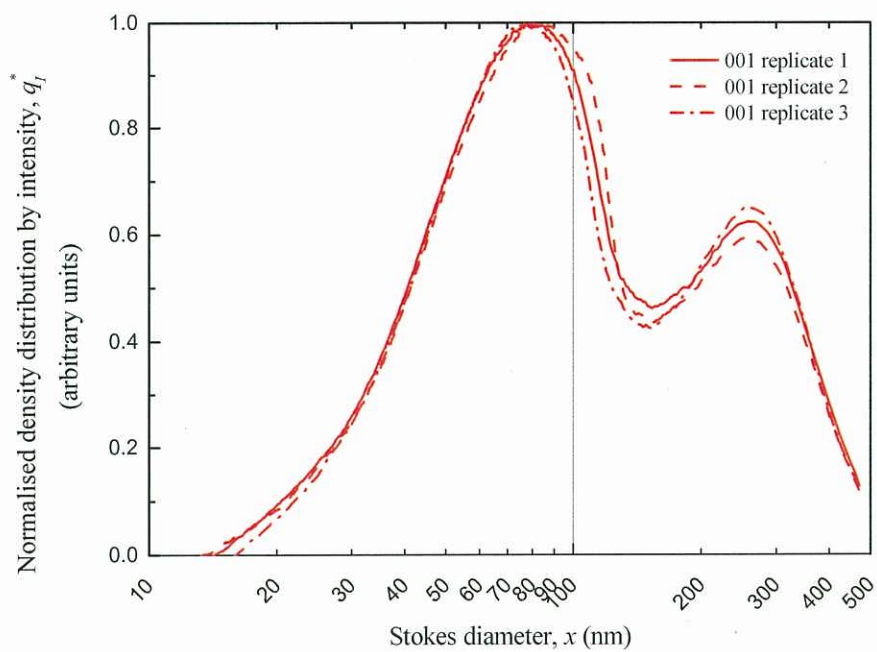
The distributions by volume and number are summarised in Table 4. Stated errors represent the standard deviations of the triplicate sets of measurements.

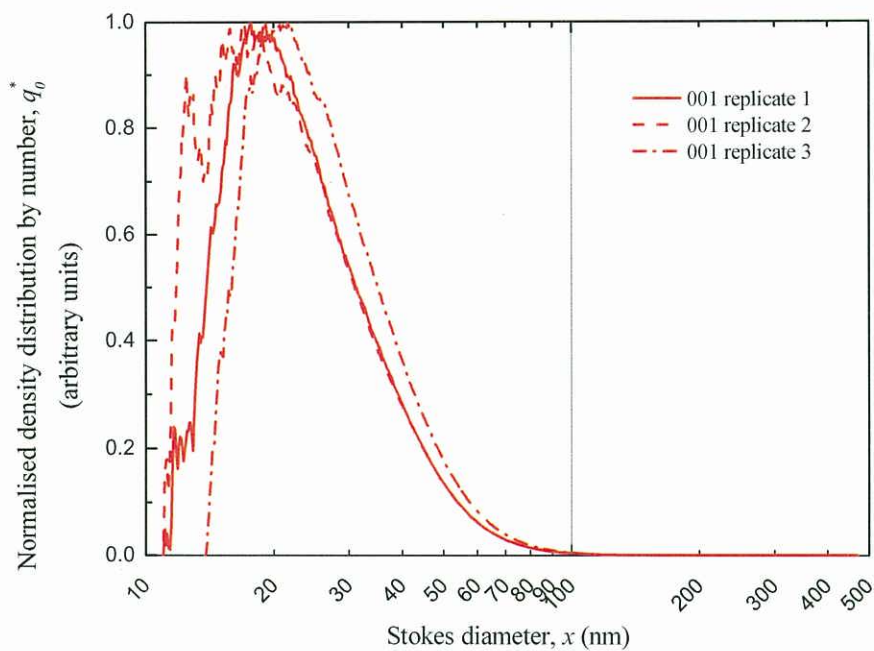
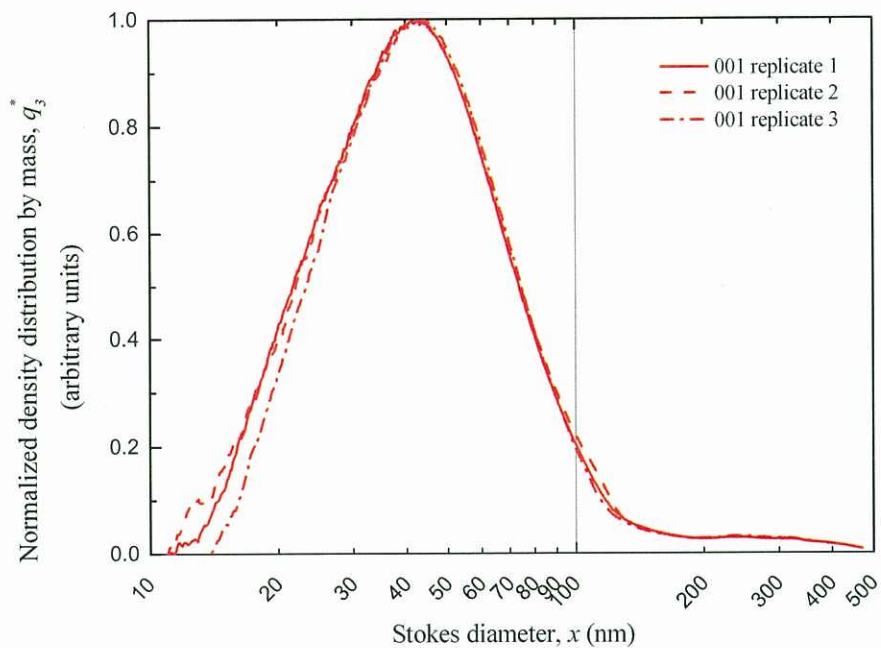
Table 4: Summary of DCS data.

	$x_{3,10}$ (nm)	$x_{3,50}$ (nm)	$x_{3,90}$ (nm)	$x_{0,10}$ (nm)	$x_{0,50}$ (nm)	$x_{0,90}$ (nm)
M122949_001_1	26	48	85	16	25	46
M122949_001_2	26	48	86	14	24	45
M122949_001_3	27	49	85	12	26	47
Average M122949_001	26 ± 1	48 ± 1	85 ± 1	14 ± 2	25 ± 1	46 ± 1
M122949_002_1	14	46	86	11	13	24
M122949_002_2	13	49	97	10	13	19
M122949_002_3	18	39	62	11	14	35
Average M122949_002	15 ± 3	45 ± 5	80 ± 20	11 ± 1	13 ± 1	26 ± 9

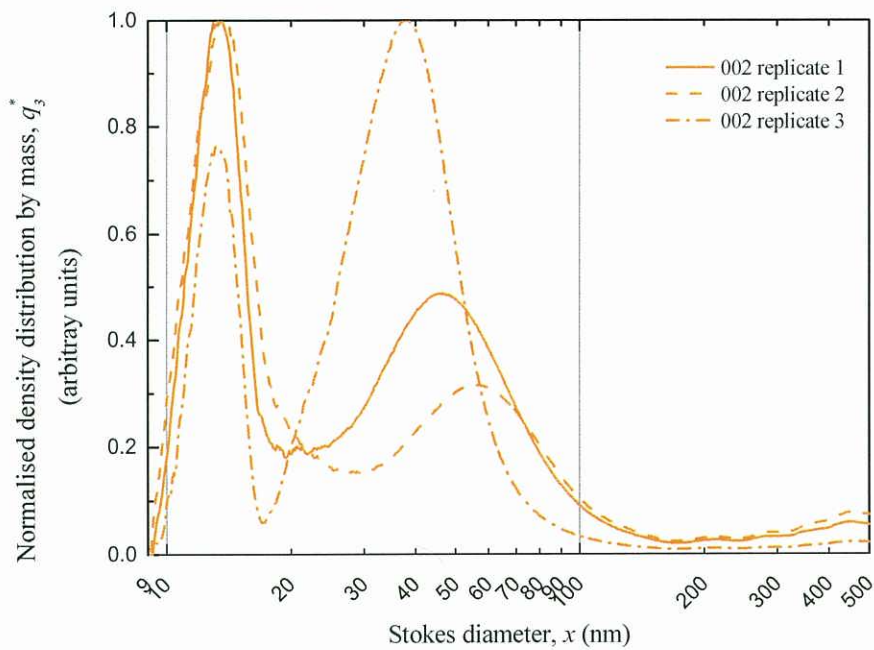
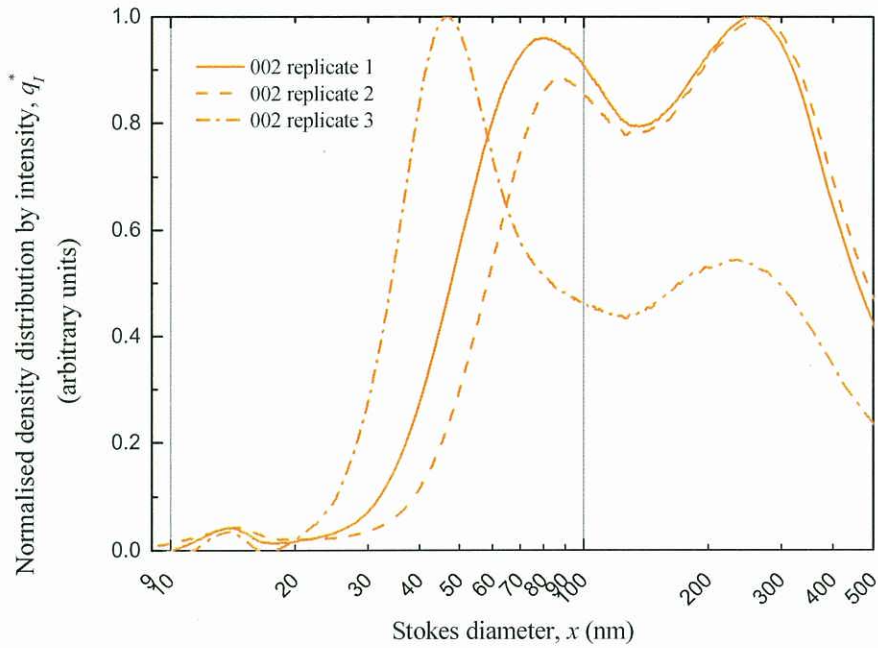
	$x_{3,10}$ (nm)	$x_{3,50}$ (nm)	$x_{3,90}$ (nm)	$x_{0,10}$ (nm)	$x_{0,50}$ (nm)	$x_{0,90}$ (nm)
M122949_006_1	20	39	68	13	20	36
M122949_006_2	21	40	69	13	20	37
M122949_006_3	21	40	69	13	20	37
Average M122949_006	21 ± 1	40 ± 1	69 ± 1	13 ± 0	20 ± 0	37 ± 1
M122949_007_1	15	50	88	12	15	22
M122949_007_2	15	54	91	11	15	20
M122949_007_3	15	51	89	11	15	21
Average M122949_007	15 ± 0	52 ± 2	89 ± 2	11 ± 1	15 ± 0	21 ± 1

