Characterization of engineered nanomaterials for health studies

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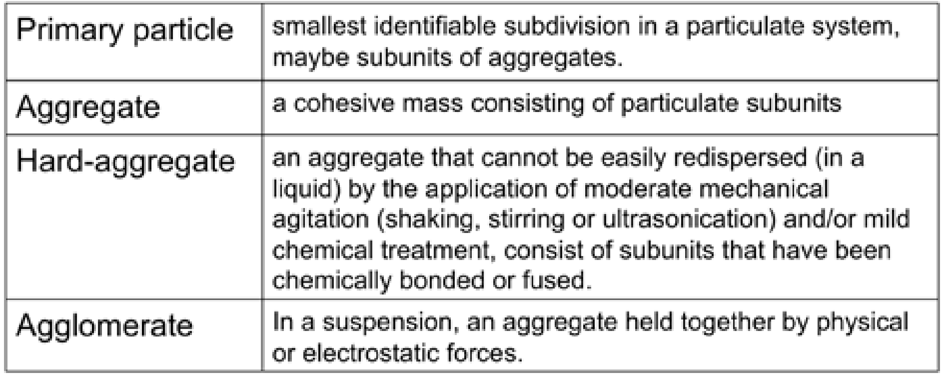
In Vitro Studies of the Effects of Engineered Nanomaterials (ENMs)
In Vivo Studies of the Effects of Engineered Nanomaterials (ENMs)

Nanomaterials & human health +
Instrumentation, metrology and analytical methods

3rd NNI Workshop on nanoEHS research needs
17-18 November 2009
Arlington, VA

Definitions

Dispersions of TiO_2 nanoparticles in EtOH.
2 wt% titania.
All primary particles:
10 < D < 50 nm.

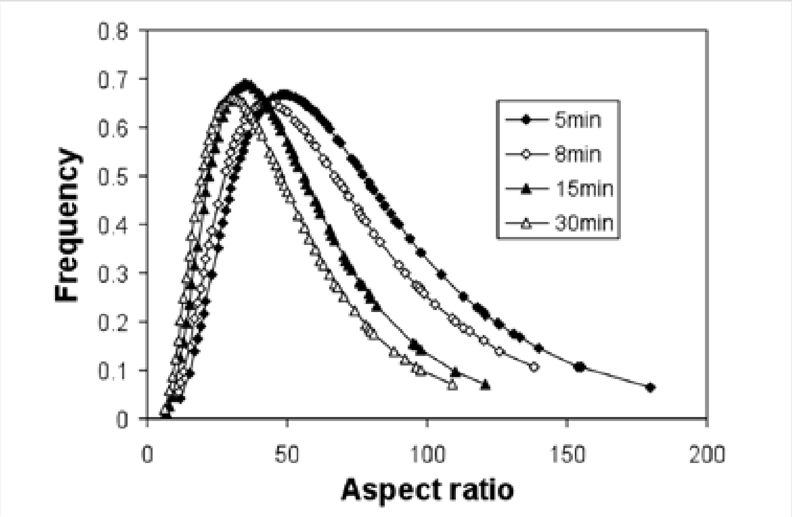


Primary particle	smallest identifiable subdivision in a particulate system, maybe subunits of aggregates.
Aggregate	a cohesive mass consisting of particulate subunits
Hard-aggregate	an aggregate that cannot be easily redispersed (in a liquid) by the application of moderate mechanical agitation (shaking, stirring or ultrasonication) and/or mild chemical treatment, consist of subunits that have been chemically bonded or fused.
Agglomerate	In a suspension, an aggregate held together by physical or electrostatic forces.

NIST guide (*The Use of Nomenclature in Dispersion Science and Technology, V. A. Hackley and C. F. Ferraris, National Institute of Standards and Technology, Special Publication 960-3, August 2001, 72 pp 5*)

- Engineered nanomaterials (ENMs): at least one dimension between 1 to 100 nm.
- These dimensions can lead to novel catalytic, optical and other properties, but raise concerns about their potential environmental and health risks.
- The physico-chemical properties of ENMs, such as size and size distribution, shape and surface chemistry, are critical to achieve accurate and reliable toxicity evaluation.

Differential lognormal frequency distribution of MWNT aspect ratios for different sonication times. Thermal and rheological properties of carbon nanotube-in-oil dispersions, Yang et al., *J. Appl. Phys.*, **99** (11), 114307/1-114307/8, 2006.



OECD nanomaterials list, typical endpoints

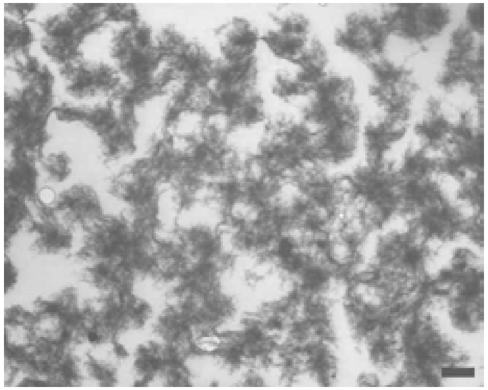
Materials approach to NP characterization: mapping hazard assessment needs to physico-chemical properties to analytical methods

Characterization examples

Research ‘gap’ analysis

OUTLINE

OECD ENM list for testing



MWNTs dispersed in poly(a-olefin). 3 min ultrasonication. Solid bar is 200 mm.

- Carbon-based
  - Fullerenes (C60)
  - SWCNTs
  - MWCNTs
  - Carbon black
- Silver nanoparticles
- Iron nanoparticles
- Nanoclays

- TiO_2
- Al_2O_3
- CeO_2
- ZnO
- SiO_2
- Polystyrene
- Dendrimers

OECD Endpoints

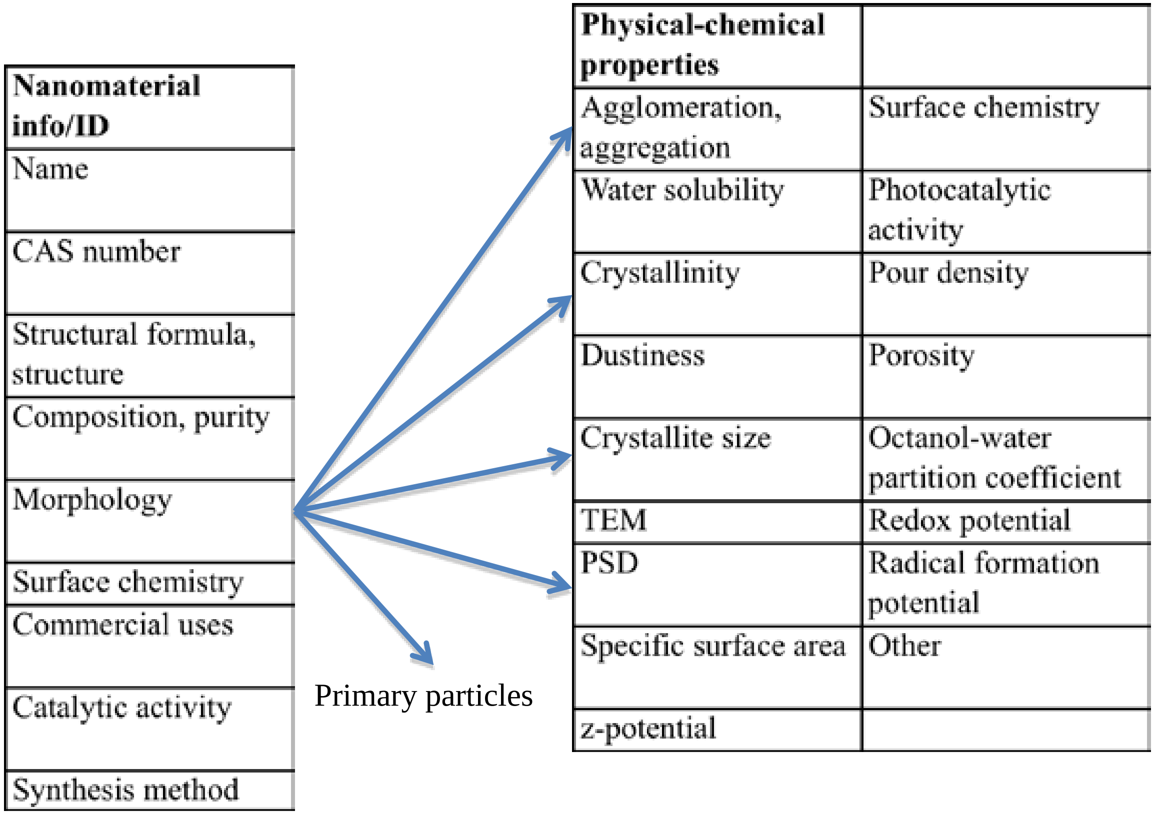
- Nanomaterial information (9)
- Physico-chemical properties (17)
- Material safety (3)
- Environmental fate (15)
- Environmental toxicity (6)
- Mammalian toxicity (7)

