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DCS measurements and representative TEM images of a sub-set of sunscreen samples

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For:	Dr Gregory Crocetti Friends of the Earth Australia 312 Smith Street Collingwood VIC 3066
Reference:	Q122949, Q123217
Description:	DCS measurements and representative TEM images of a sub-set of sunscreen samples
Maker:	Various. Please see Section 2 of report for sample listing.
Date of Receipt:	All samples received on 06 September 2012 except Coco Islands, received on 14 September 2012
Serial Number:	M122949
Previous Examination:	XRD phase analysis of the samples was conducted previously as described in 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.

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[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 of the solid fraction of material extracted from one sunscreen sample, and representative transmission electron microscopy (TEM) images of the solid fraction of material extracted from three 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 TiO_2 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 (%)
KEY SUN *WHITE ZINKE* SPF 30+ 4HRS Water Resistance UVA Broad Spectrum UVB 50 g NET	68007B	M122949_003	32	4
NIVEA SUN *Kids Swim & Play* *Protective Lotion* SPF 30+ VERY HIGH PROTECTION Broad Spectrum UVA+UVB Filters With Panthenol Protects the skin barrier 4 h water resistant 150 mL	B12470577	M122949_004	—	6
Coco Island SPF 30+ Broad Spectrum 4 Hours Water resistant WHITE ZINC CREAM 50 g Aust.	848WI	M122949_005	32	4

In addition to the client's samples, TEM imaging of NIST Reference Material 8013[c] (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.

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[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] 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 three 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_003	organic	yes	Both nano and non-nanomaterial observed in TEM
M122439_004	aqueous	yes	—
M122439_005	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.

Samples M122949_003 and M122949_005 contained two principal metal oxide components (TiO_2 and ZnO) and were therefore not suitable for DCS analysis.

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 MΩ cm, filtered through final filter of 0.22 μm) 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 ≥ 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 ≥ 97.0 %, Lot# SZBC085CV) was added and the separating funnel was stoppered and shaken vigorously for ~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.

Sample M122949_004 contained particles in the aqueous phase (as indicated by the turbidity of the phase and by DCS measurements).

Samples M122949_003 and M122949_005 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 ≥ 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 MΩ cm, filtered through final filter of 0.22 μm) was added and the separating funnel was stoppered and shaken vigorously for ~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.

In the case of samples M122949_003 and M122949_005, the organic layer remained clear after settling. However a thick white layer, presumed to contain particles, accumulated at the bottom of the organic phase. This layer was agitated with a glass pipette and sampled for analysis.

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 water (MilliQ, 18 MΩ 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 a 8-240 mg g^{-1} density gradient solution of sucrose (Sigma, 99.5% Lot# 029k0010) in ultrapure water (MilliQ, 18 MΩ cm, filtered through final filter of 0.22 μm). Subsequently, 0.5 mL of dodecane (Sigma, Reagent Plus, ≥ 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 sample was 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.

	TiO_2	Gradient fluid[d] (aqueous)
Refractive index	2.59[e]	1.072
Absorption coefficient	0.0015[e]	—
Density (g cm^{-3})	4.0[f]	1.361
Dynamic viscosity (mPa s)	—	1.2

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[d] 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.

[e] Value for rutile TiO_2 at 27 °C and a wavelength of 405 nm. Taken from: http://www.luxpop.com/ accessed on 23 November 2012. The value of rutile was used for both anatase and rutile because an equivalent value for anatase could not be found.

[f] Density calculated as a weighted average density between anatase 3.9 g cm^{-3}, assumed to be 750 mg g^{-1} and rutile 4.2 g cm^{-3}, assumed to be 250 mg g^{-1}. The values for the density of anatase and rutile were obtained from the CRC Handbook, 90th Edition.

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

Image J software[g] 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 $(\text{pixel})^2$, 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 $(1.159 \pm 0.012)\ \text{nm}\ (\text{pixel})^{-1}$ 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.

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[g] http://rsbweb.nih.gov/ij/

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, 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 the sample contains a detectable fraction of primary particles or aggregates/agglomerates with a Stokes diameter below 100 nm.

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

The $q_0^*(x)$ distribution by number is 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.

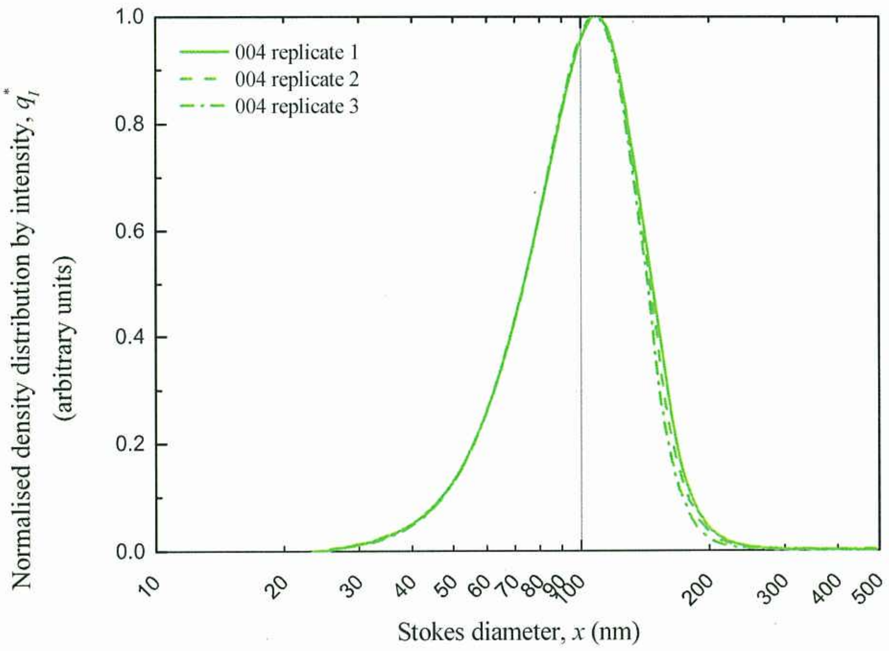
The distributions by volume and number are summarised in Table 4. Stated errors represent the standard deviations of the triplicate sets of measurements.

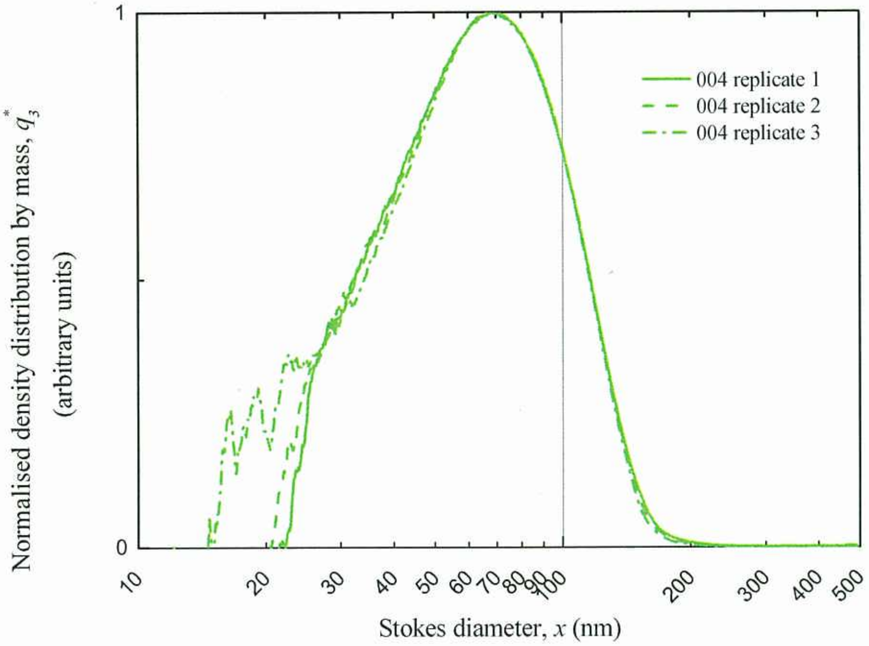
Table 4: Summary of DCS data.

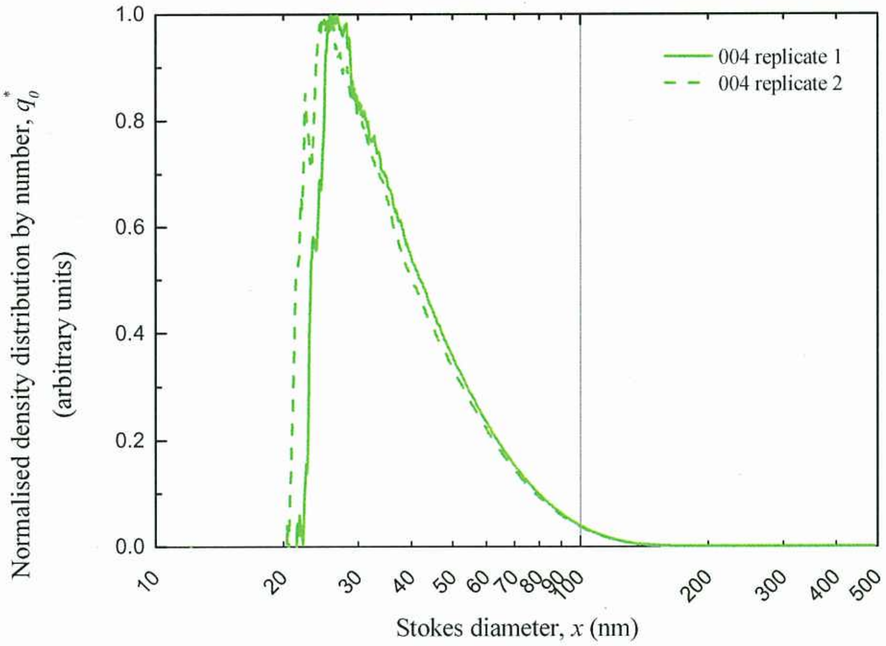
	$x_{3,10}$ (nm)	$x_{3,50}$ (nm)	$x_{3,90}$ (nm)	$x_{0,10}$ (nm)	$x_{0,50}$ (nm)	$x_{0,90}$ (nm)
M122949_004_1	33	67	109	13	17	52
M122949_004_2	28	65	108	9	12	31
M122949_004_3	29	66	108	–	–	–
Average M122949_004	30 ± 3	66 ± 1	108 ± 1	11 ± 2	15 ± 3	42 ± 15

Note that replicate 3 was very noisy and was excluded from number distribution analysis as the noise would dominate the measurement, giving anomalous results.