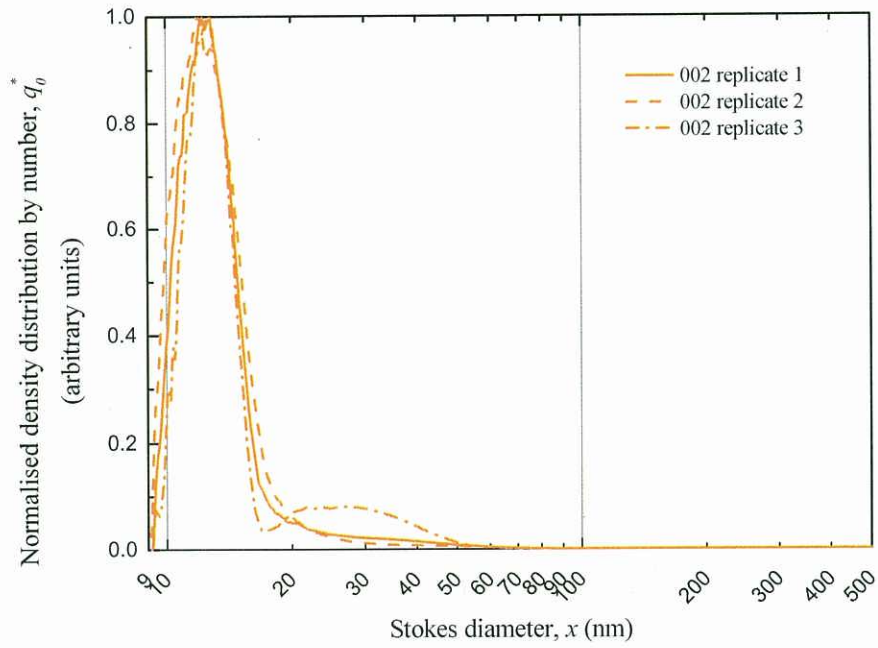
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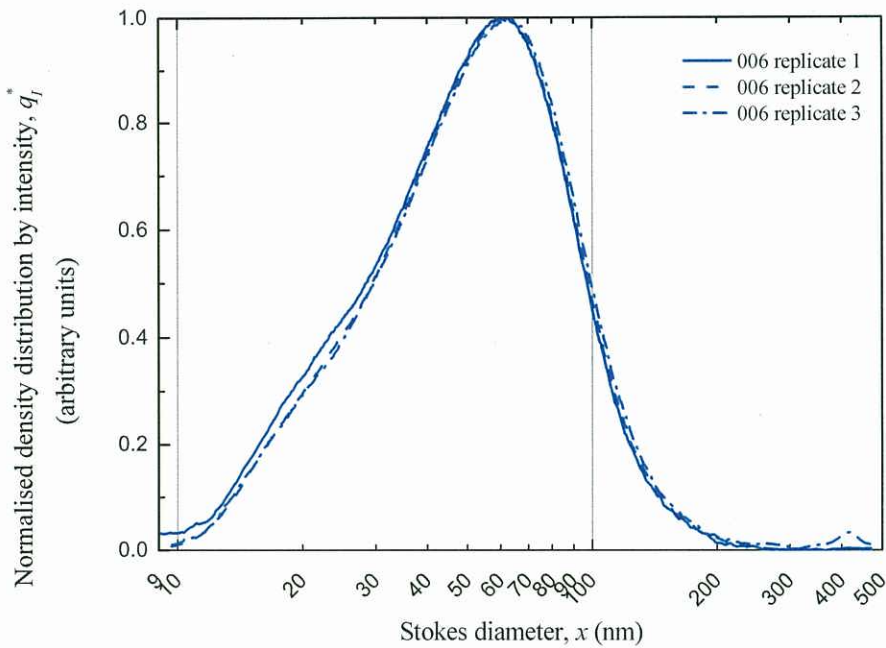


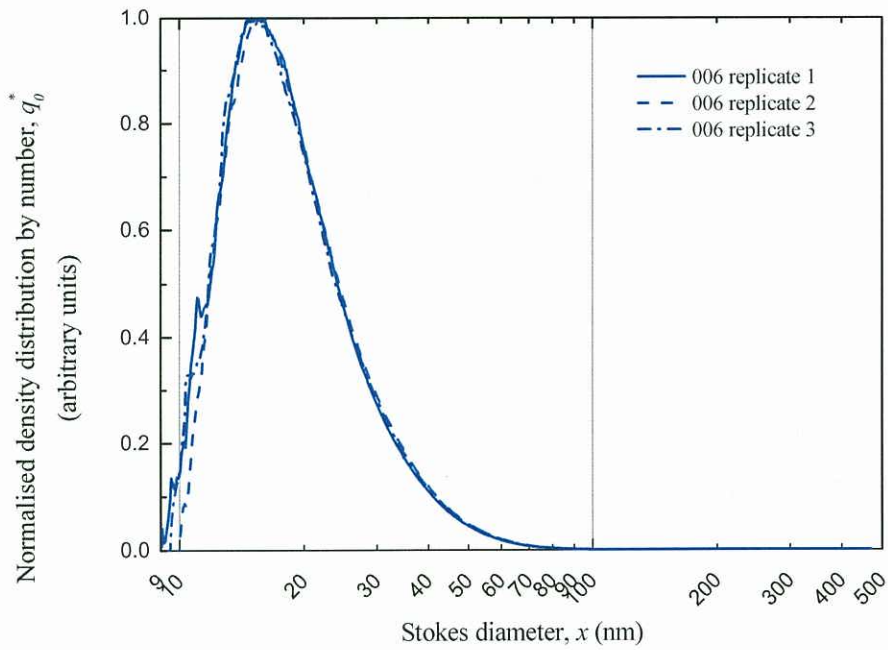
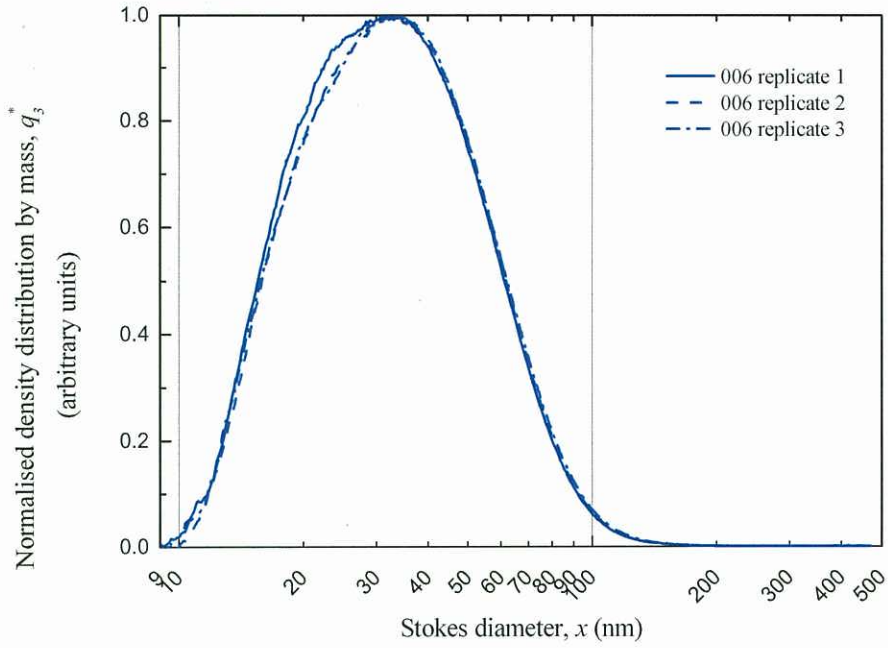
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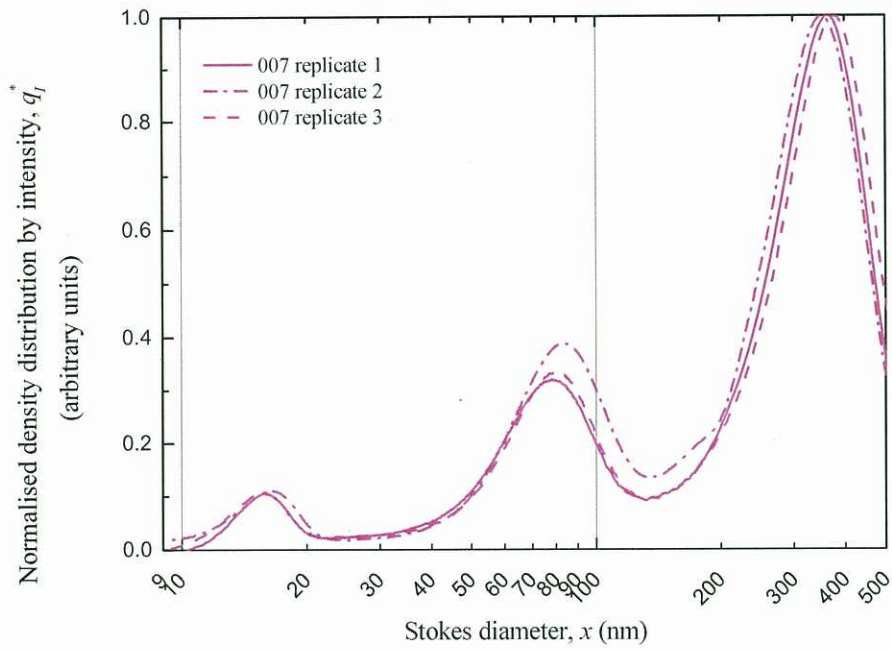