OECD endpoints

Many physico-chemical properties are established in the manufacturing process and the post-synthesis processing for a specific application.

<u>Example: CNTs.</u>

Carbon nanotubes are not readily dispersible in most fluids. Dispersion is improved by chemical etching, coupling agents, surfactants, dispersants, polymer wrapping, etc… These modifications often change the transport properties of the nanotubes.



Nanomaterial info/ID
Name
CAS number
Structural formula, structure
Composition, purity
Morphology
Surface chemistry
Commercial uses
Catalytic activity
Synthesis method

Primary particles

Physical-chemical properties	
Agglomeration, aggregation	Surface chemistry
Water solubility	Photocatalytic activity
Crystallinity	Pour density
Dustiness	Porosity
Crystallite size	Octanol-water partition coefficient
TEM	Redox potential
PSD	Radical formation potential
Specific surface area	Other
z-potential	

Some endpoints map to multiple PC properties.
<u>Example: morphology</u>

Properties list includes test methods (TEM, surface area, z-potential) + properties.

MATERIALS APPROACH TO NP CHARACTERIZATION

Hazards assessment of nanoparticles

Mapping physico-chemical properties to the hazards assessment

Mapping analytical methods to physico-chemical properties

Materials Science

- BIOMATERIALS

- Composites
- MAGNETIC MATERIALS
- METALS
- Electronic, optical, photonic
- Superconducting materials
- POLYMERS
- Catalysts
- Nanomaterials

HAZARD ASSESSMENT FOR NANOPARTICLES

- Extensive physico-chemical characterization
- Capacity for macromolecular perturbation
- Potential for transport of toxic molecules
- Translocation
- Agglomeration state
- Chemical composition

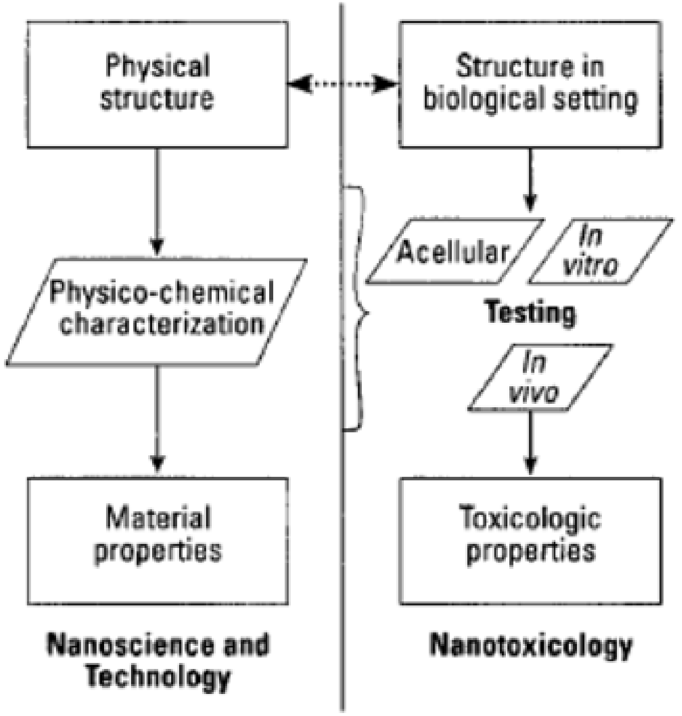
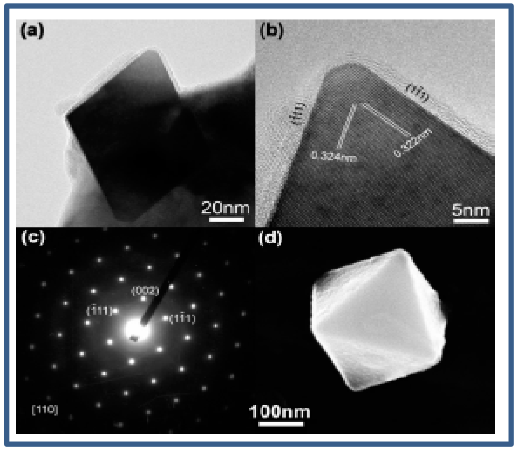


Figure 1. The parallel relationship between material design and material testing (nanotechnology and nanotoxicology).

Meeting report: hazard assessment of nanomaterials-report from an interdisciplinary workshop, Balbus, J. M., et al., *Environmental Health Perspectives*, **115**(11), 1654-1659 (2007)