M122949_004



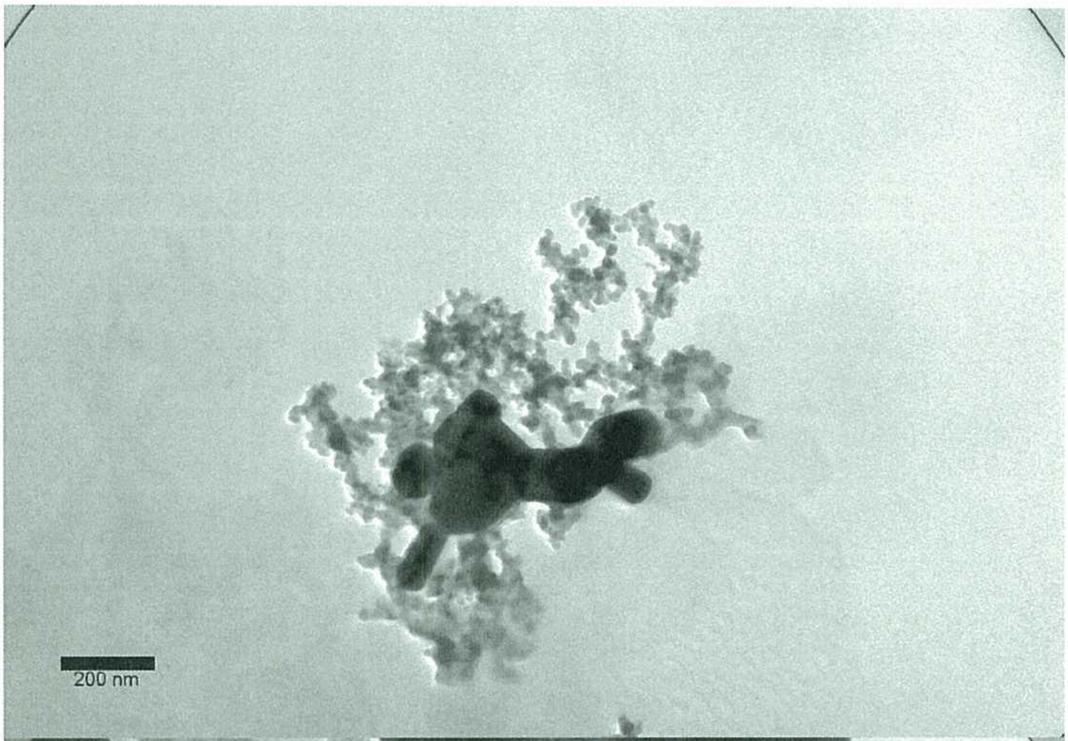


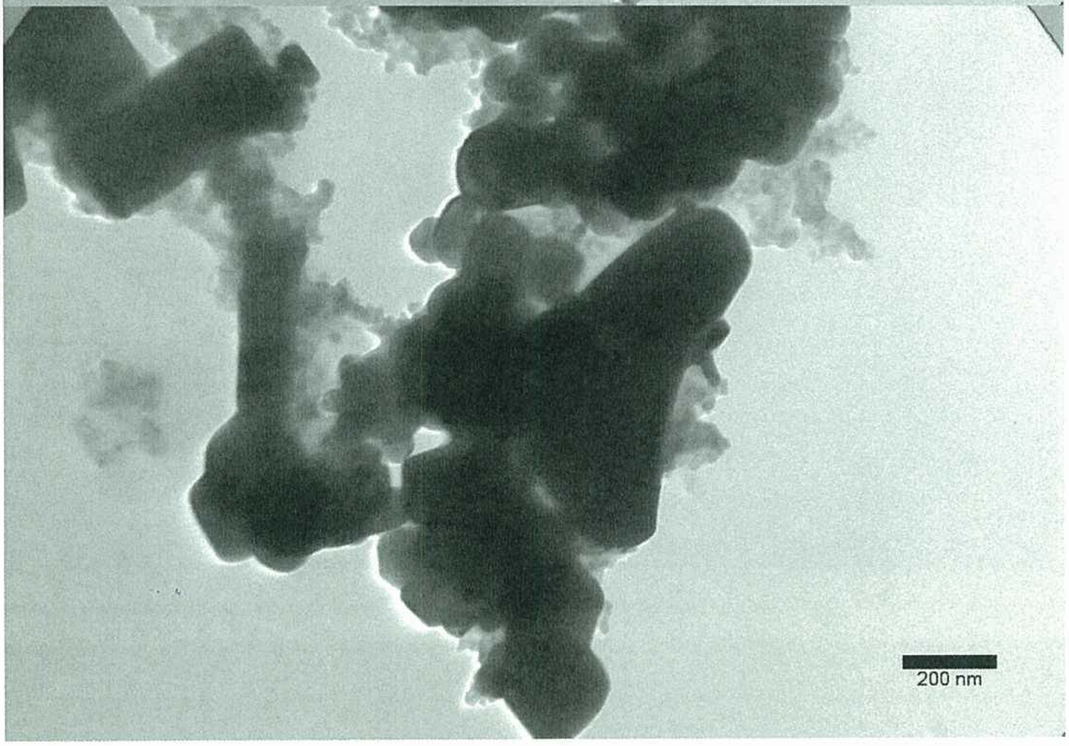


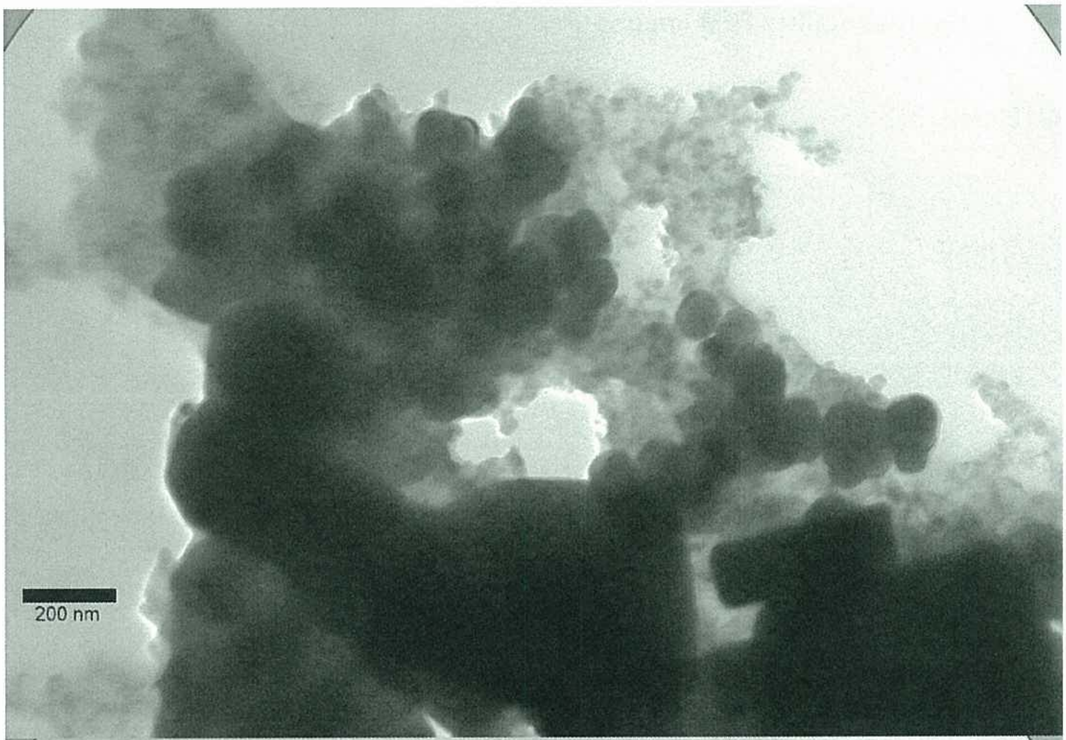
Note that replicate 3 was very noisy and was excluded from number distribution analysis as the noise would dominate the measurement, giving anomalous results.