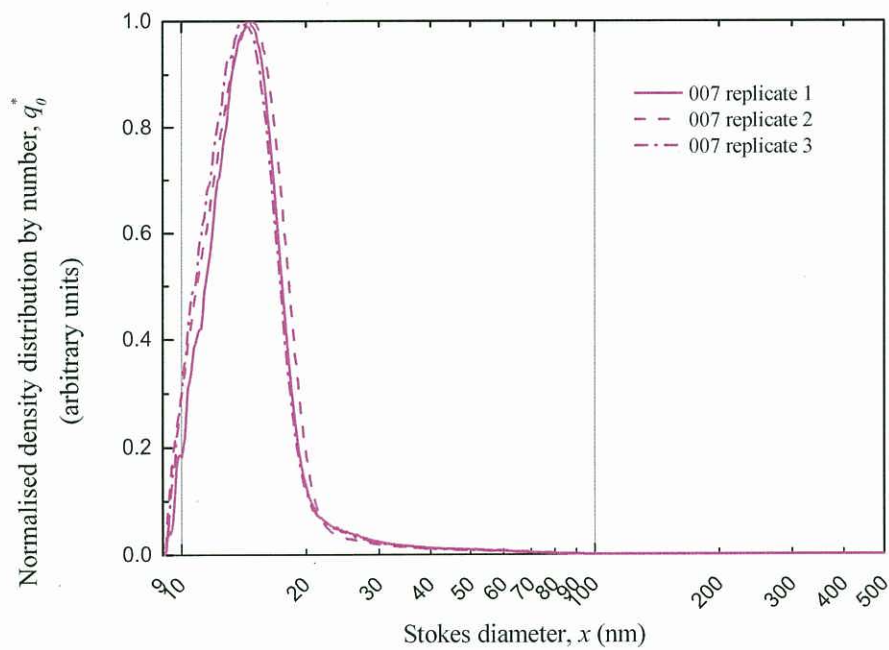
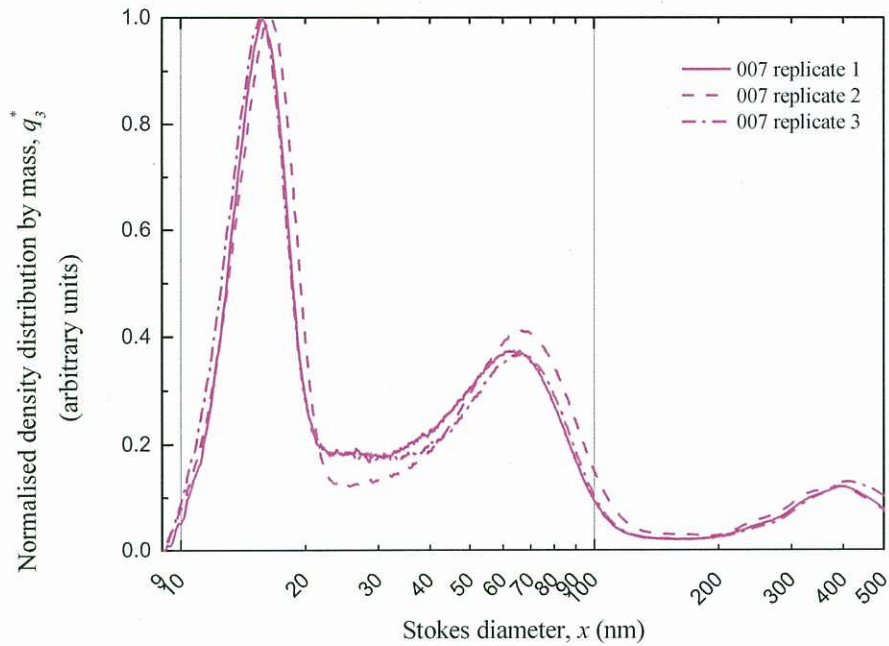
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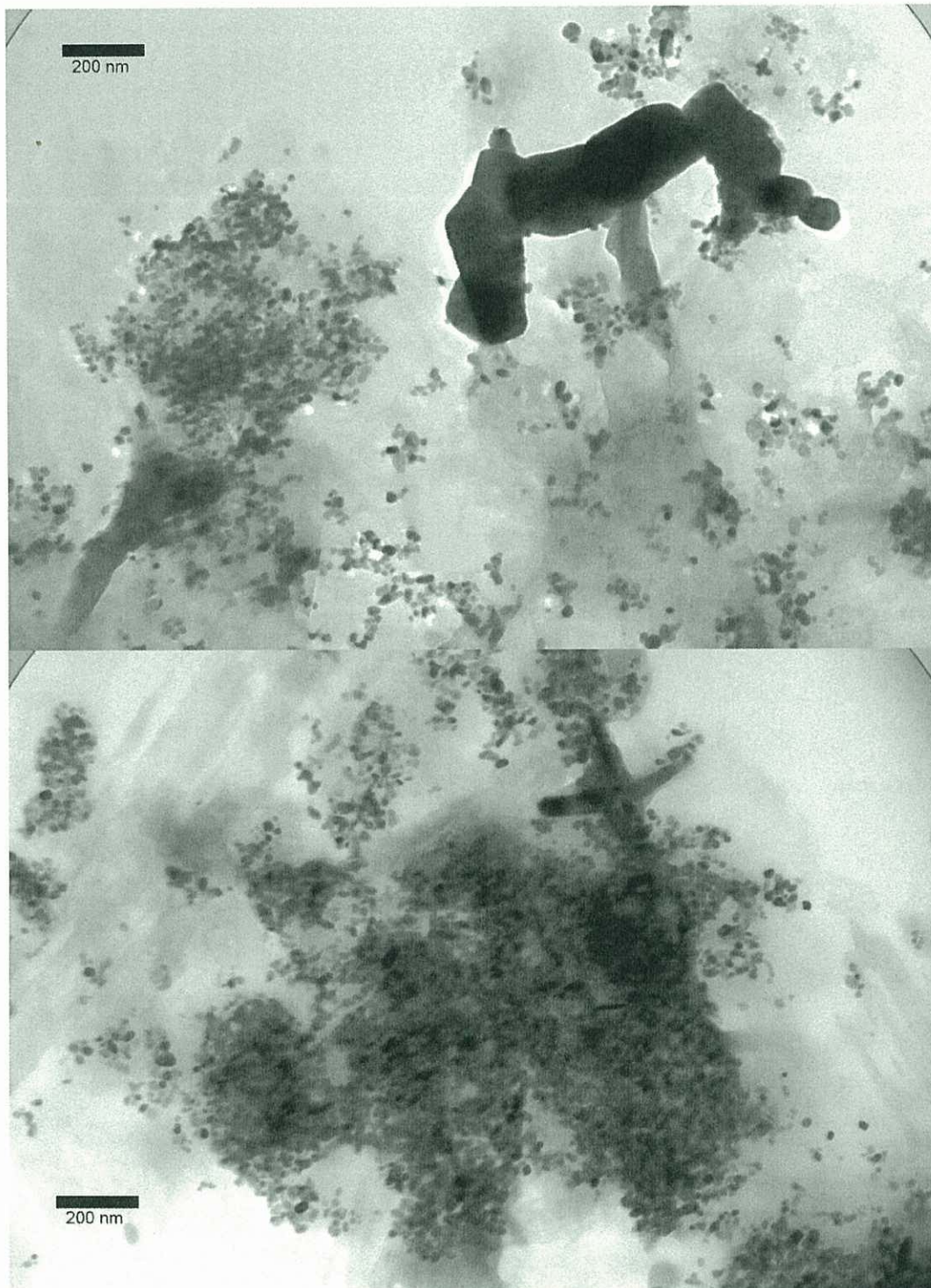
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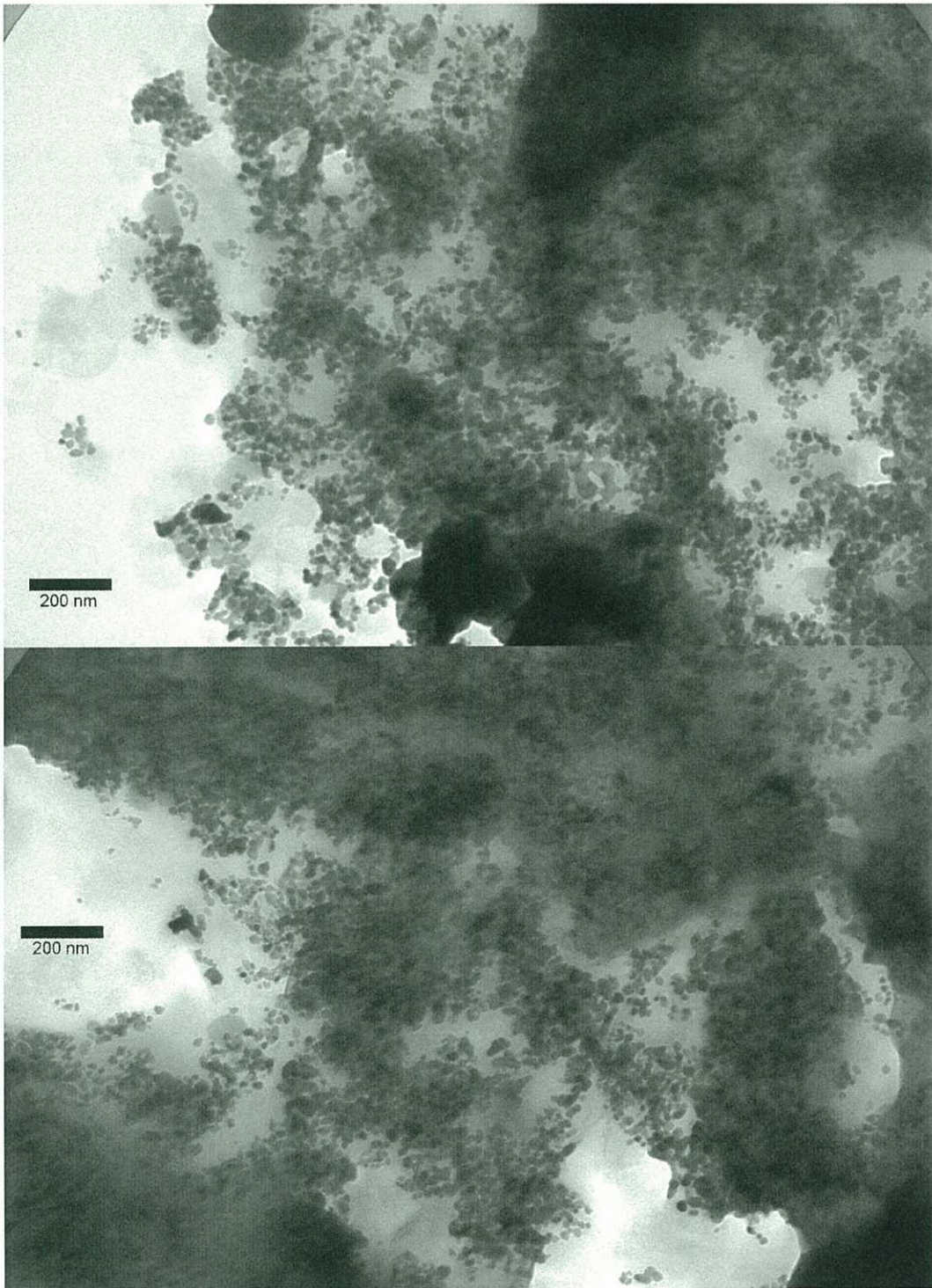