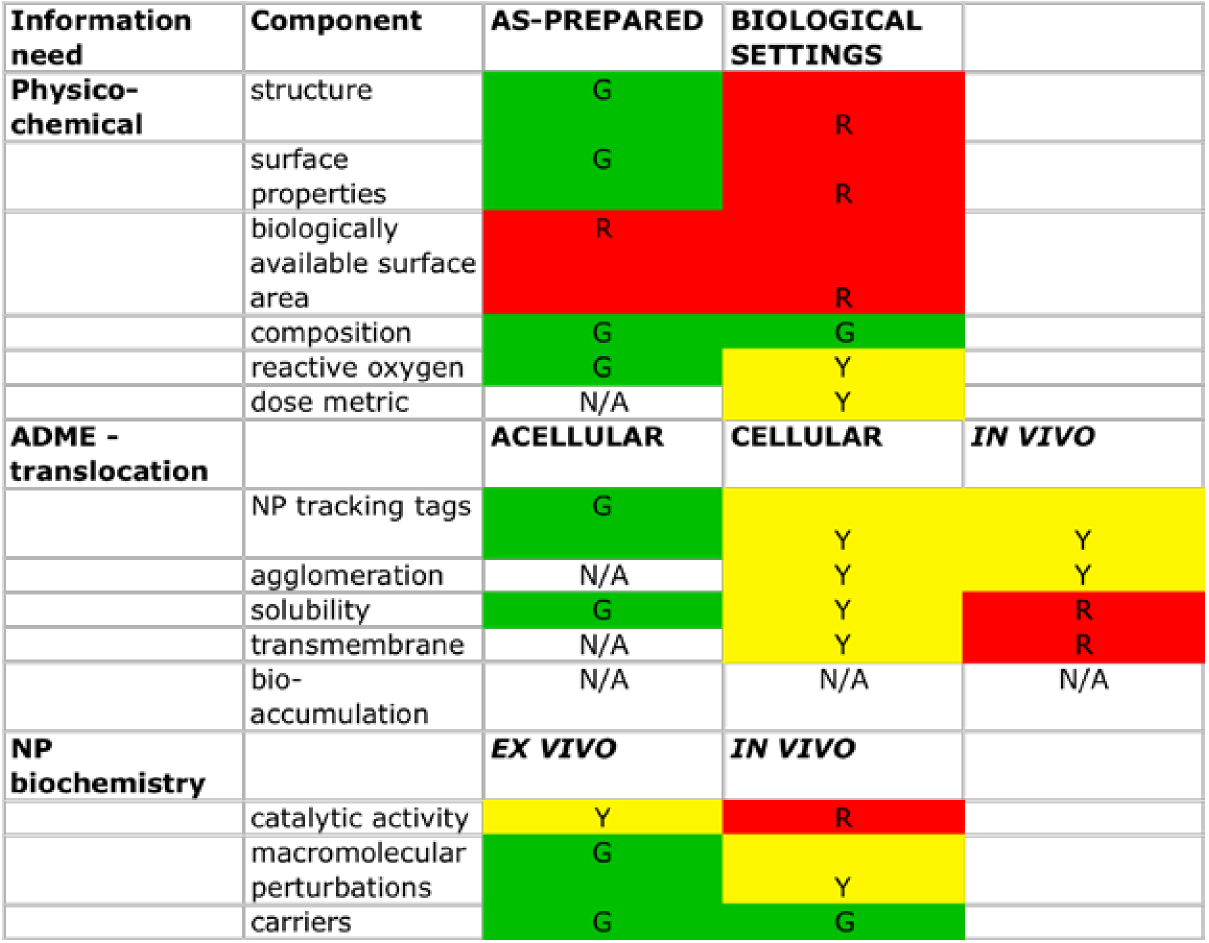
Difference between chemicals and nanomaterials in toxicological studies

Template-Free Hydrothermal Synthesis of CeO2 Nano-octahedrons and Nanorods: Investigation of the Morphology Evolution by Lai Yan, Ranbo Yu, Jun Chen, and Xianran Xing, CRYSTAL GROWTH & DESIGN 2008 VOL. 8, NO. 5 1474–1477



Difference in toxicological study	*Molecule*	*Solubility*	*Shape*	*Dose*	*Interaction with biological system*	*Alteration of property in biological environment*
Chemicals	1	good (uniform solution)	N/A (in solution)	mass, volume	at molecular level / whole molecule	N/A
Nanomaterials	>1	poor (colloid)	edges, corners and exposure surfaces	surface area, particle number and surface activity	surface first (exposure surface, coating et al.)	aggregates/ agglomerates; absorbance of protein

Hazard assessment mapped to physico-chemical properties



Information need	**Component**	**AS-PREPARED**	**BIOLOGICAL SETTINGS**	
Physico-chemical	structure	G	R	
	surface properties	G	R	
	biologically available surface area	R	R	
	composition	G	G	
	reactive oxygen	G	Y	
	dose metric	N/A	Y	
ADME - translocation		**ACELLULAR**	**CELLULAR**	***IN VIVO***
	NP tracking tags	G	Y	Y
	agglomeration	N/A	Y	Y
	solubility	G	Y	R
	transmembrane	N/A	Y	R
	bio-accumulation	N/A	N/A	N/A
NP biochemistry		***EX VIVO***	***IN VIVO***	
	catalytic activity	Y	R	
	macromolecular perturbations	G	Y	
	carriers	G	G	

RED: no agreed upon test method; high priority for development.

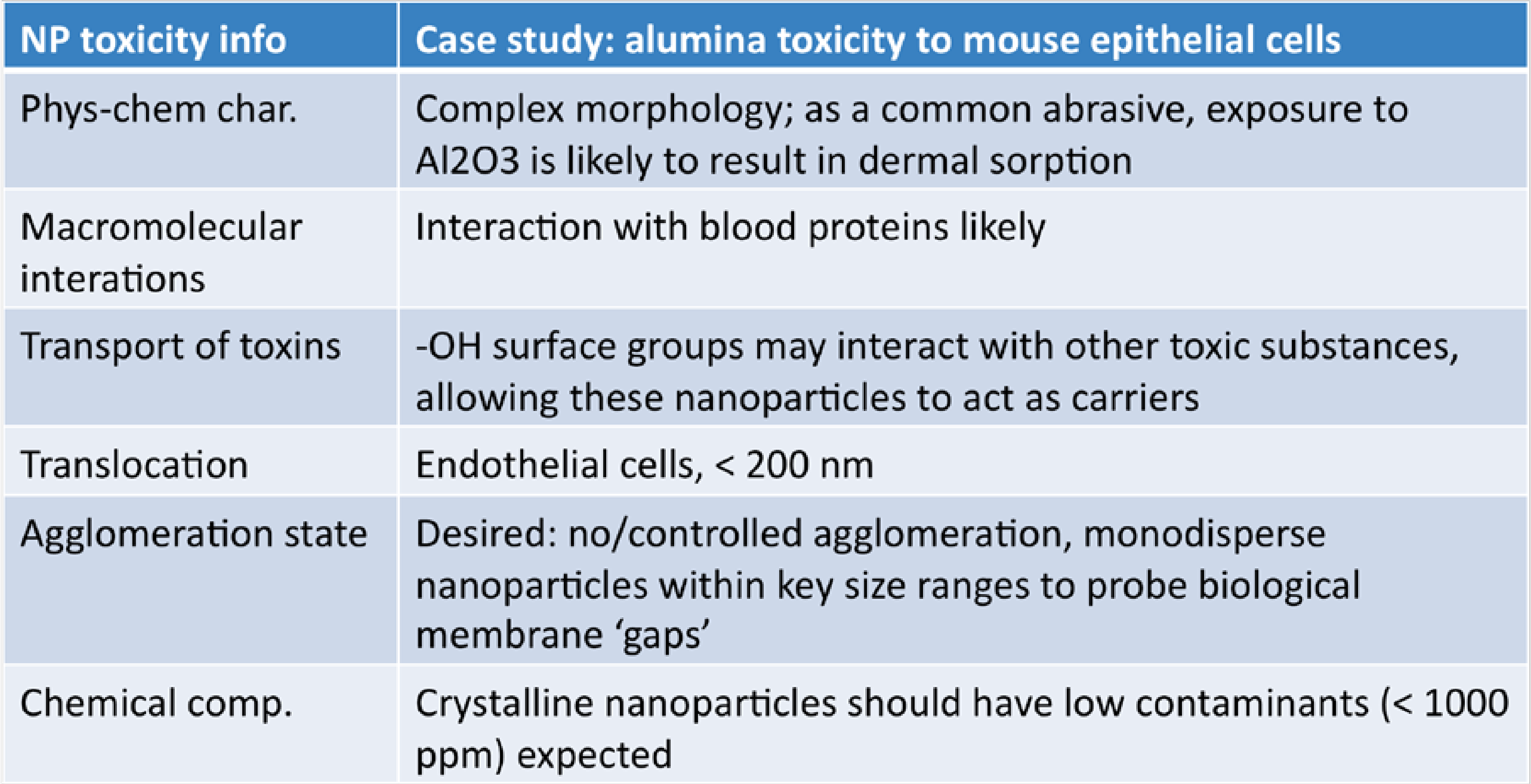
YELLOW: test methods available, no consensus on proper method.