5.2 Representative TEM images

M122949_003



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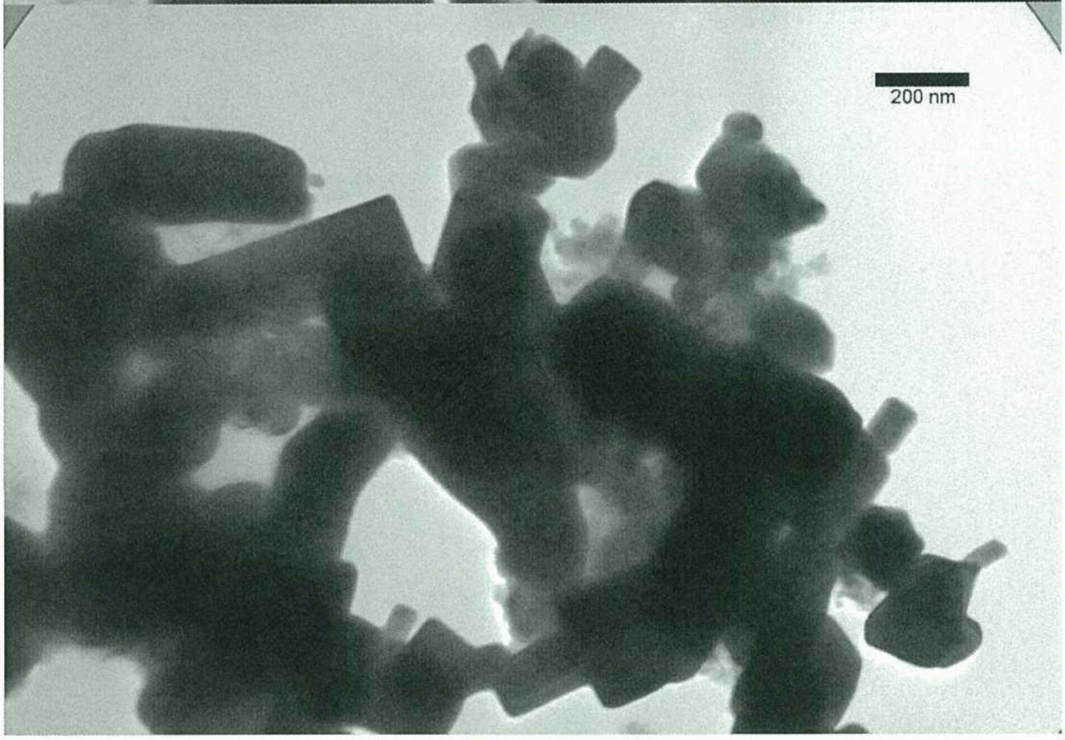
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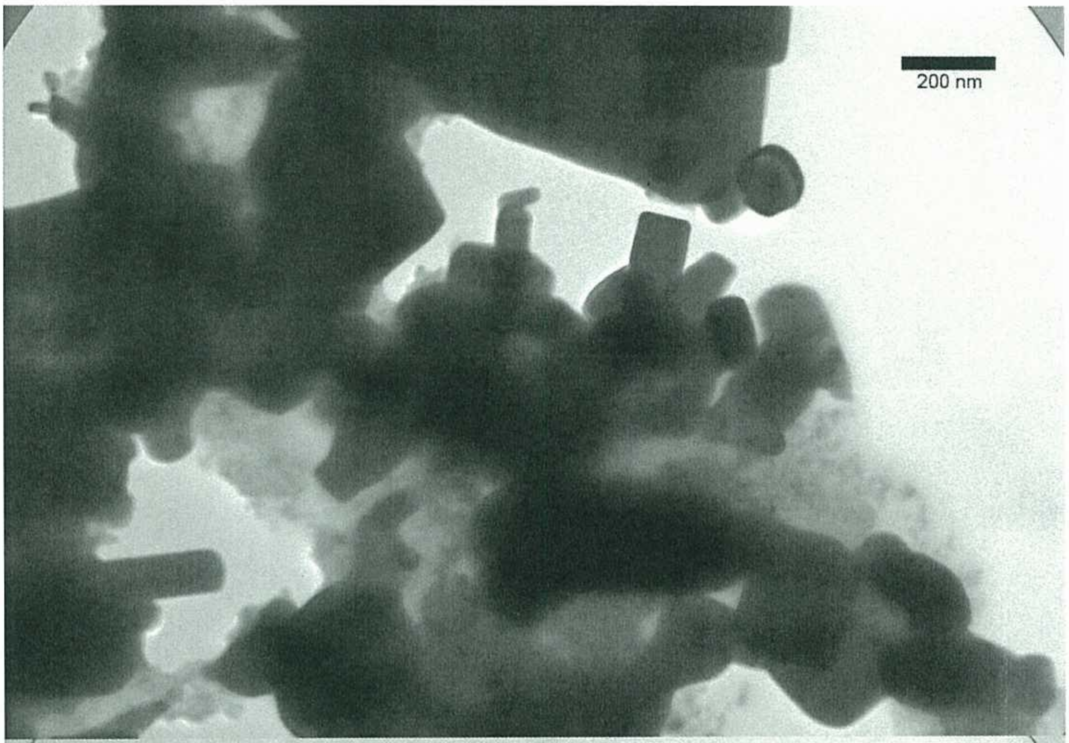
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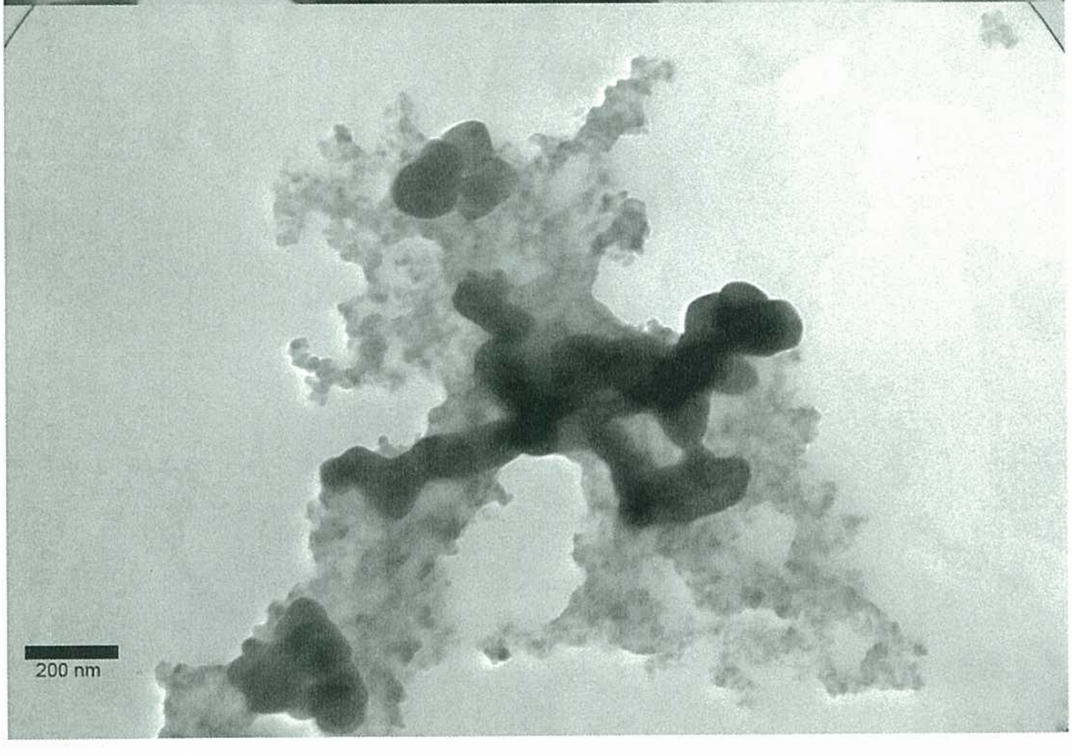
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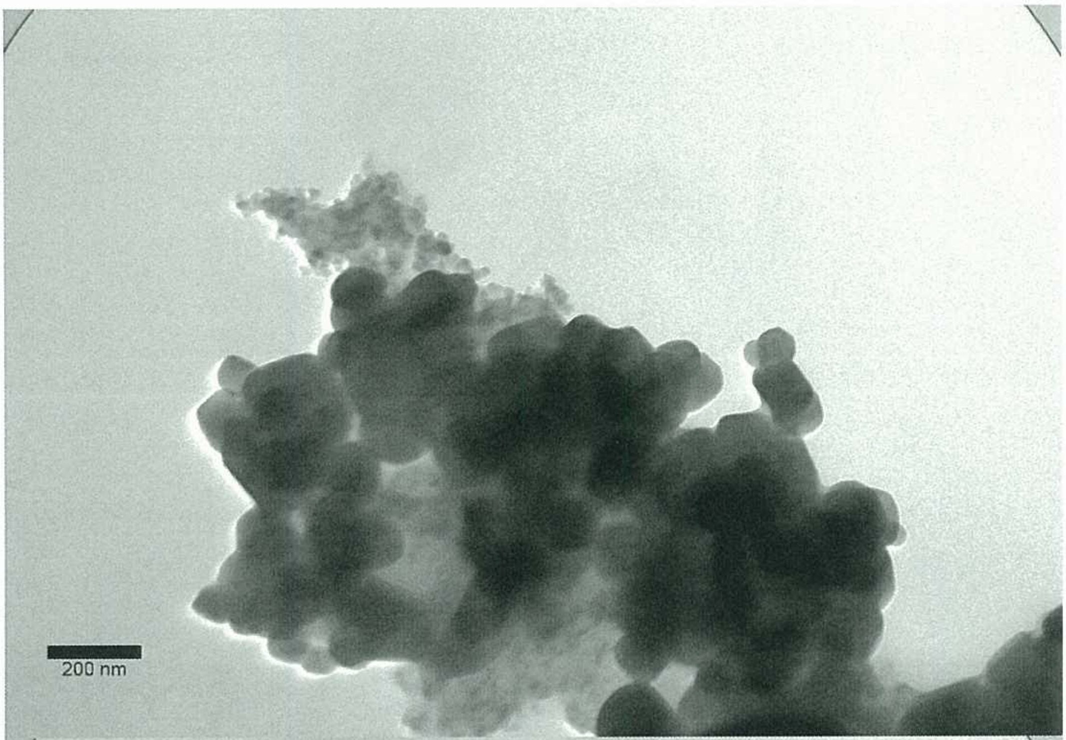