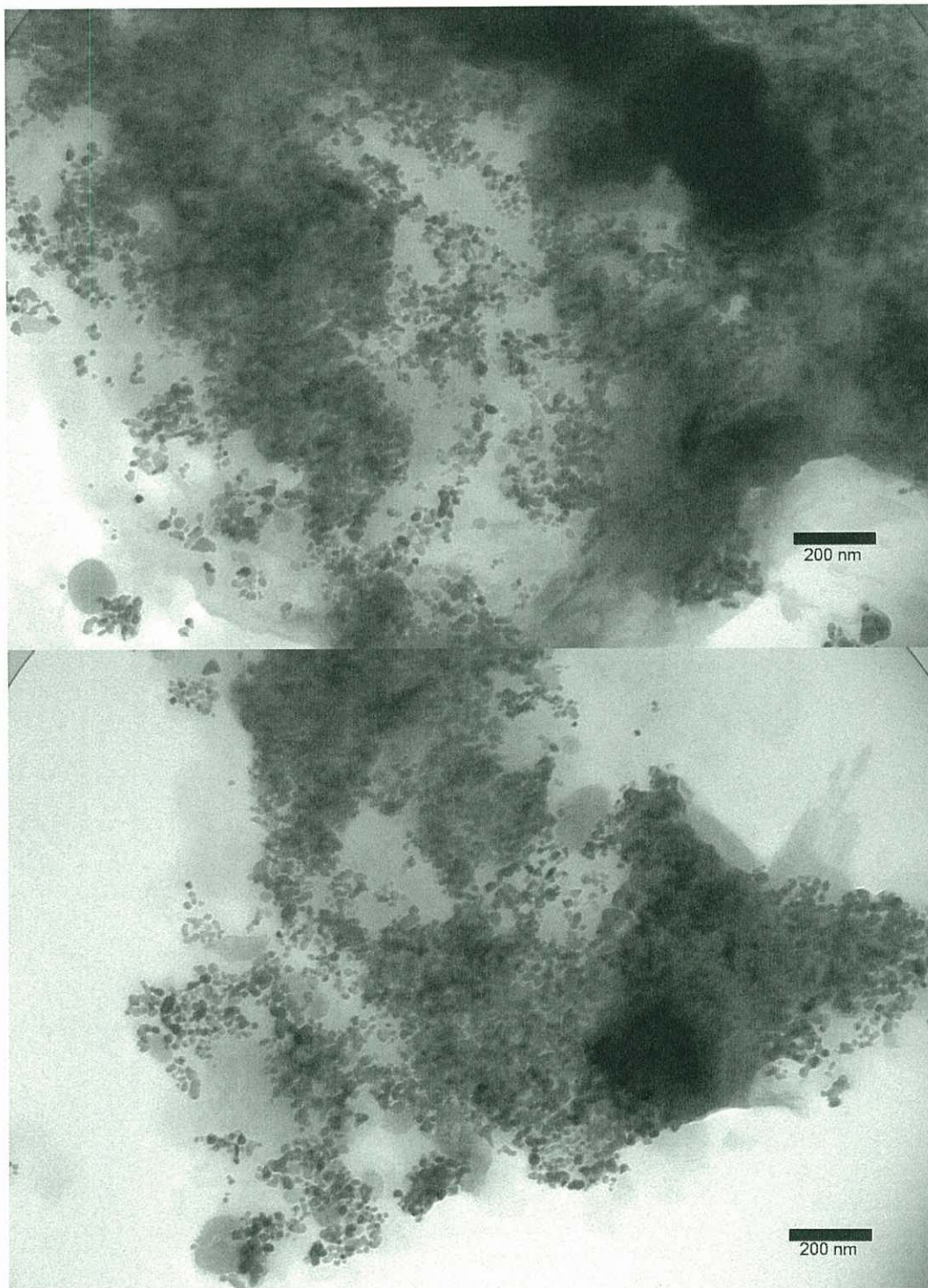


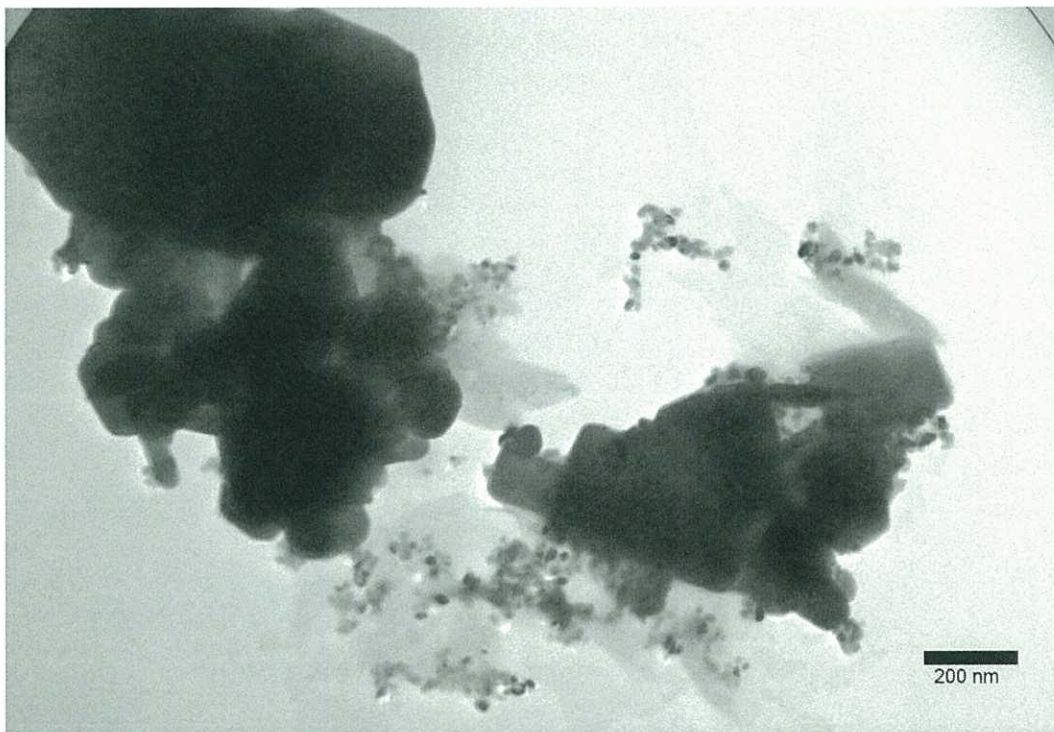
5.2 Representative TEM images

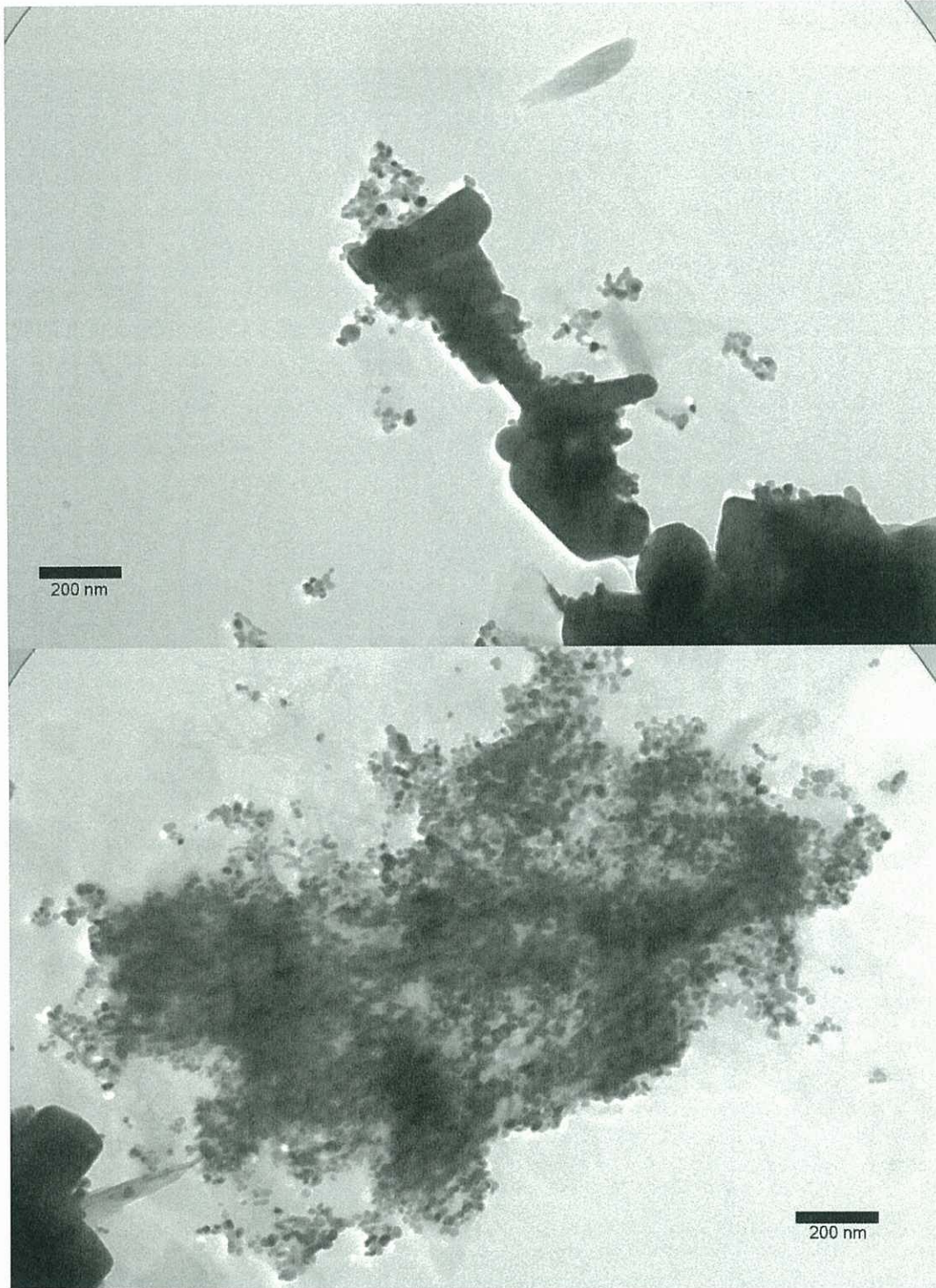
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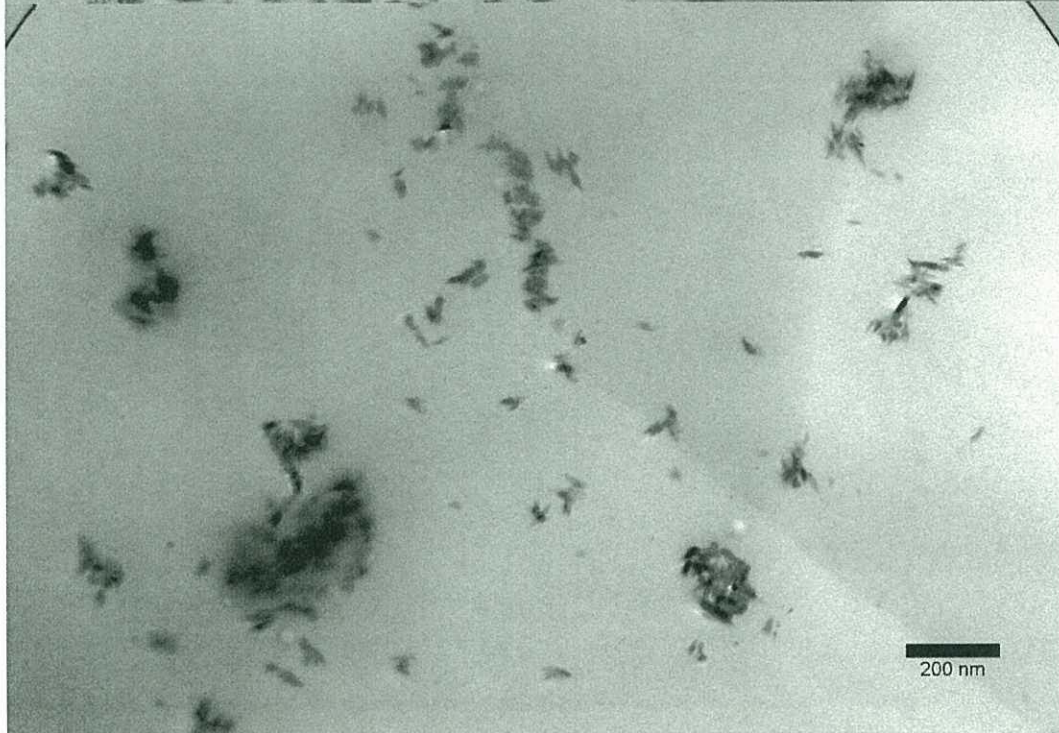