: common usage that could lead to standardization

Meeting report: hazard assessment of nanomaterials-report from an interdisciplinary workshop, Balbus, J. M., et al., *Environmental Health Perspectives*, **115**(11), 1654-1659 (2007)

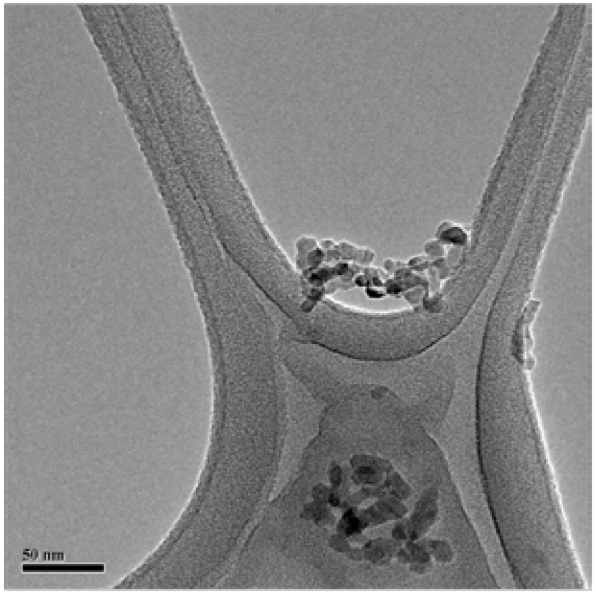
Short case study:

alumina toxicity to mouse epithelial cells

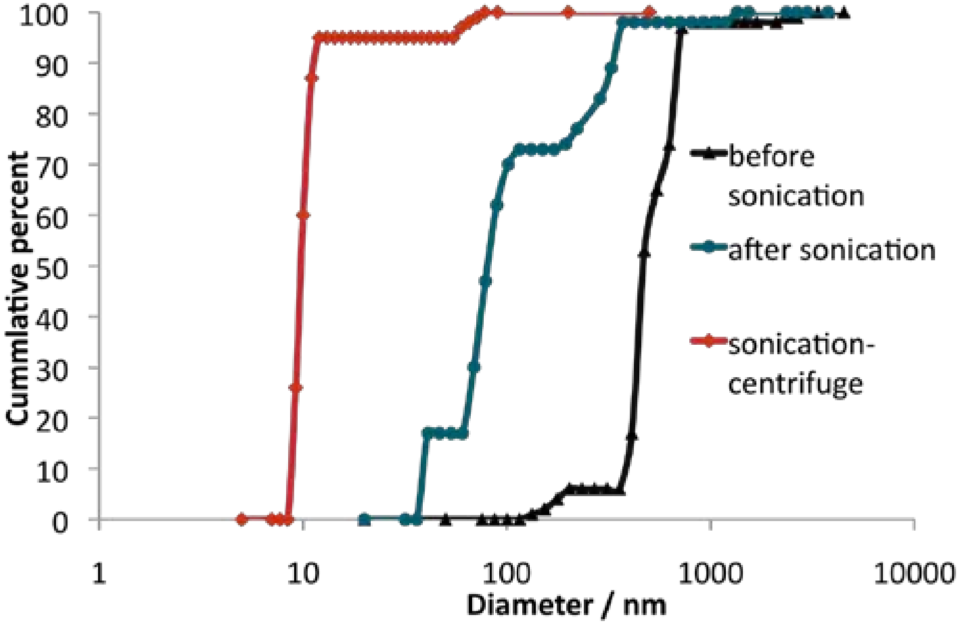


NP toxicity info	Case study: alumina toxicity to mouse epithelial cells
Phys-chem char.	Complex morphology; as a common abrasive, exposure to Al2O3 is likely to result in dermal sorption
Macromolecular interations	Interaction with blood proteins likely
Transport of toxins	-OH surface groups may interact with other toxic substances, allowing these nanoparticles to act as carriers
Translocation	Endothelial cells, < 200 nm
Agglomeration state	Desired: no/controlled agglomeration, monodisperse nanoparticles within key size ranges to probe biological membrane ‘gaps’
Chemical comp.	Crystalline nanoparticles should have low contaminants (< 1000 ppm) expected

Defined dose with known PSD

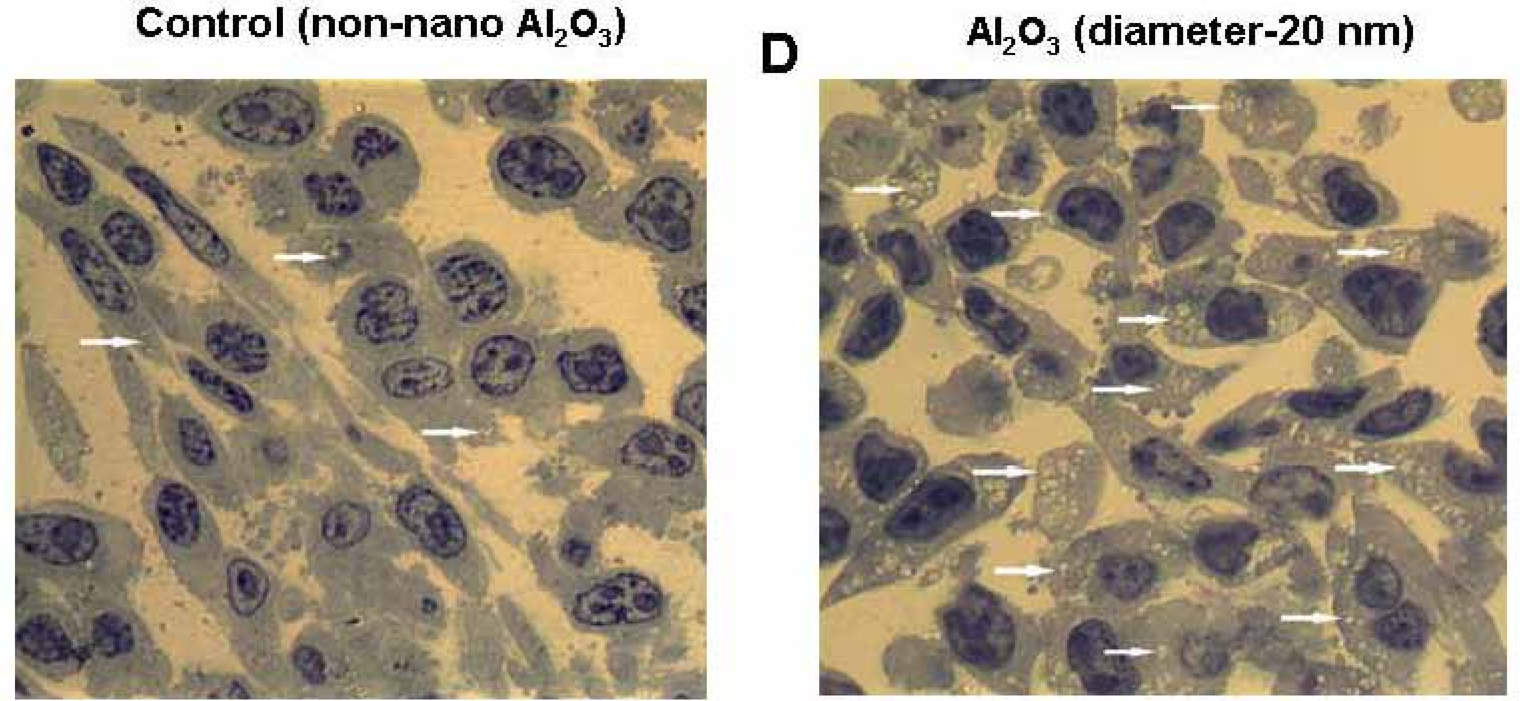


Alumina, CAS# 1344-28-1, g-a, 99.98 %, primary particle size of 10-20 nm, commercial supplier. TEM confirmation of PSD after sonication.