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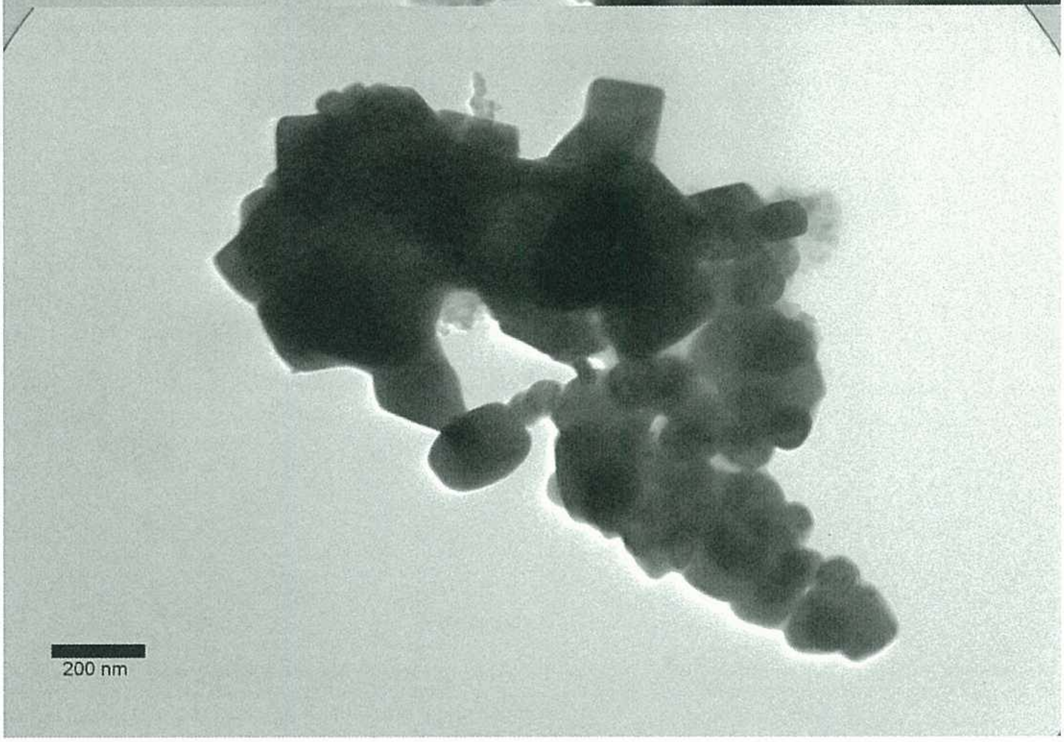
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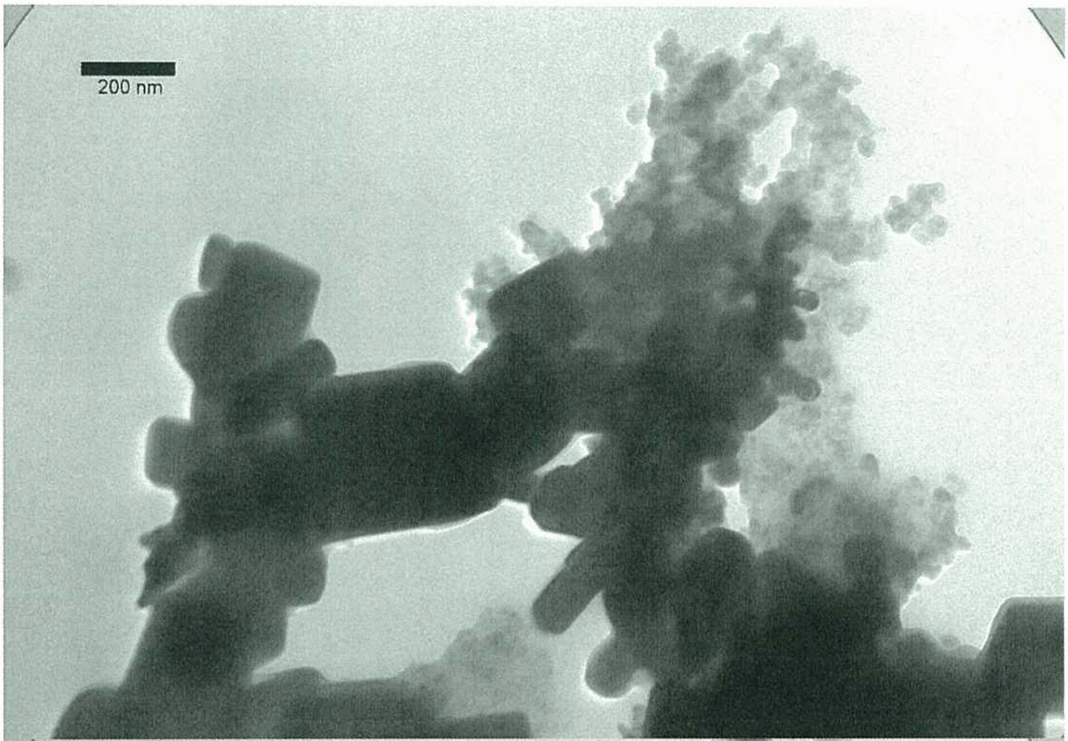
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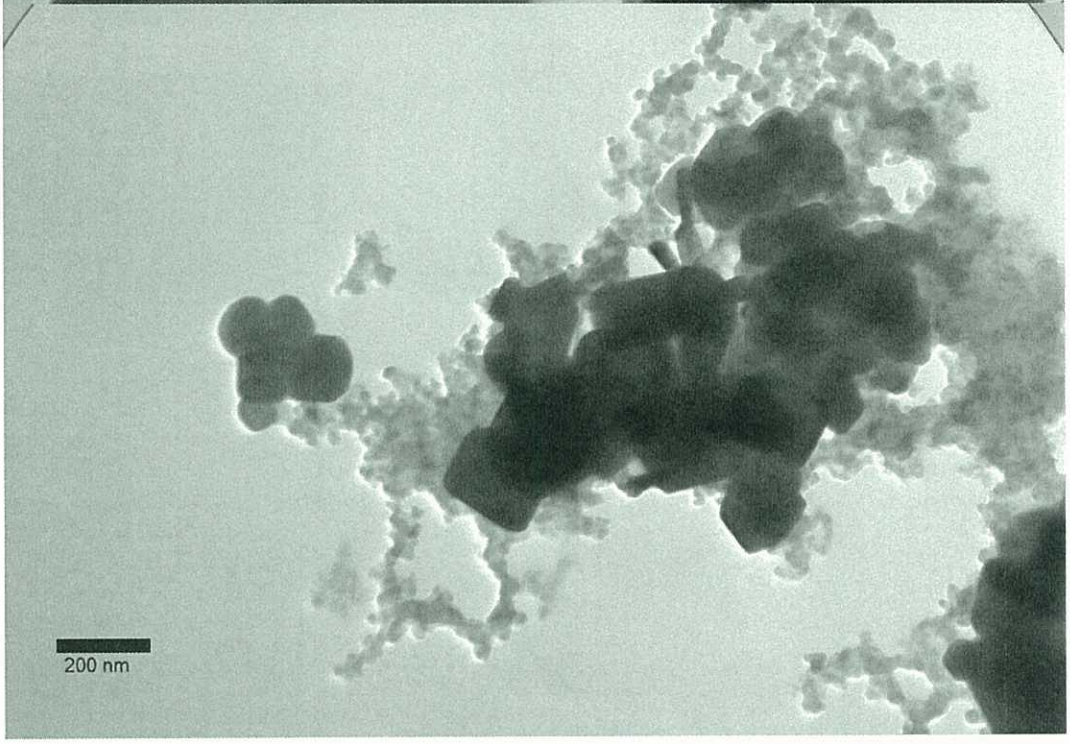
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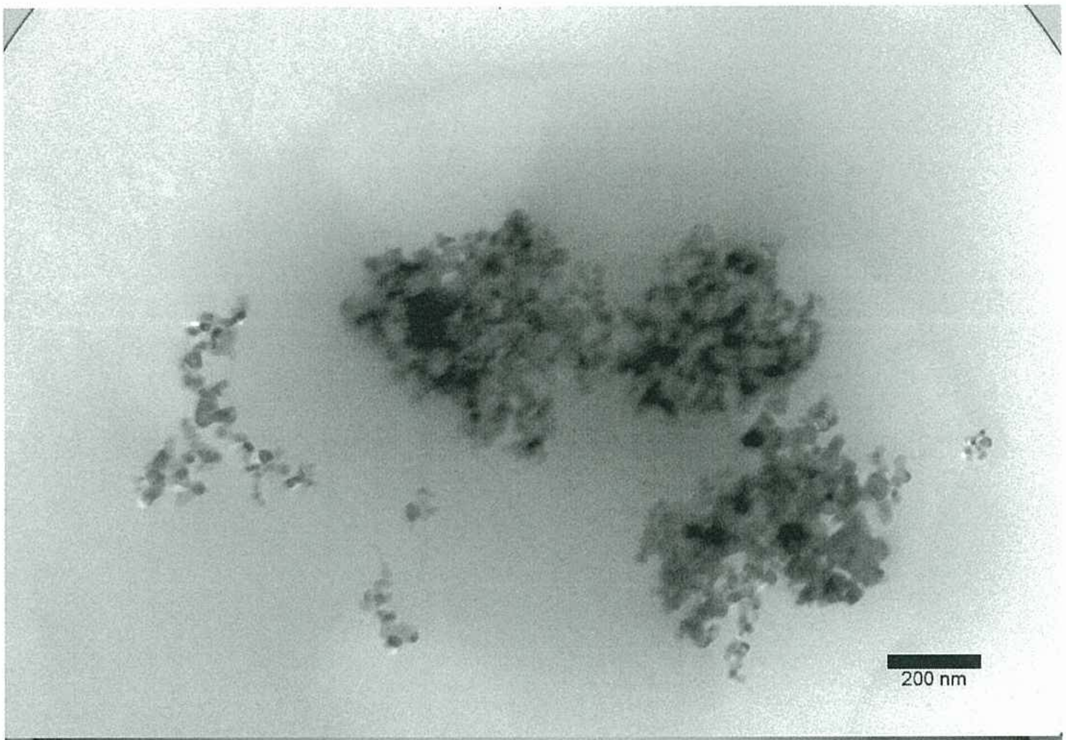