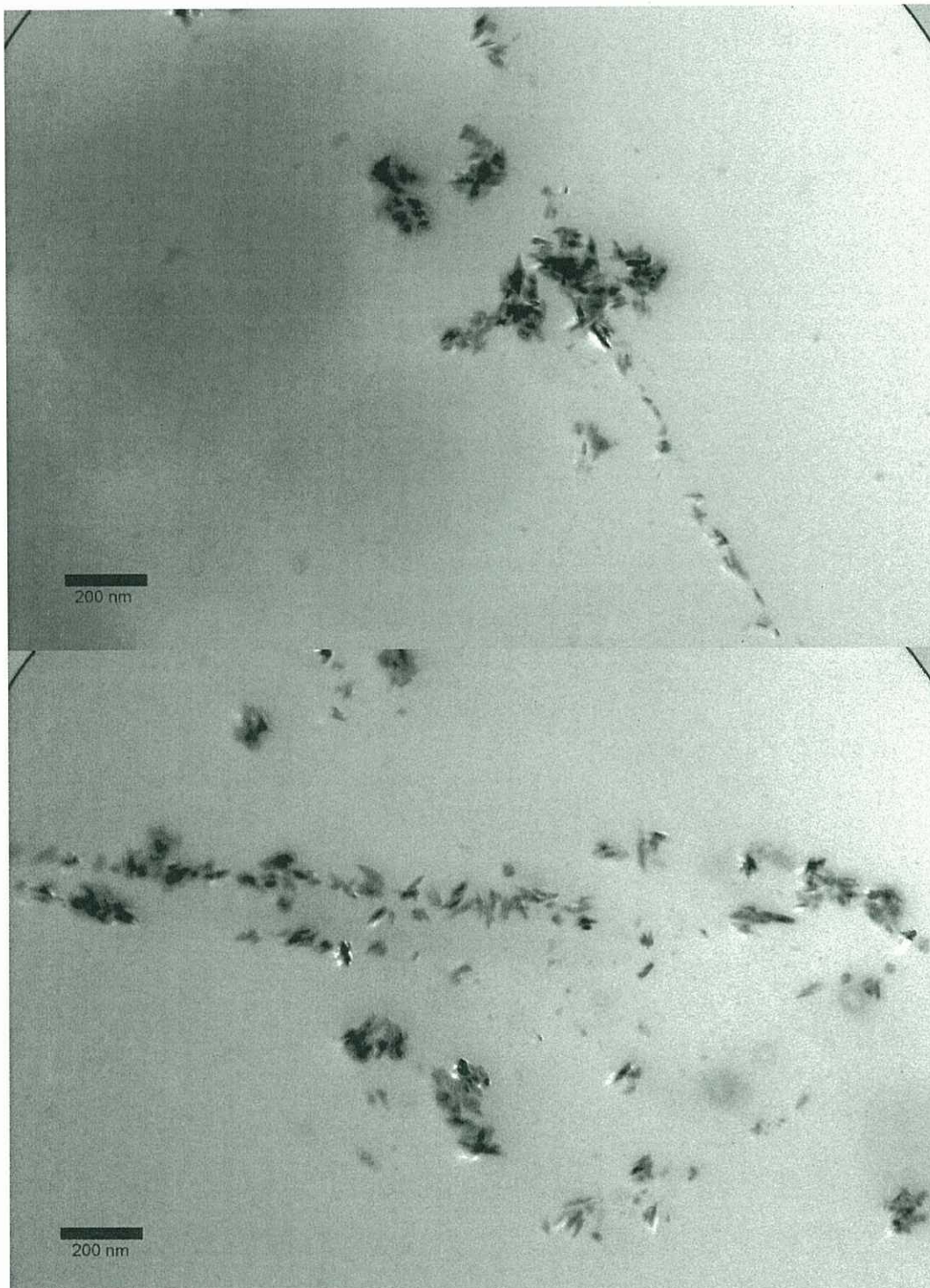


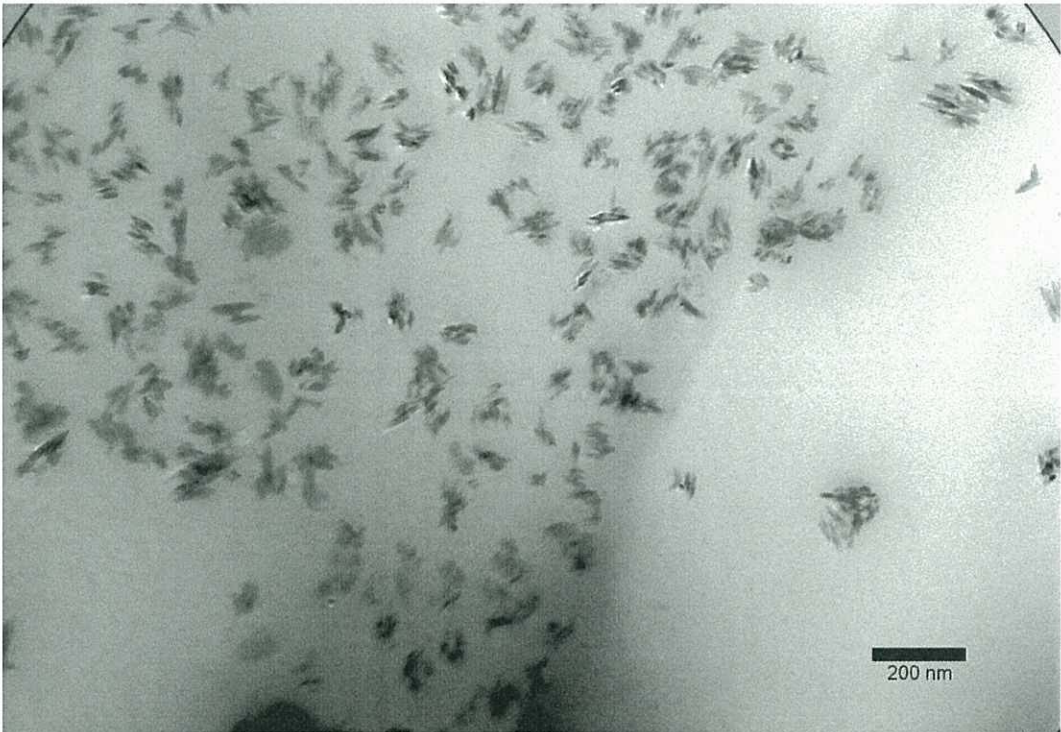


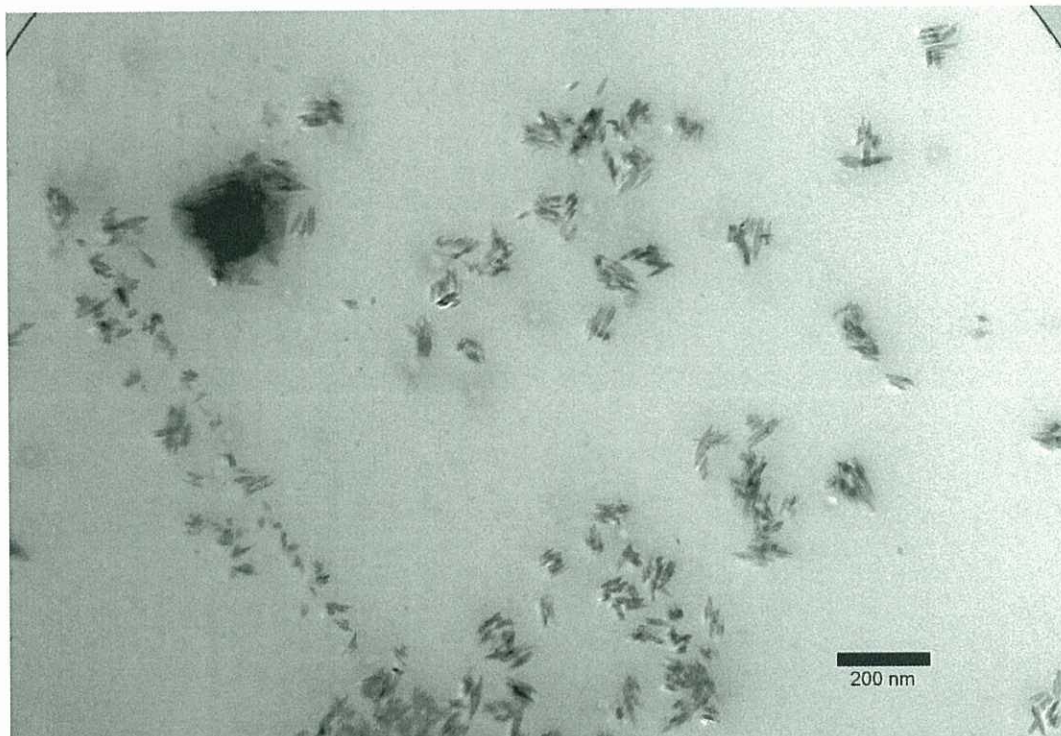