DLS cumulative vol% distribution of sonicated, centrifuged alumina sample. This sample has a known dose: **0.6 wt% alumina with all particles < 100 nm.**

In-vitro study of alumina NPs

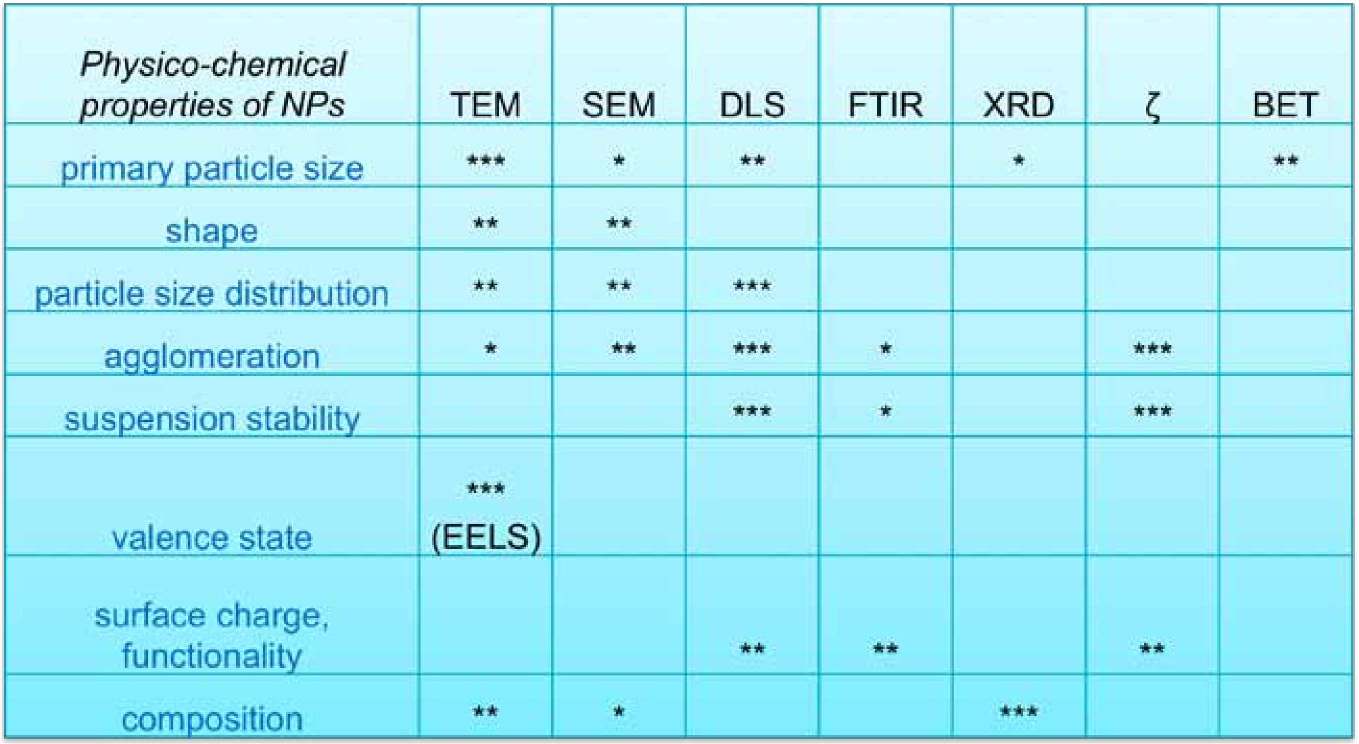


Mouse epithelial cells (JB6) treated with non-sonicated particles showing uptake of particles in **some** cells.

Treated with sonicated/dispersed particles, virtually **all** cells contain refractory inclusions (arrows).

S. Dey, V. Bakthavatchalu, M. T.Tseng, P. Wu, R. L.Florence, E. A.Grulke, R. A.Yokel, S. K. Dhar, H. Yang, Y. Chen and D. K.St Clair, Carcinogenesis vol.29 no.10 pp.1920–1929, 2008.

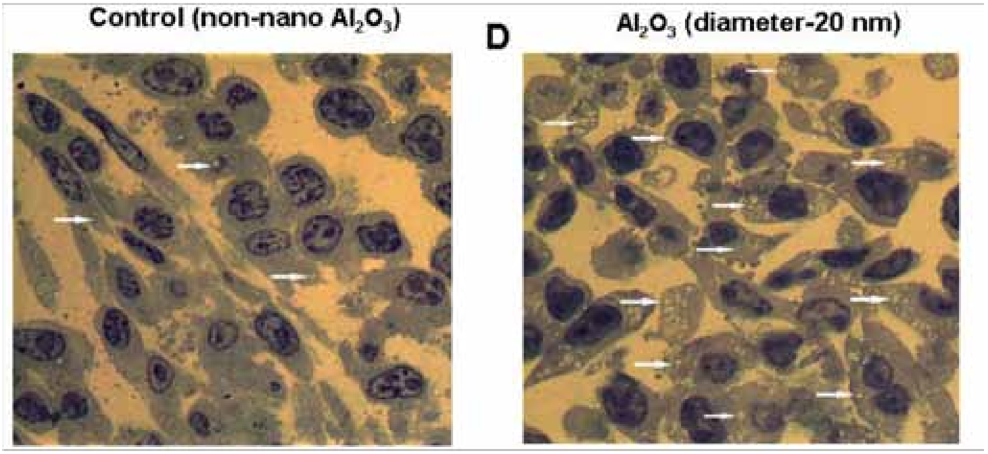
***-primary method;
** - secondary method;
*-correlating method



Physico-chemical properties of NPs	TEM	SEM	DLS	FTIR	XRD	ζ	BET
primary particle size	***	*	**		*		**
shape	**	**					
particle size distribution	**	**	***				
agglomeration	*	**	***	*		***	
suspension stability			***	*		***	
valence state	*** (EELS)						
surface charge, functionality			**	**		**	
composition	**	*			***		

MAPPING PHYSICO-CHEMICAL PROPERTIES TO ANALYTICAL METHODS

This mapping of physico-chemical properties to analytical methods has been used to characterize nanoparticles for nanocomposite applications.



Biological structure ‘gaps’
Expanded ‘toolkit’ for *in vitro*, *in vivo* systems
Morphology
Composition
Surface properties
Aqueous dispersions