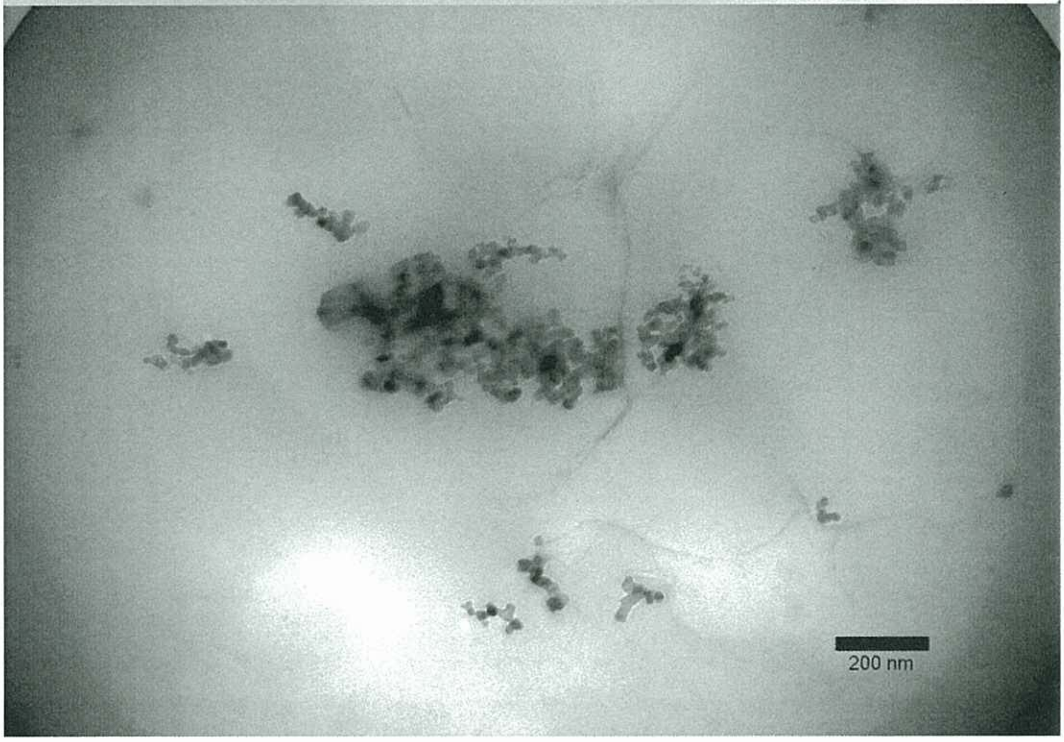

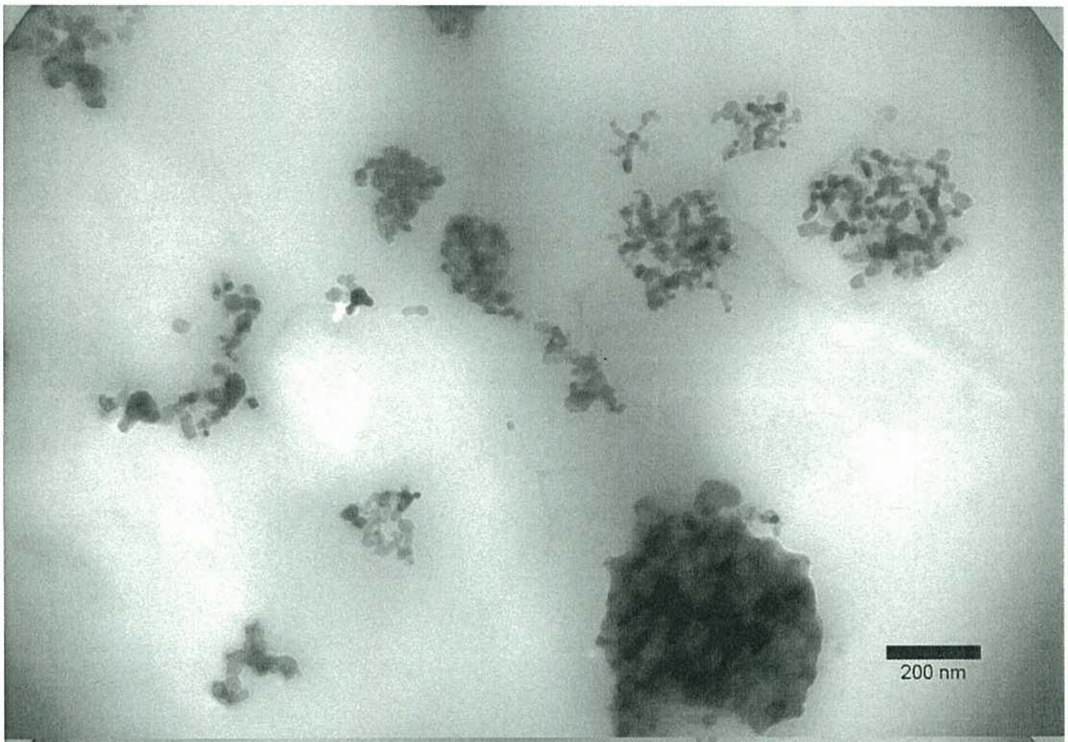
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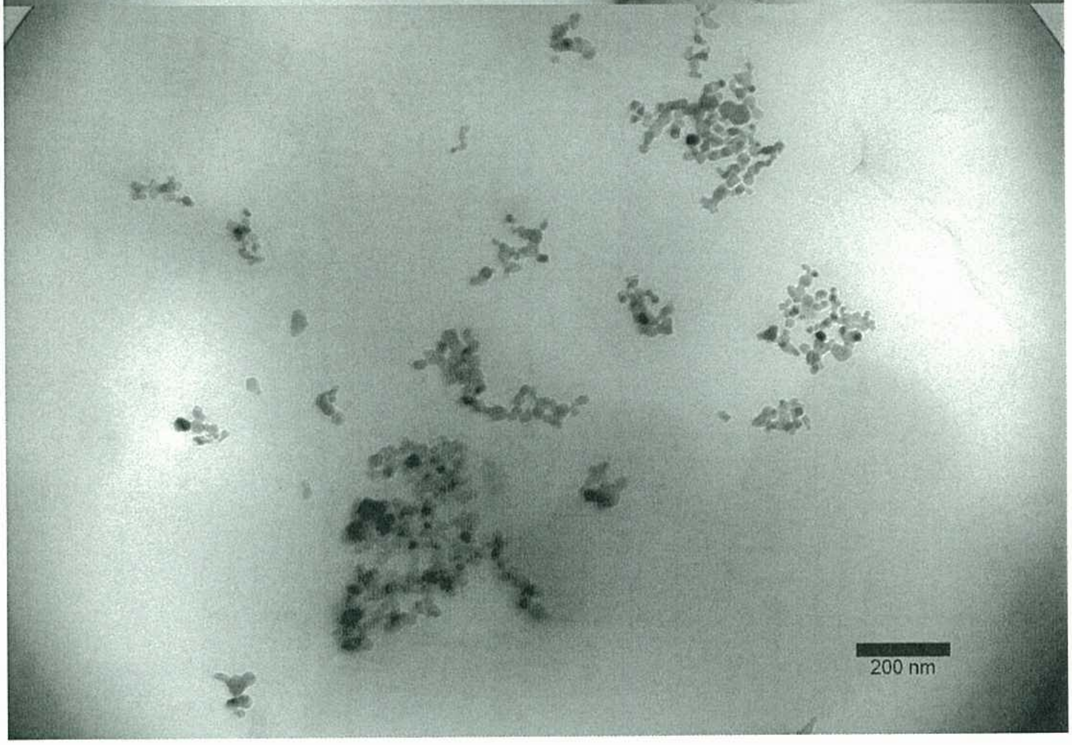
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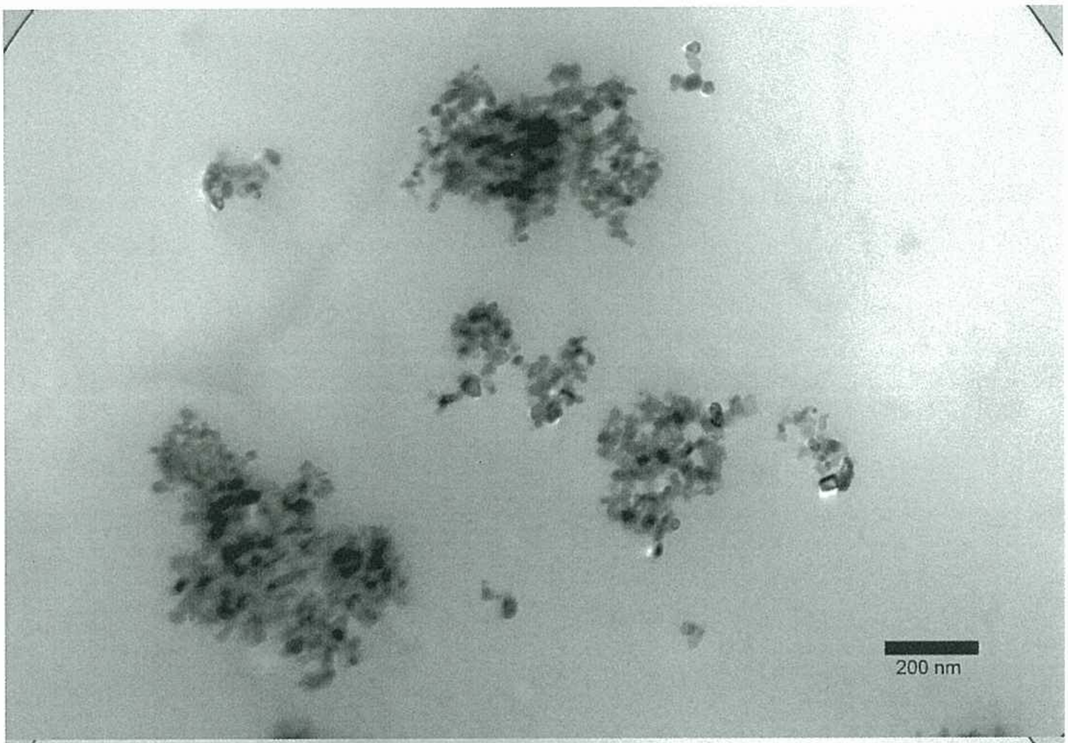
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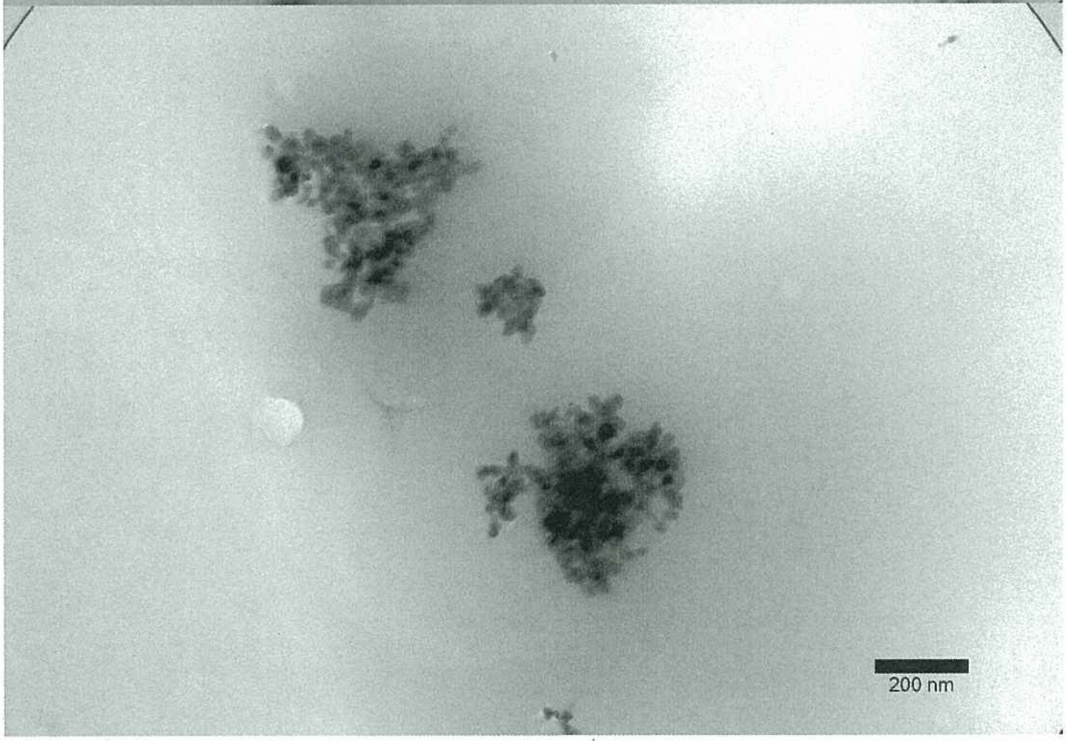
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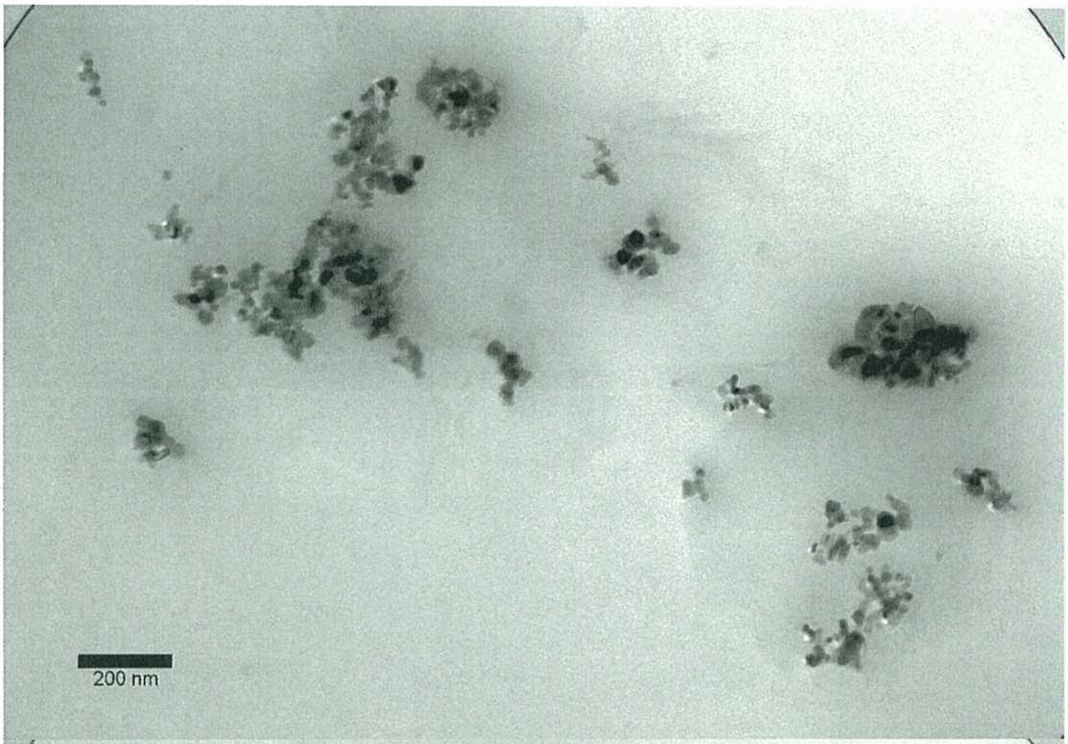