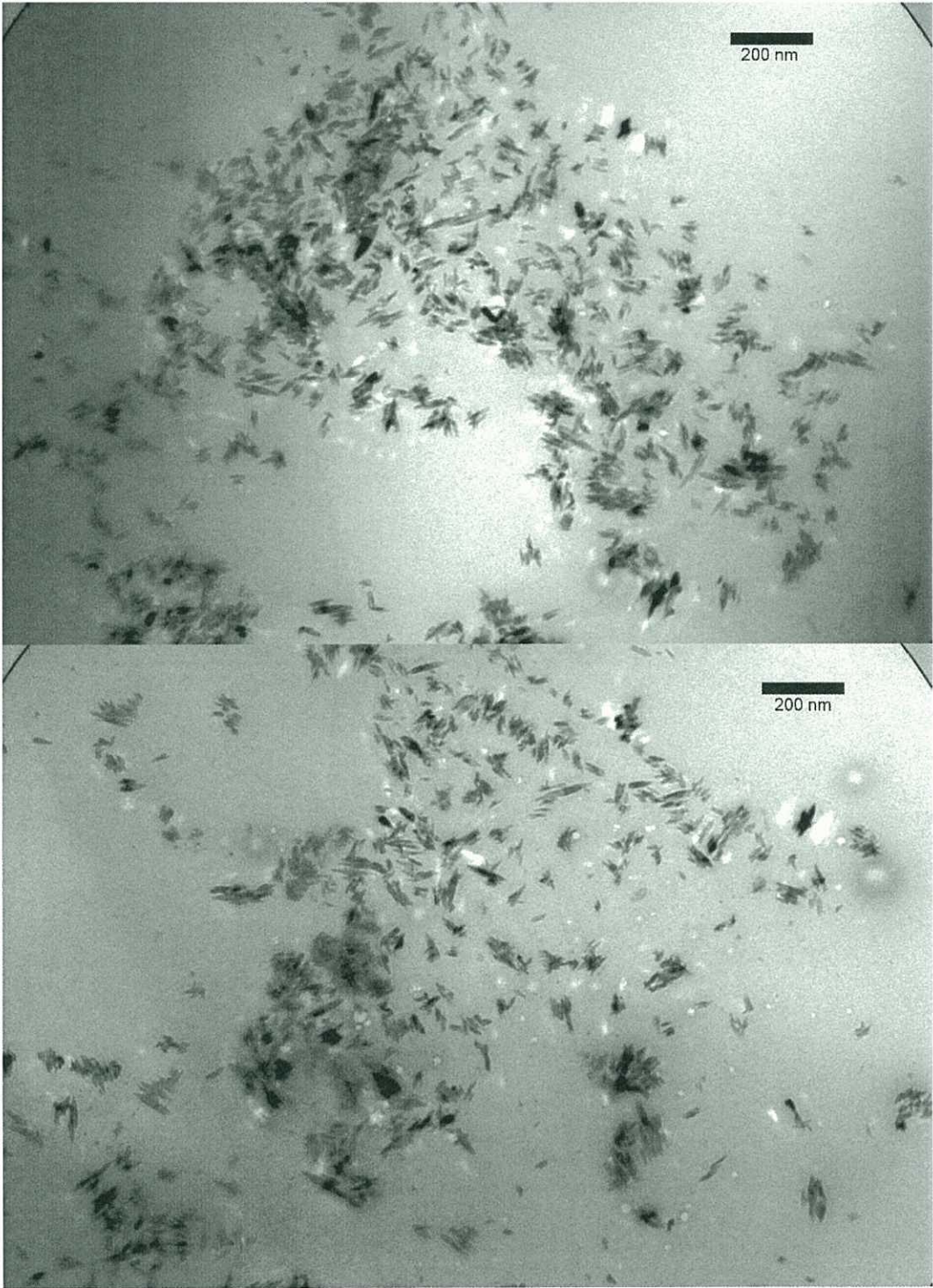
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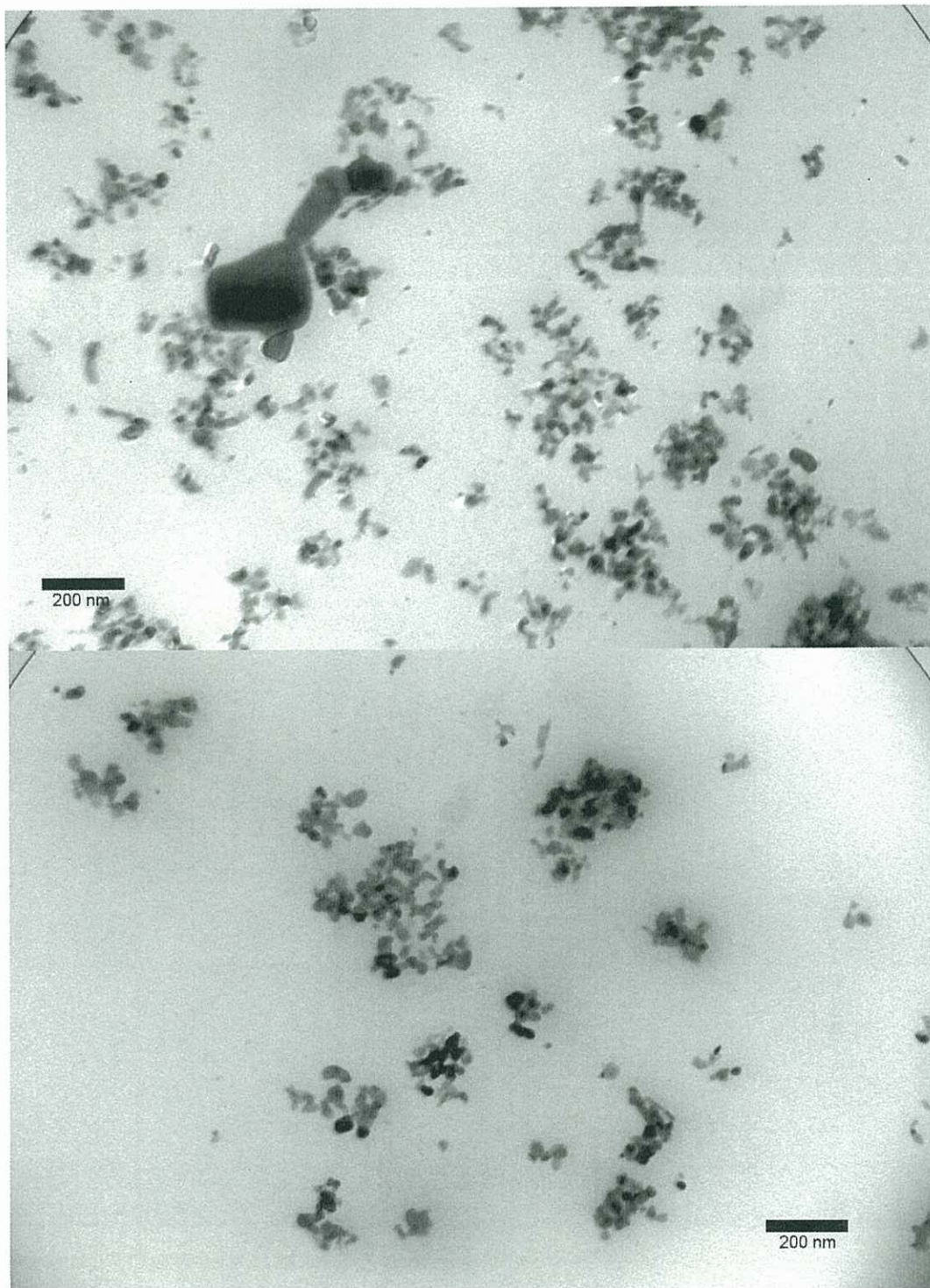