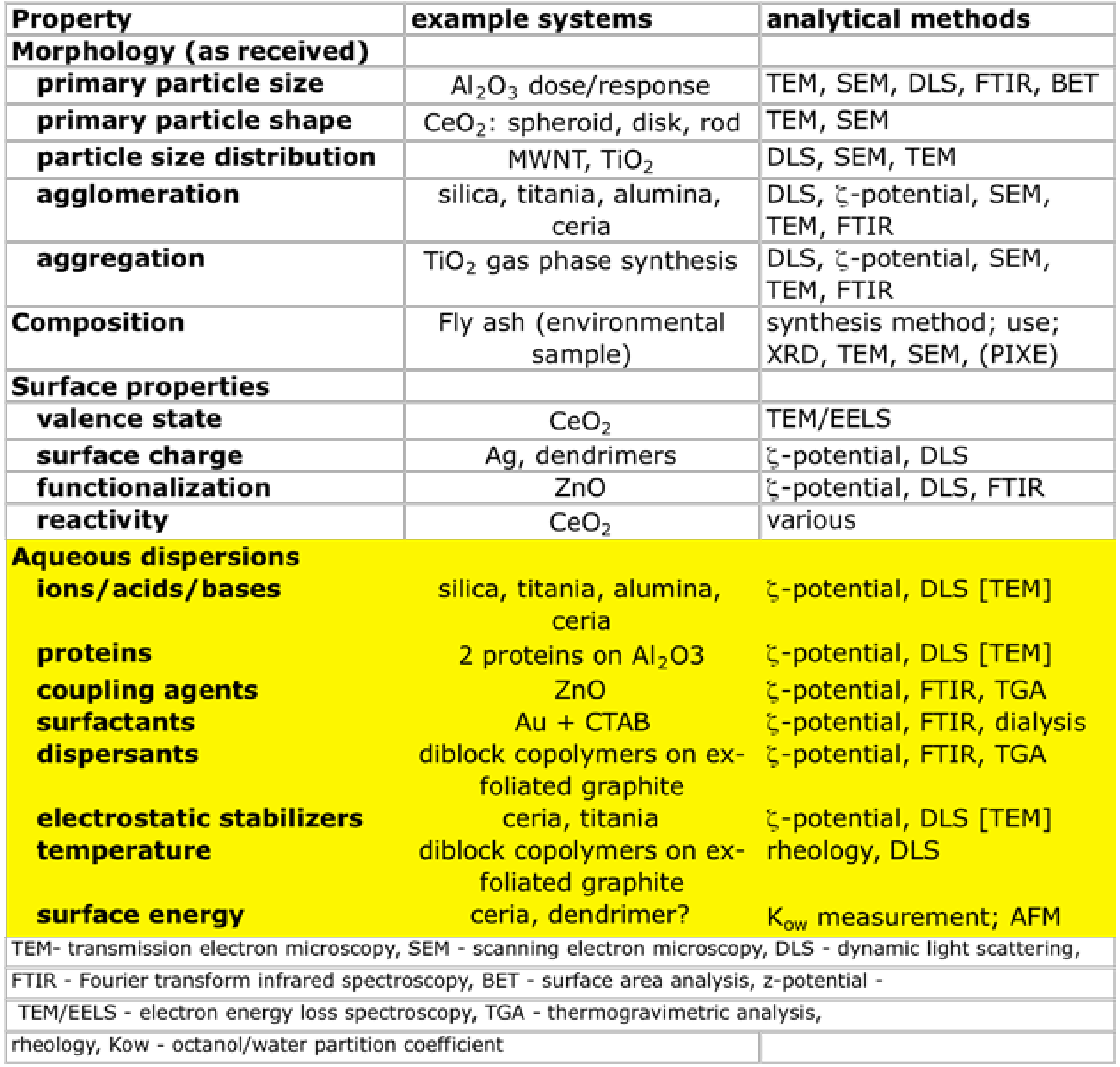
CHARACTERIZATION EXAMPLES

Biological structure ‘gaps’

Biological structure	Diameter or junction space	Size criteria to physically go through the structure
capillary [1]	5-10 µm in diameter	< 5 µm
phagosome [2]	>0.25 µm in diameter	£ 250 nm
gap junctions between cells [3]	3 - 25 nm	< 25 nm or <3 nm
blood-brain barrier (tight junction) [4]	~4 nm	< 4 nm

[1]. Wikipedia, the free encyclopedia, item ‘capillary’
[2] The Cell: A molecular approach’, fourth edition, 2007 by Geoffrey M. Cooper, p556-557
[3] ‘Review of medical physiology’, fourteenth edition, by William F. Ganong, 1989, p12
[4] Kniesel U and Wolburg H 2000. Tight junctions of the blood-brain barrier. Cellular and Molecular Neurobiology 20: 57-76.

PC properties/analytical method



Property	example systems	analytical methods
Morphology (as received)		
primary particle size	Al_2O_3 dose/response	TEM, SEM, DLS, FTIR, BET
primary particle shape	CeO_2: spheroid, disk, rod	TEM, SEM
particle size distribution	MWNT, TiO_2	DLS, SEM, TEM
agglomeration	silica, titania, alumina, ceria	DLS, ζ-potential, SEM, TEM, FTIR
aggregation	TiO_2 gas phase synthesis	DLS, ζ-potential, SEM, TEM, FTIR
Composition	Fly ash (environmental sample)	synthesis method; use; XRD, TEM, SEM, (PIXE)
Surface properties		
valence state	CeO_2	TEM/EELS
surface charge	Ag, dendrimers	ζ-potential, DLS
functionalization	ZnO	ζ-potential, DLS, FTIR
reactivity	CeO_2	various
Aqueous dispersions		
ions/acids/bases	silica, titania, alumina, ceria	ζ-potential, DLS [TEM]
proteins	2 proteins on Al_2O3	ζ-potential, DLS [TEM]
coupling agents	ZnO	ζ-potential, FTIR, TGA
surfactants	Au + CTAB	ζ-potential, FTIR, dialysis
dispersants	diblock copolymers on exfoliated graphite	ζ-potential, FTIR, TGA
electrostatic stabilizers	ceria, titania	ζ-potential, DLS [TEM]
temperature	diblock copolymers on exfoliated graphite	rheology, DLS
surface energy	ceria, dendrimer?	K_{ow} measurement; AFM

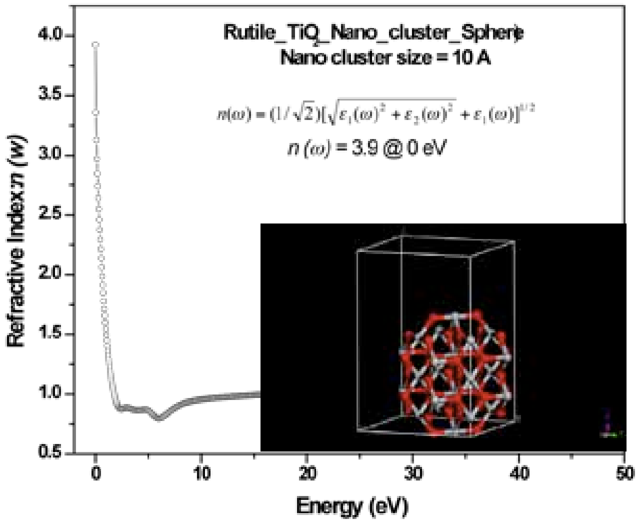
TEM- transmission electron microscopy, SEM - scanning electron microscopy, DLS - dynamic light scattering,
FTIR - Fourier transform infrared spectroscopy, BET - surface area analysis, z-potential -
TEM/EELS - electron energy loss spectroscopy, TGA - thermogravimetric analysis,
rheology, Kow - octanol/water partition coefficient

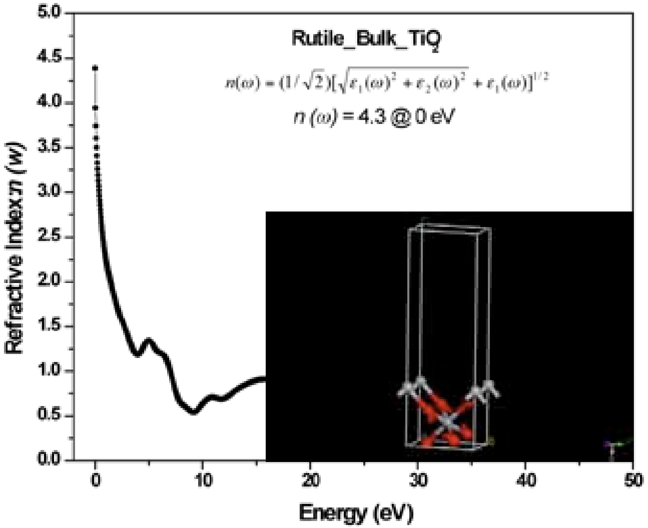
Materials Science Tools

- NANOPARTICLES: TEM, SEM, AFM, XPS, XRD, FTIR, MALDI/TOF/MS, NMR
- MOLECULAR MODELING: in some cases, molecular modeling of nanoparticle properties can identify how properties changes with particle size.
- COLLOIDS: z –potential, DLS
- DENDRIMERS: highly branched molecules with charged groups; a dendrimer with charged outer groups could be hydrophilic externally, carriying a hydrophobic molecule(s) in its interior. Example: drug delivery