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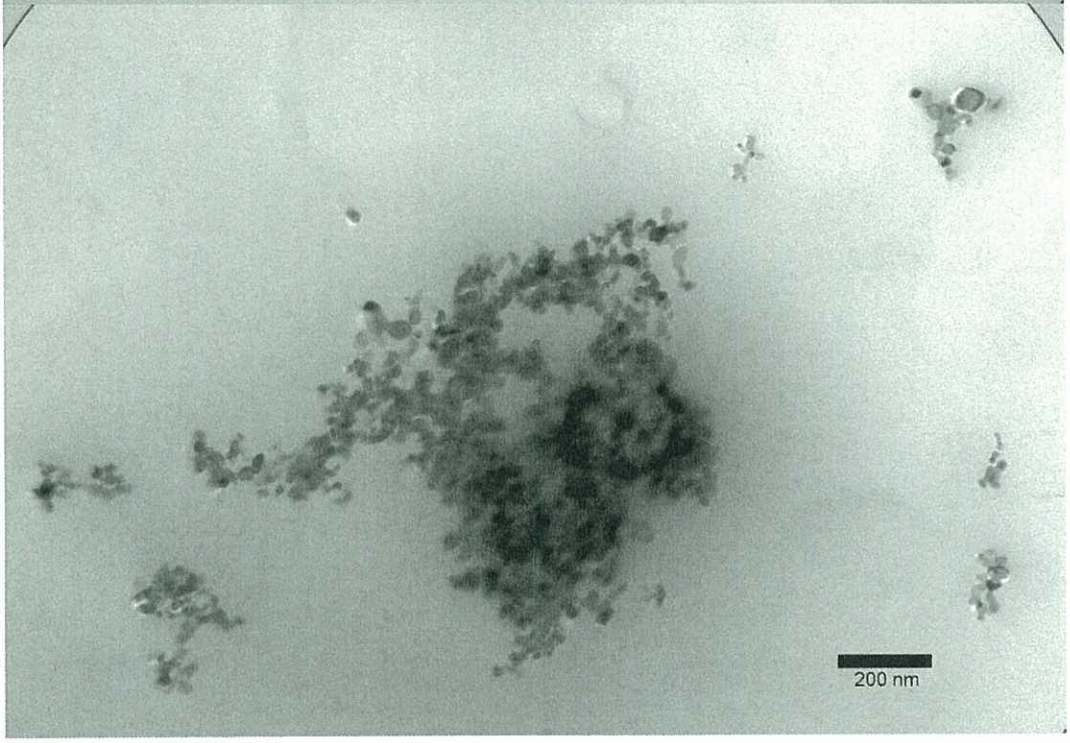
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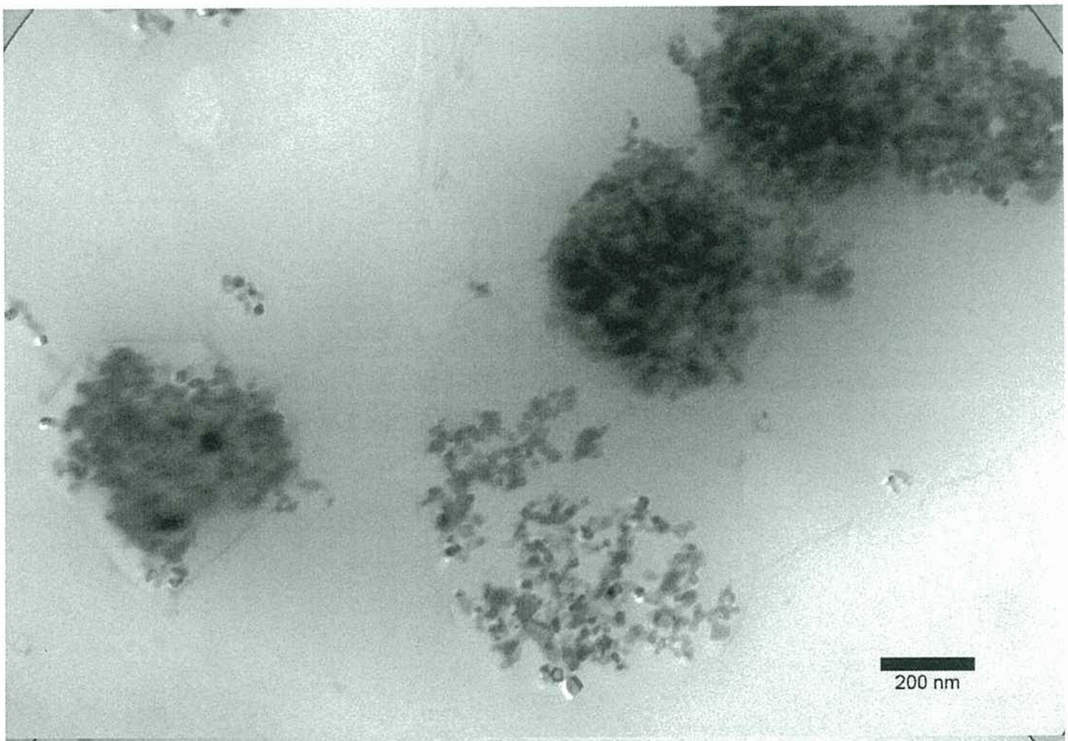
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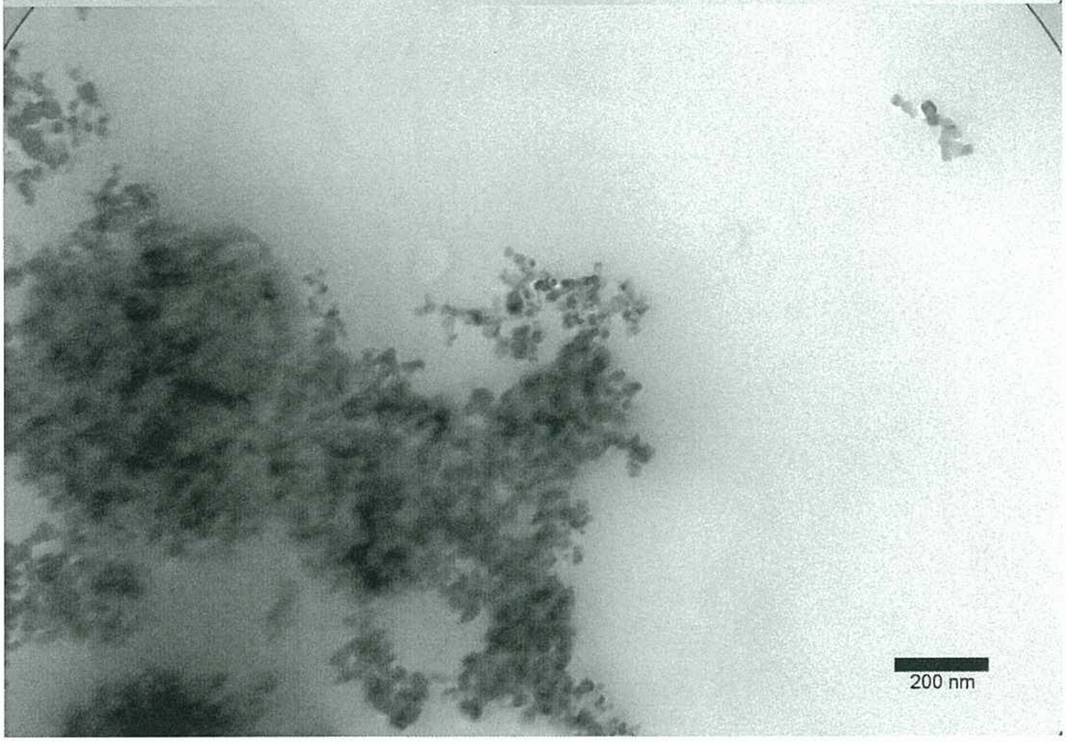
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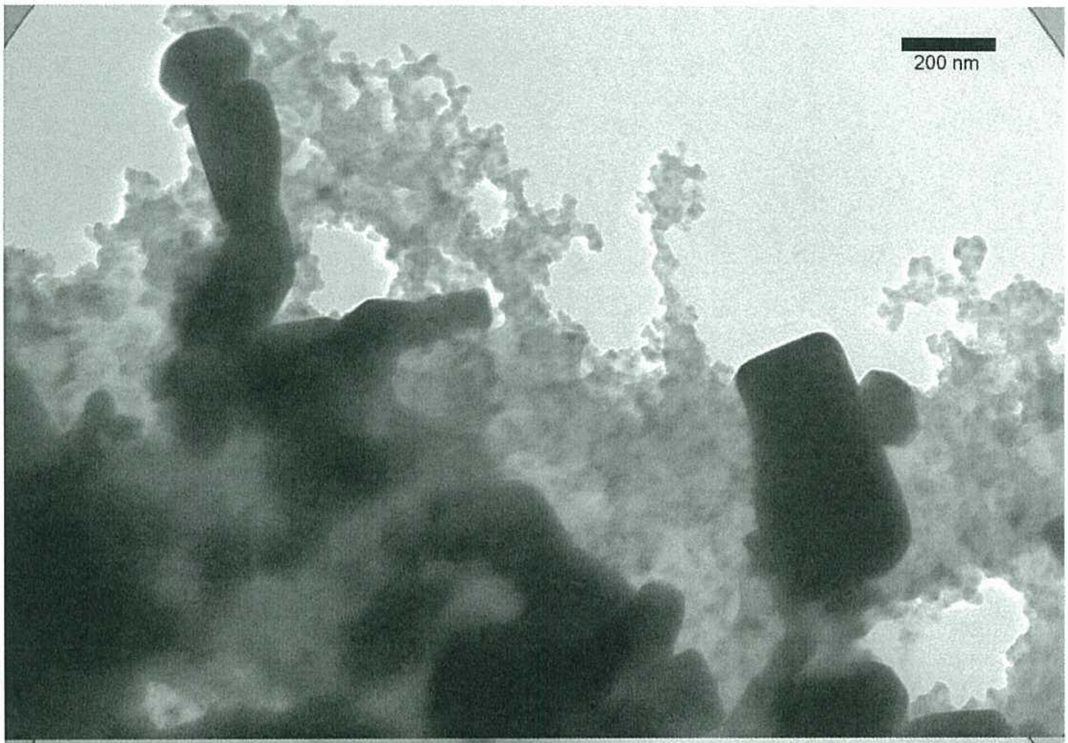