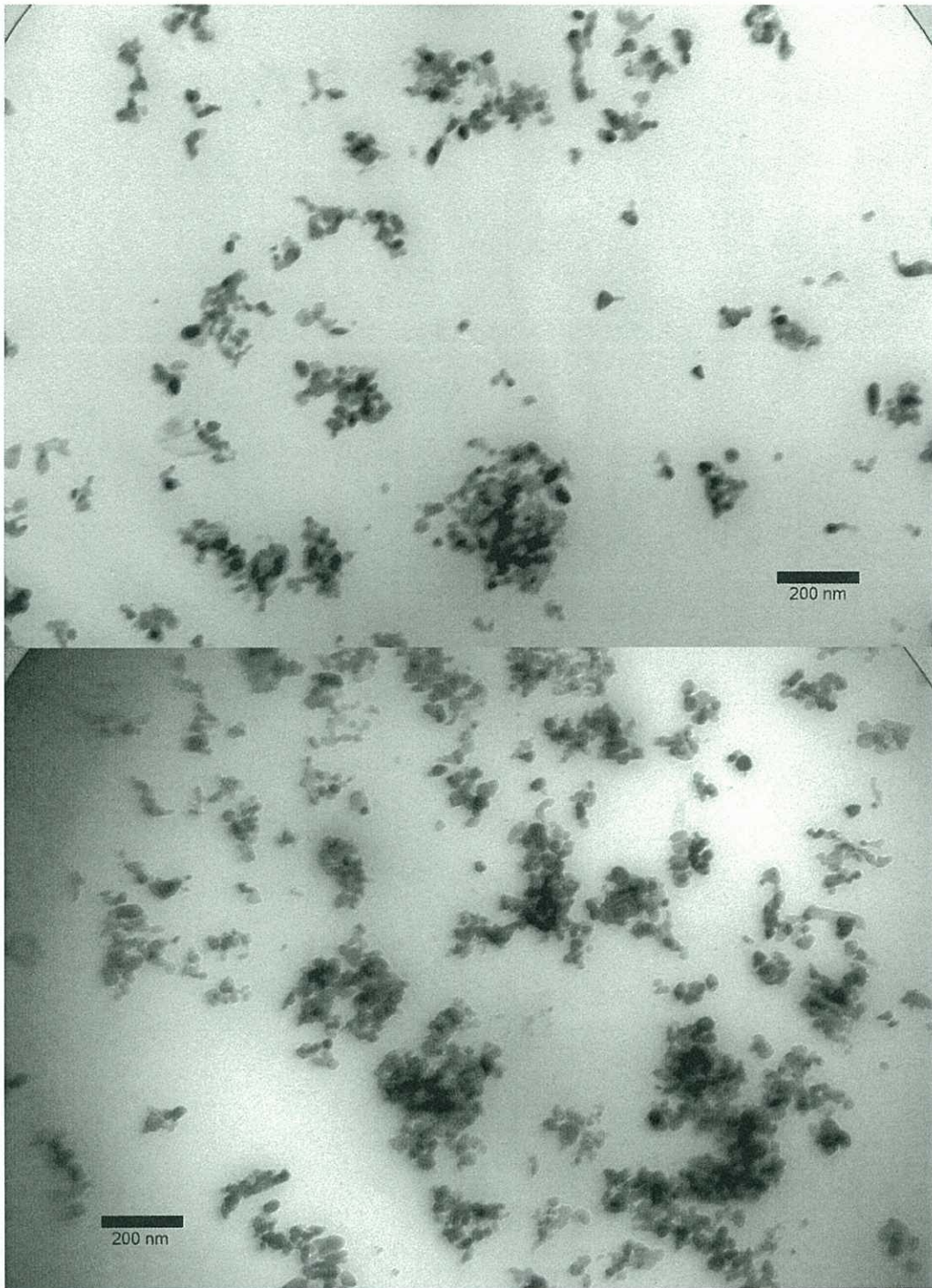


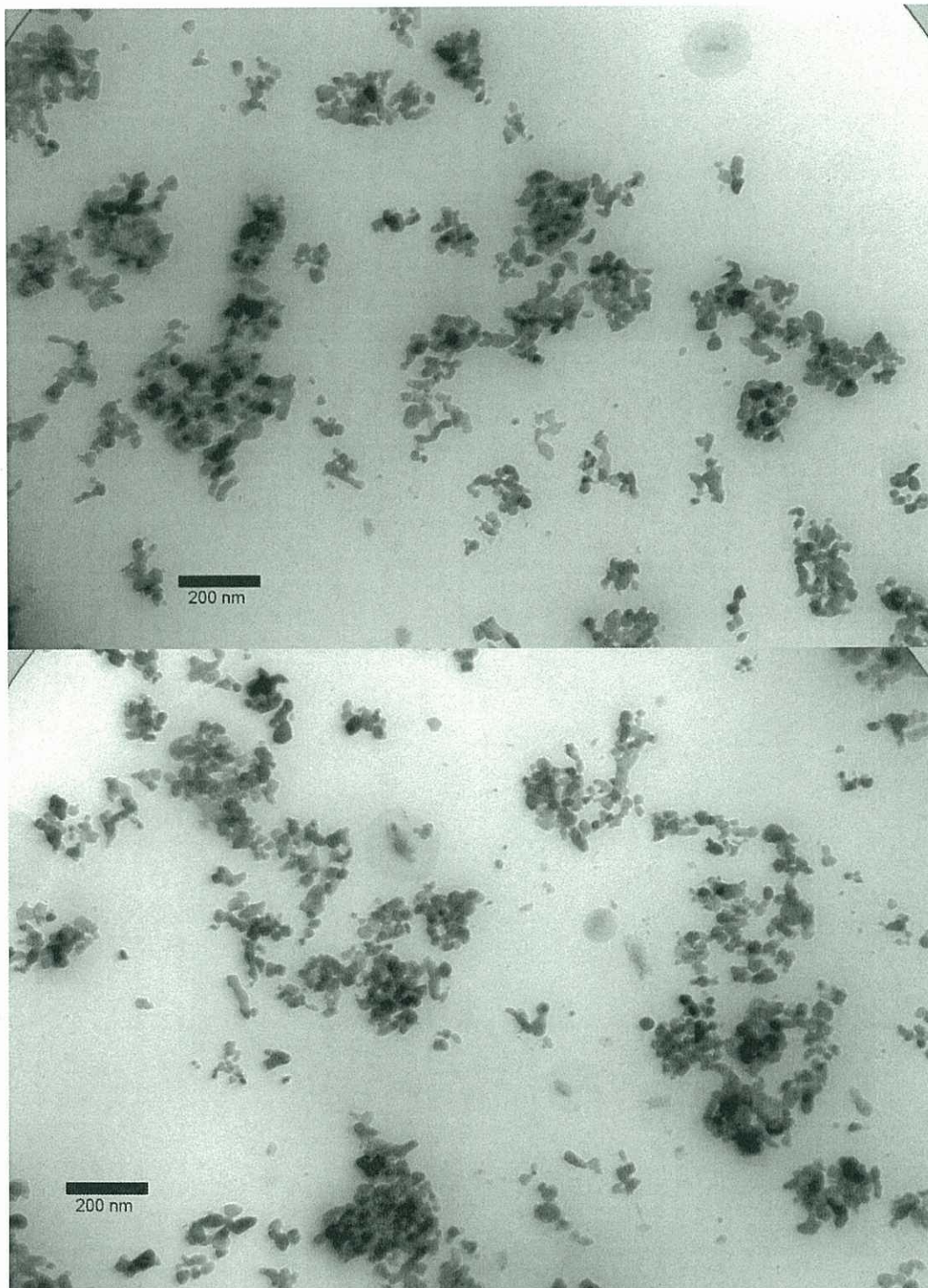


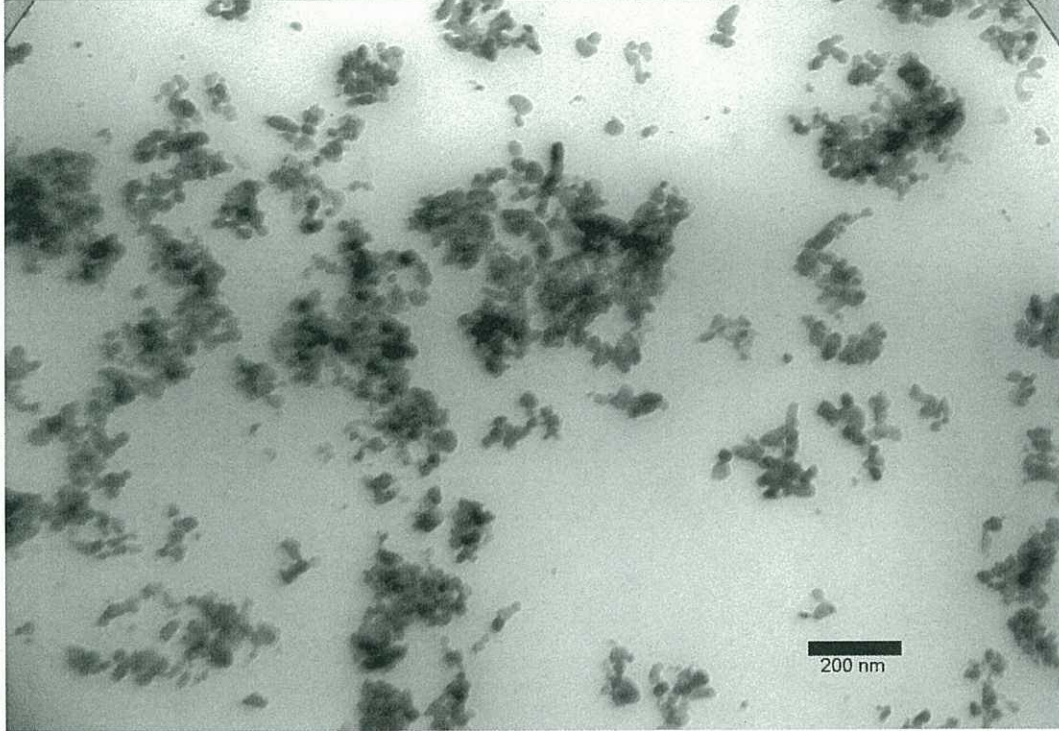
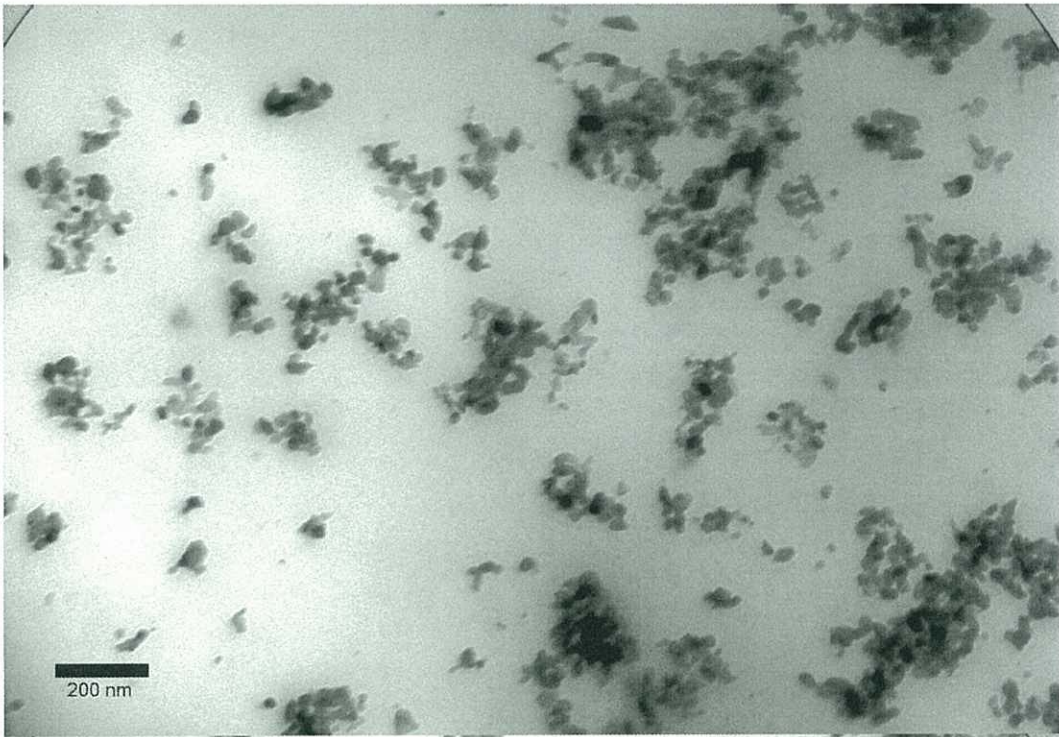
A handwritten signature in black ink, consisting of a stylized 'C' and 'J'.