MORPHOLOGY

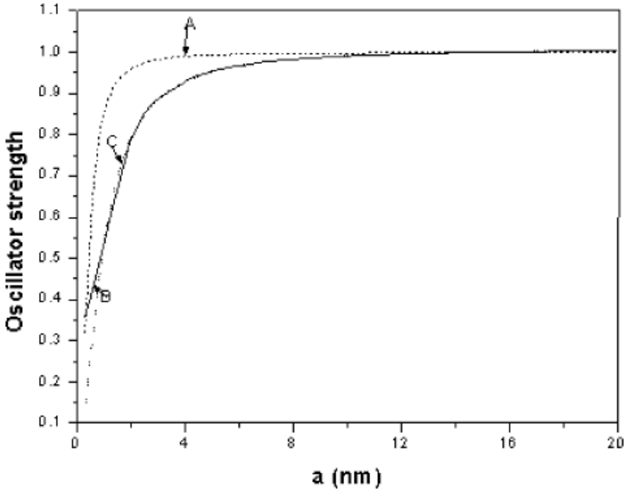
Molecular modeling can be related to EELS spectra. CASTEP example





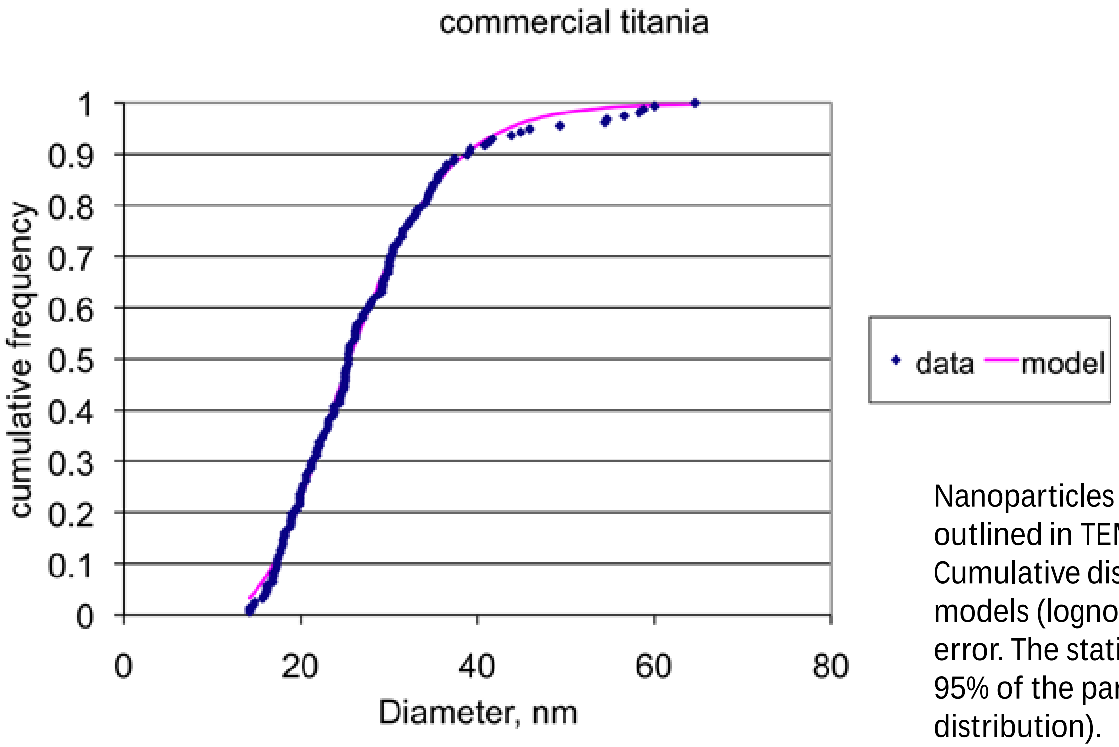
primary particle size
primary particle shape
particle size distribution (PSD)
agglomeration
aggregation
Example: lot-to-lot variation

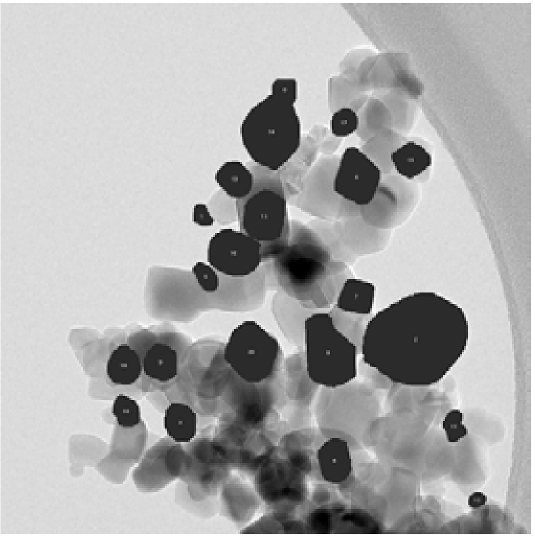
Size-dependent energy band gap and dielectric constant within the generalized Penn model applied to a semiconductor nanocrystallite, Sharma, A.C.,. Journal of Applied Physics, 2006. **100**(8): p. 084301/1-084301/8.



Primary particle size distribution: titania

95 vol % of the sample is 18 < D < 44 nm. D_{max} < 100 nm. Commercial titania.

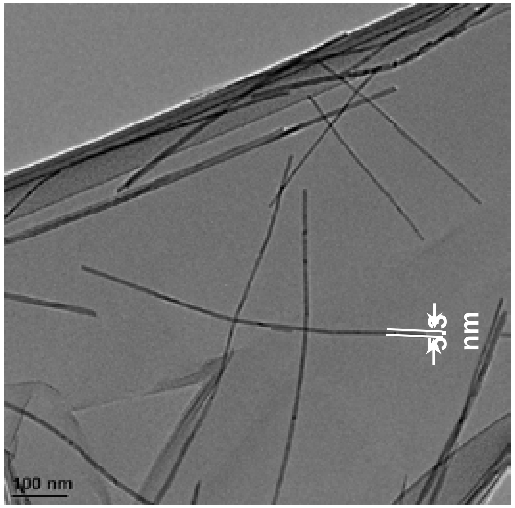


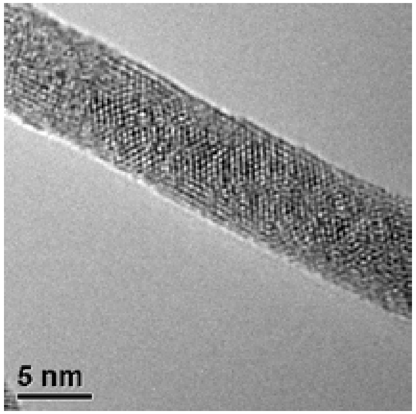


Nanoparticles (N>100) with 'clean' edges are identified and outlined in TEM images using Digital Micrograph®. Cumulative distribution data are fitted with statistical models (lognormal, etc.), identifying D_{ave} , average standard error. The statistical model is used to compute the range for 95% of the particles observed (primary particle size distribution).