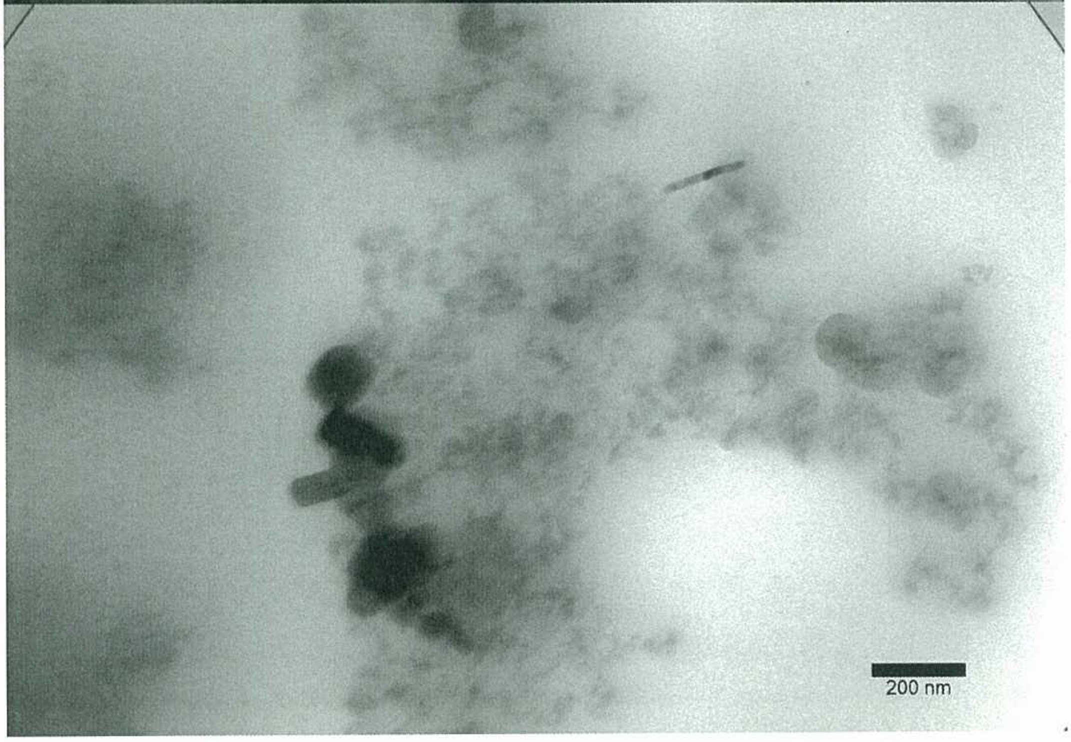

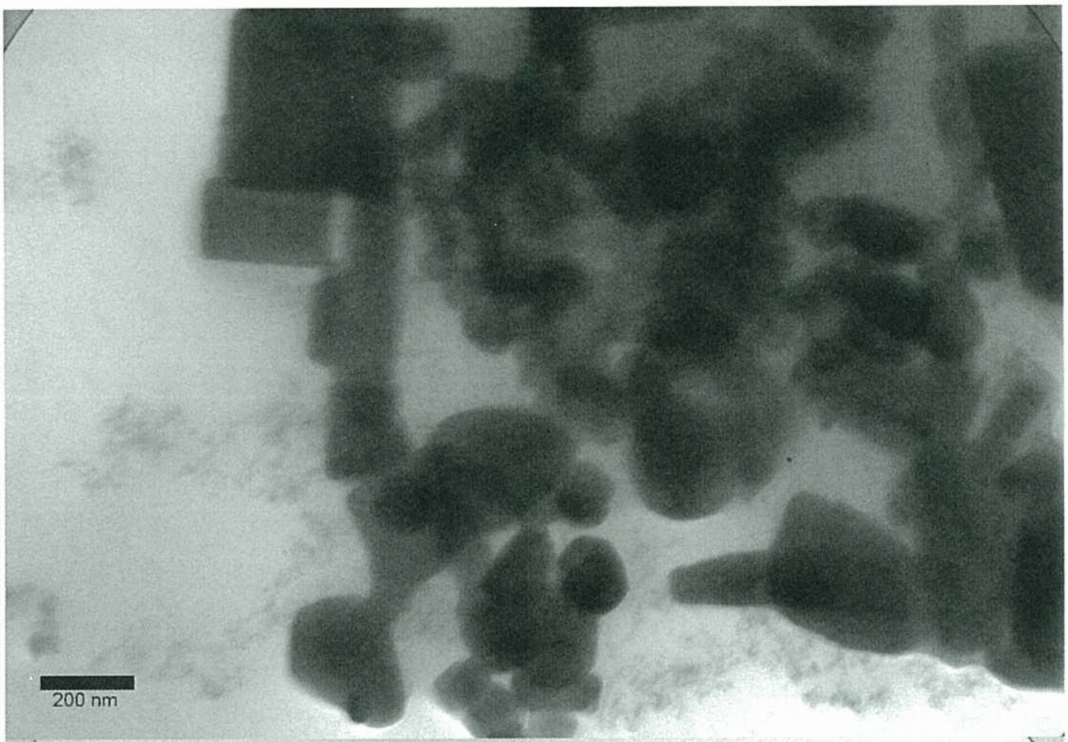
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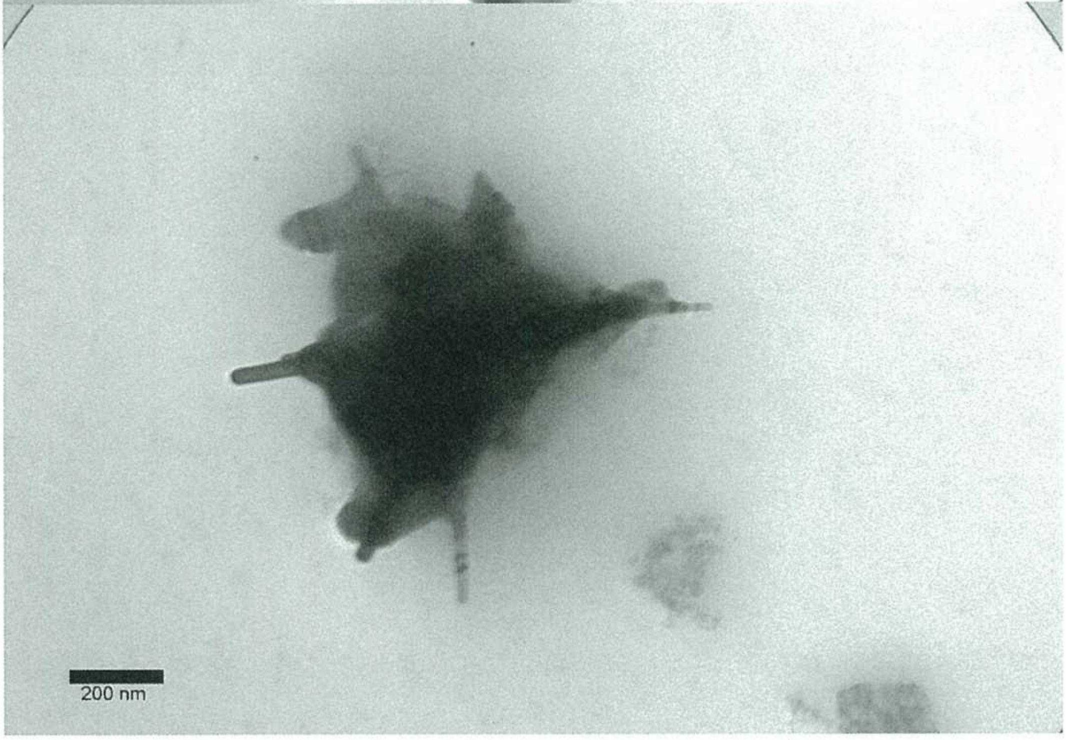
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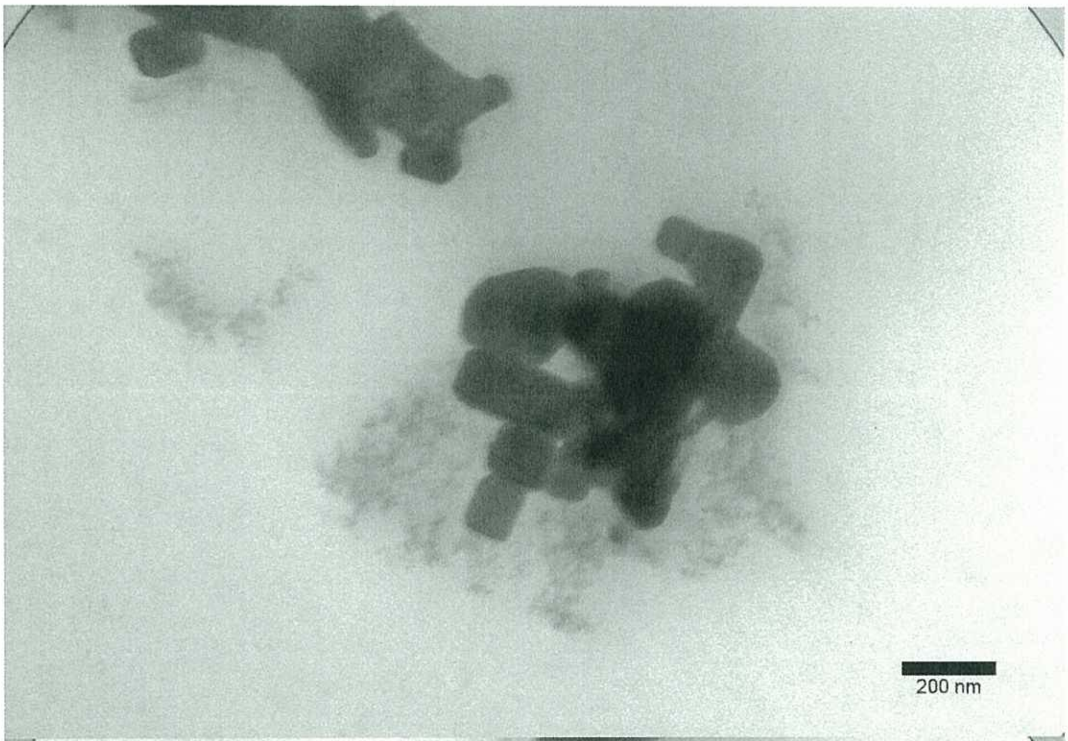
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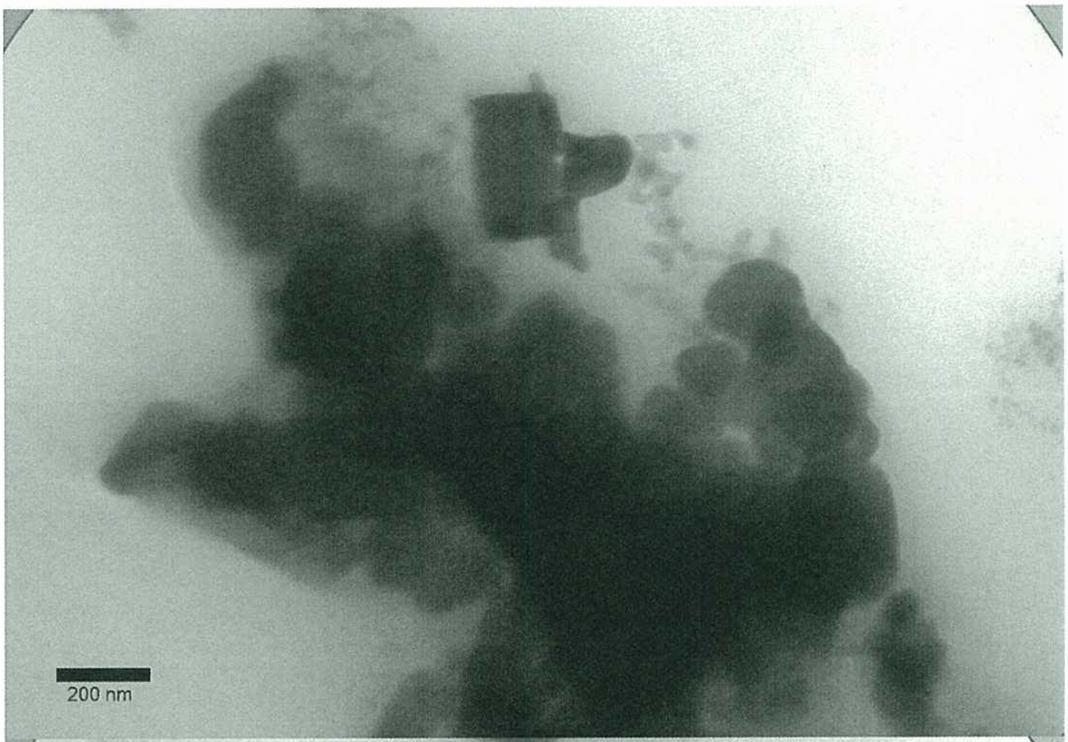
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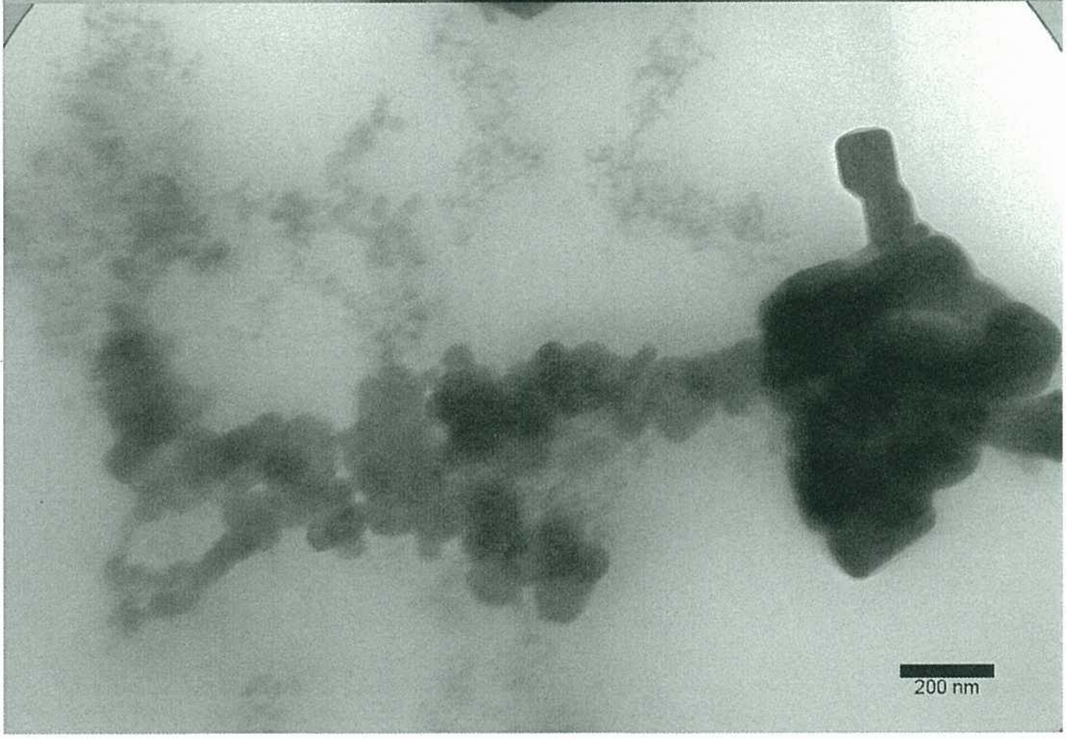
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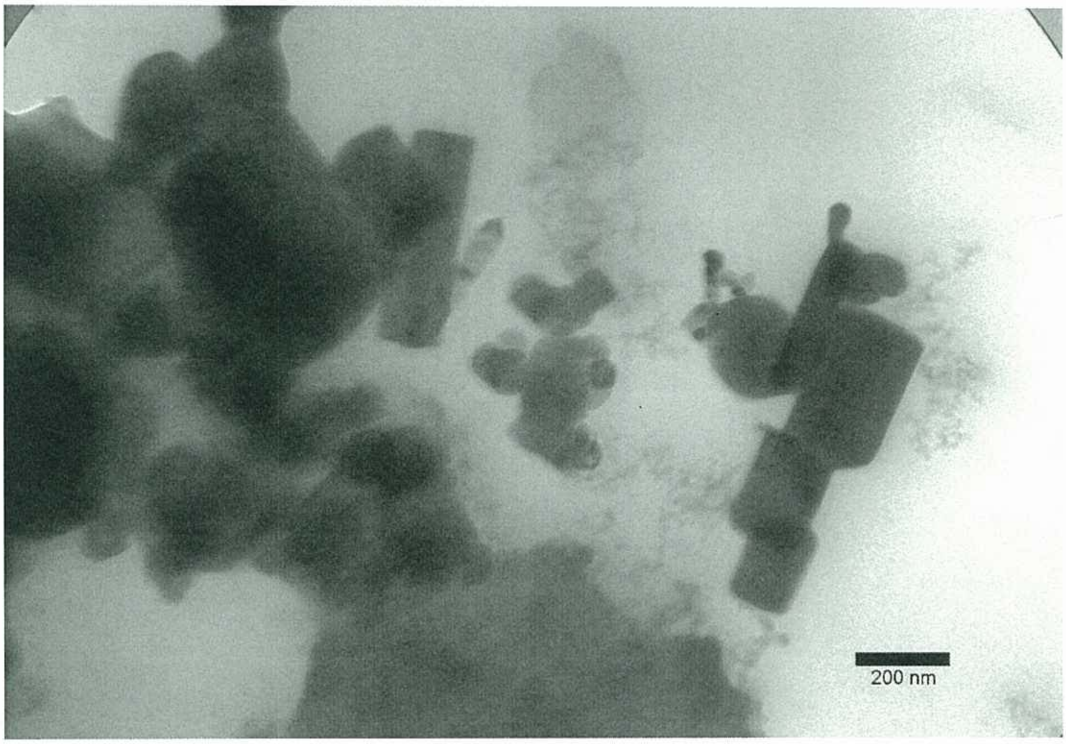

7. Conclusion

Nanomaterials (as defined by ISO[1]) were detected in samples of particulate material extracted from all of the three sunscreens.

Acknowledgements

NMI acknowledges The Australian Centre for Microscopy and Microanalysis at the University of Sydney for access to the TEM used for imaging the samples.

References

[1] ISO, in *Nanotechnologies -- Vocabulary -- Part 1: Core terms* (International Organization for Standardization, Geneva, 2010).

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