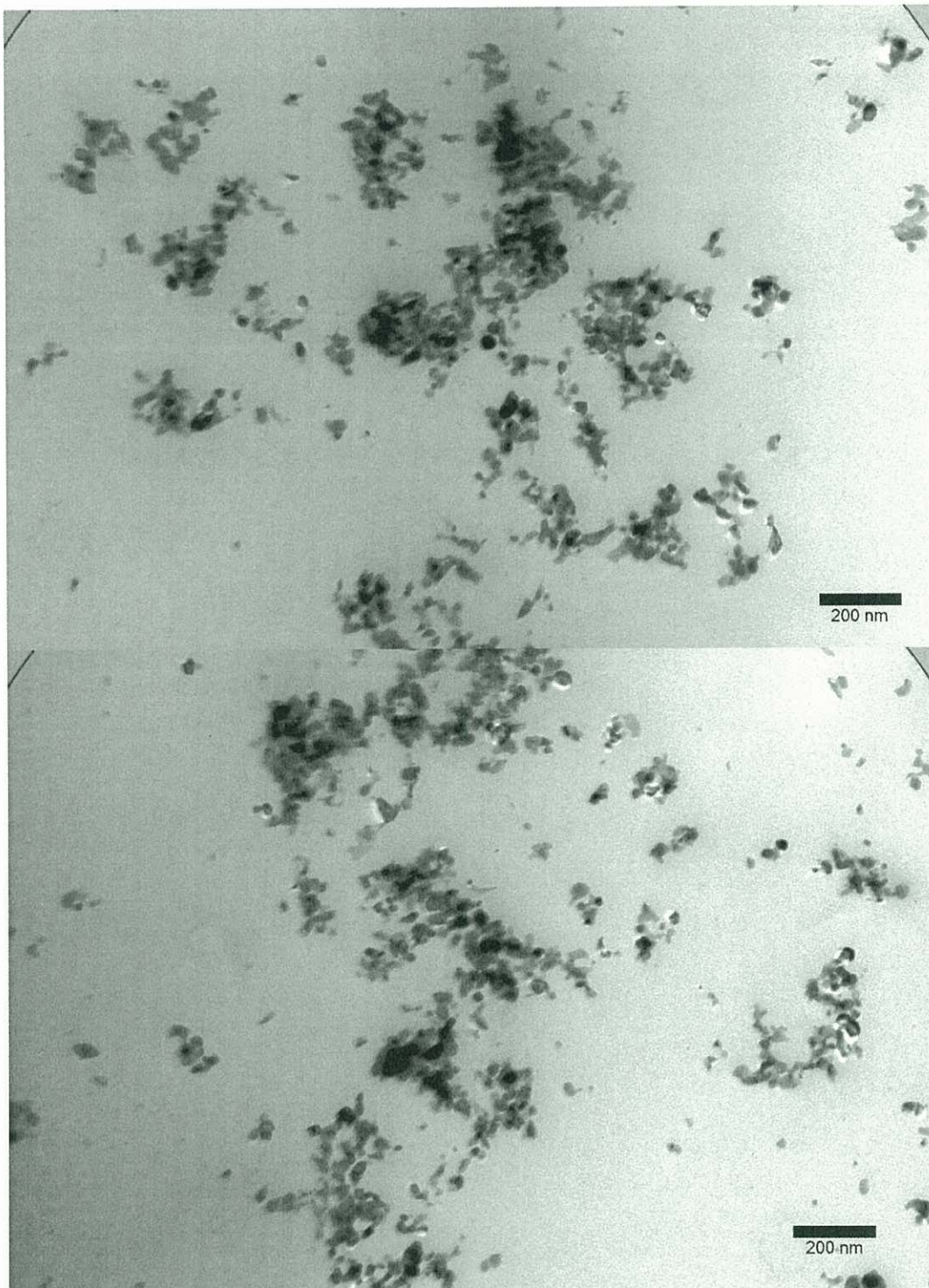
M122949_006



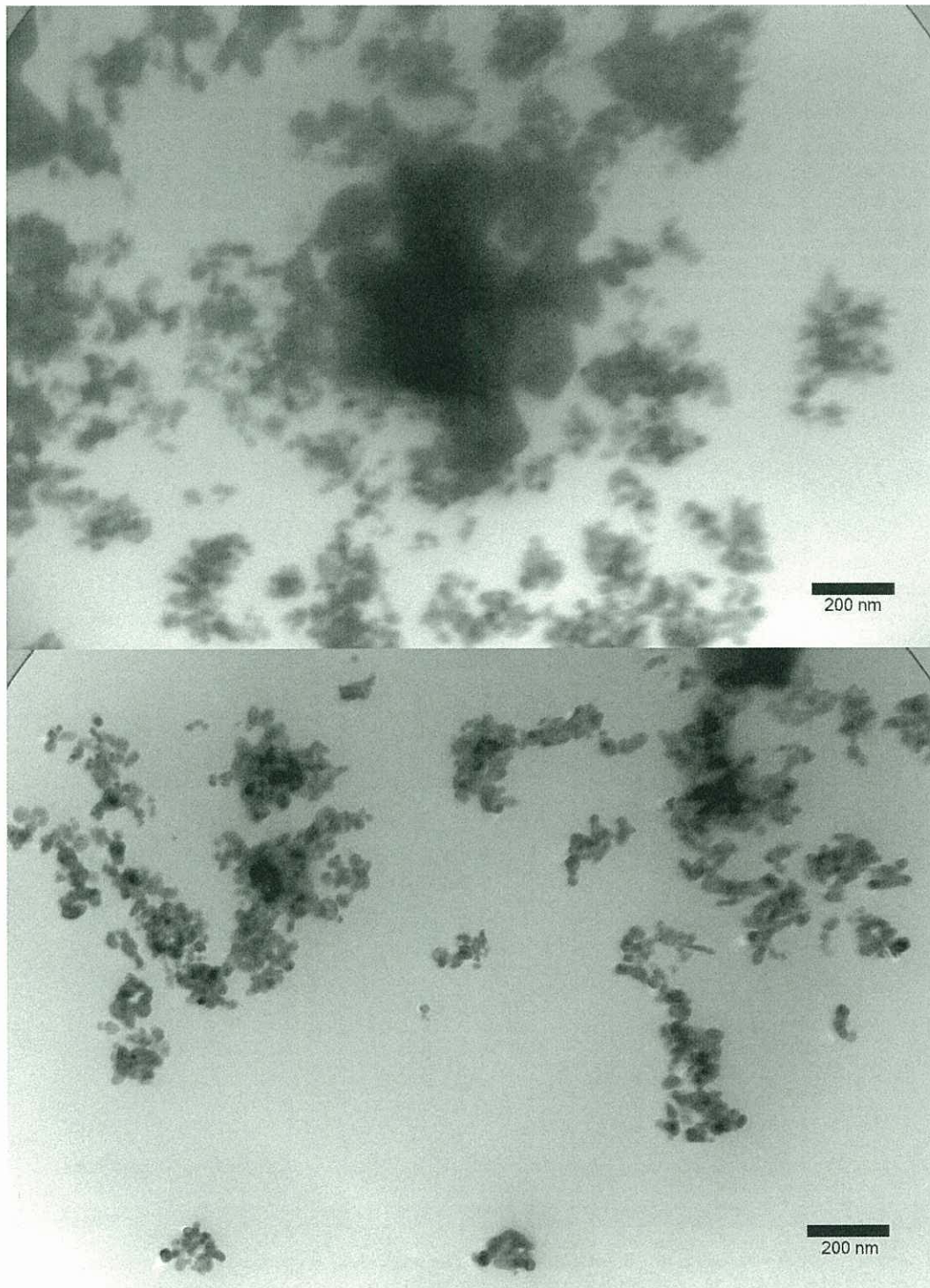




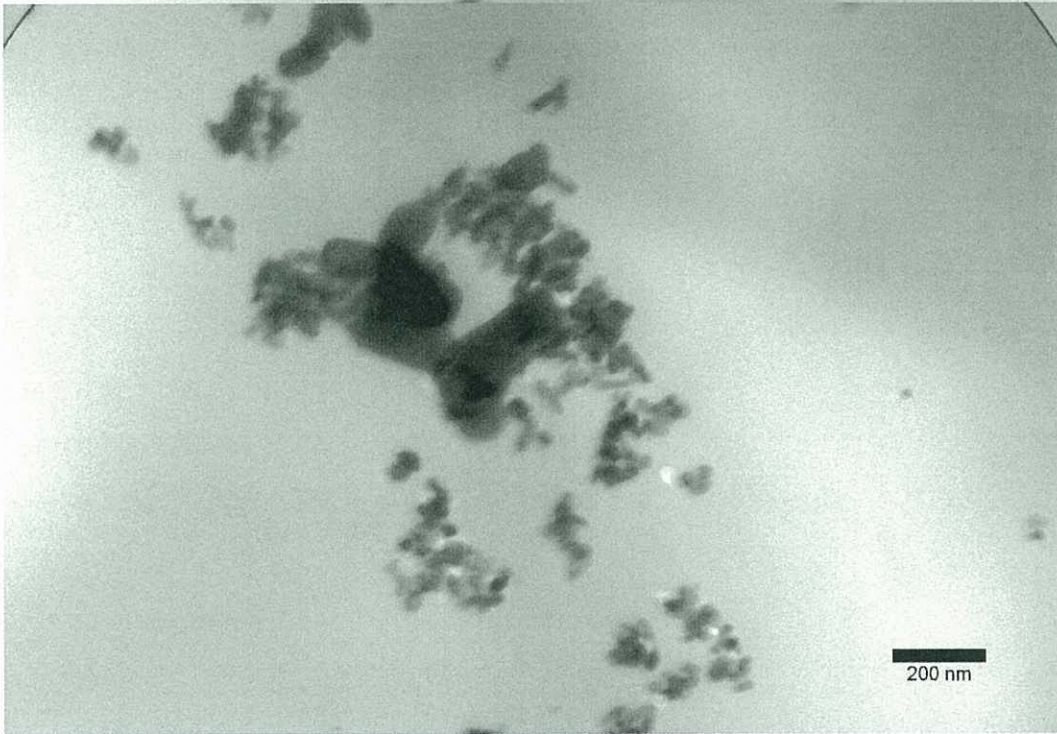
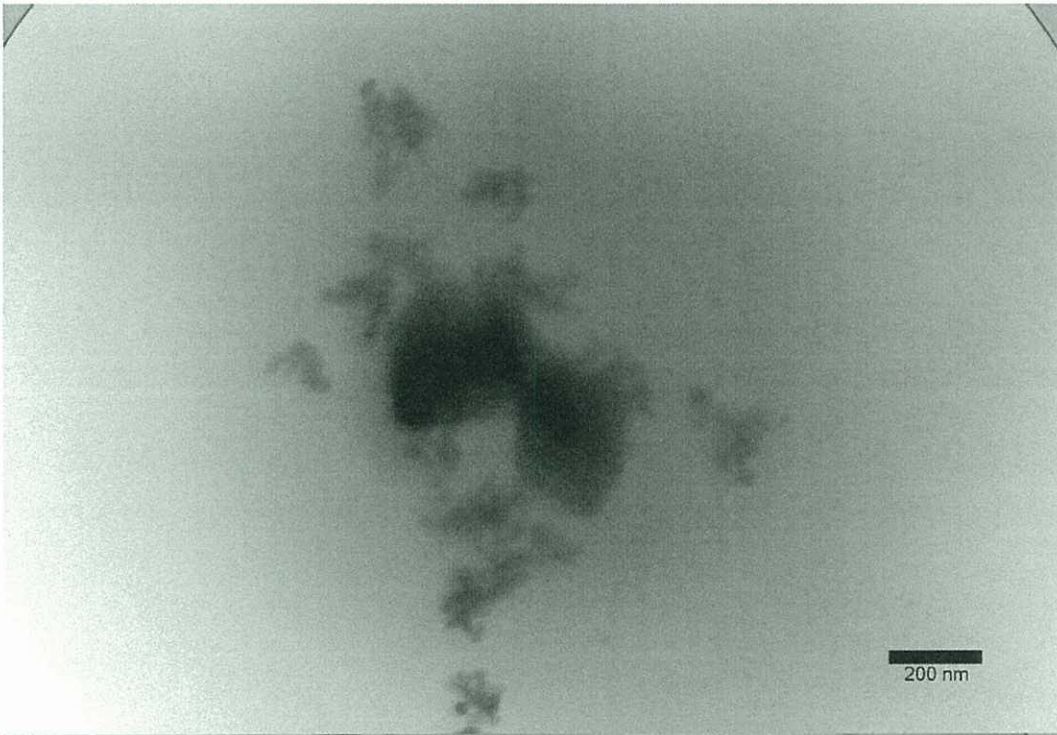




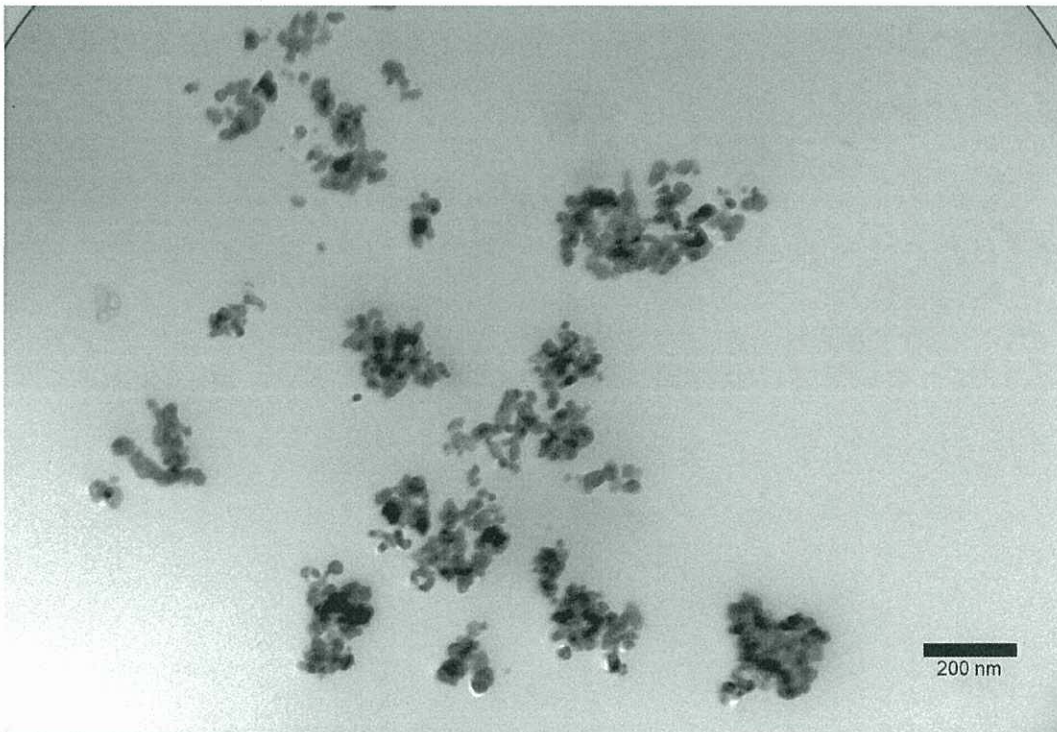
M122949_007











7. Conclusion

Nanomaterials (as defined by ISO¹) were detected in samples of particulate material extracted from all of the four sunscreens.

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References

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