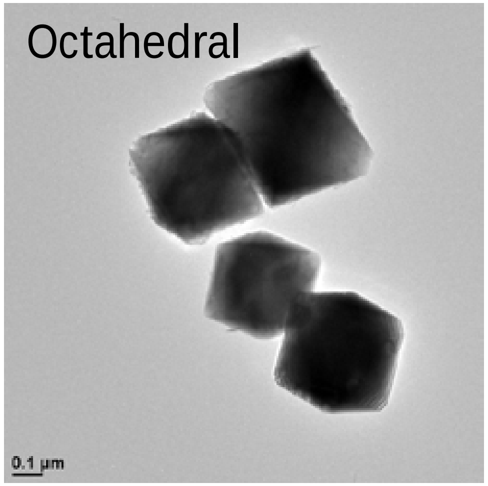
Data obtained under contract PR-NC-08-10414 for Kevin Dreher, USEPA, ORD, NHEERL, Research Triangle Park, NC

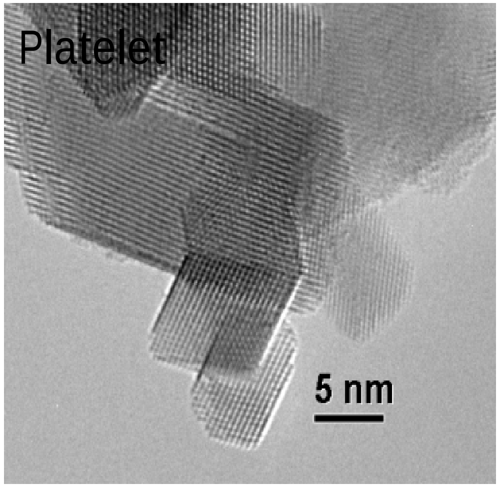
Primary particle shape: ceria

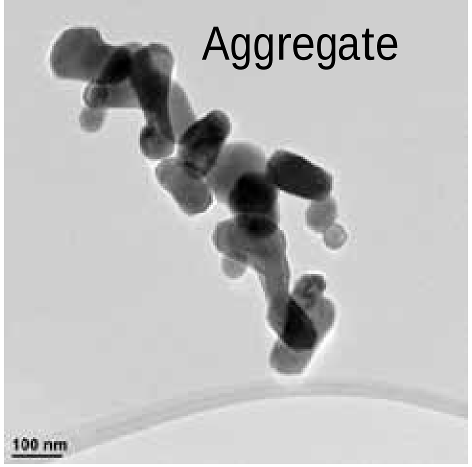




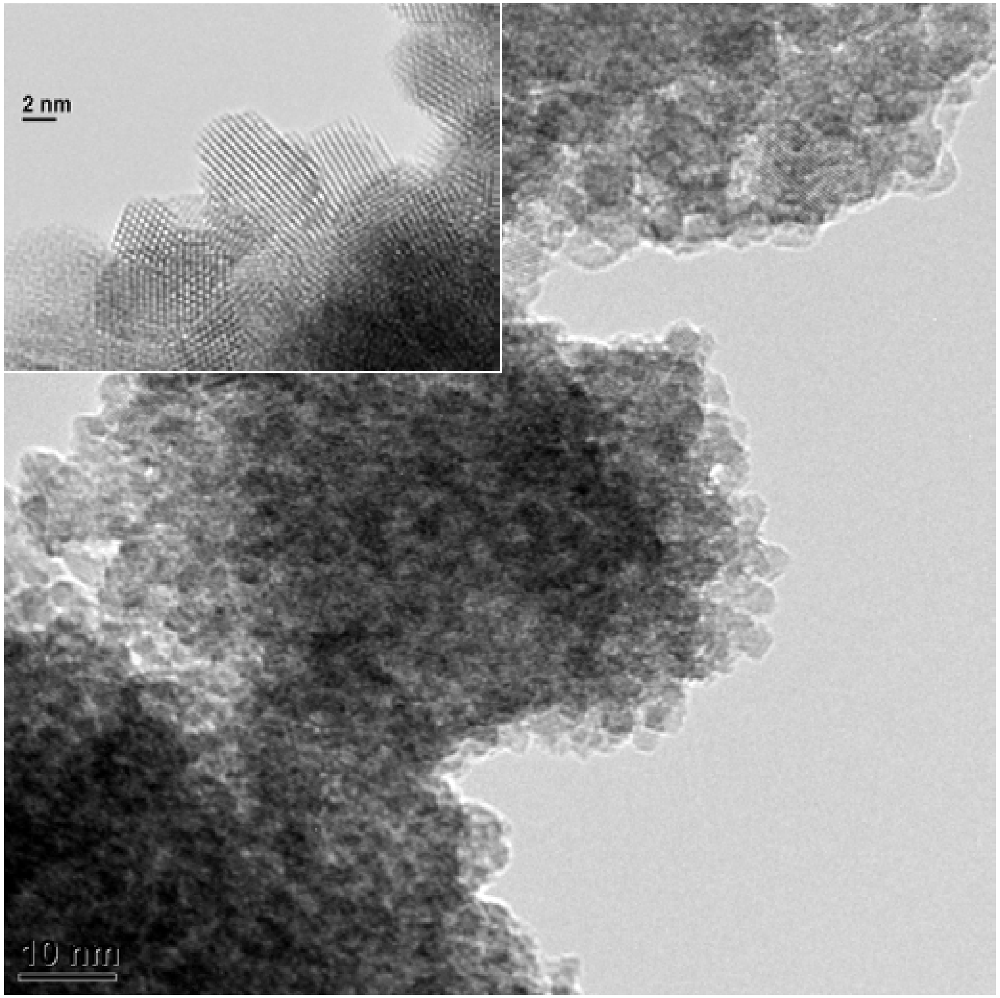
Shapes via liquid phase synthesis. Nanorods grew on octahedral ‘seeds’. Platelet and aggregate are commercial samples via vapor phase synthesis

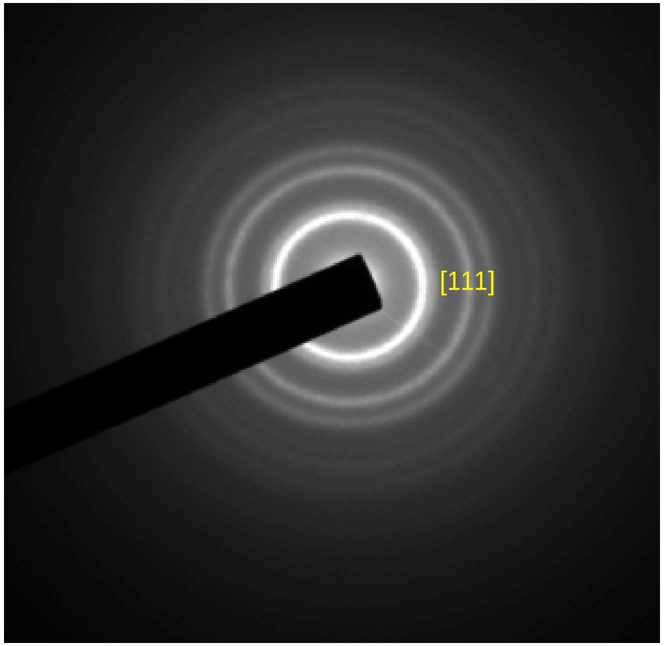






Primary particle size



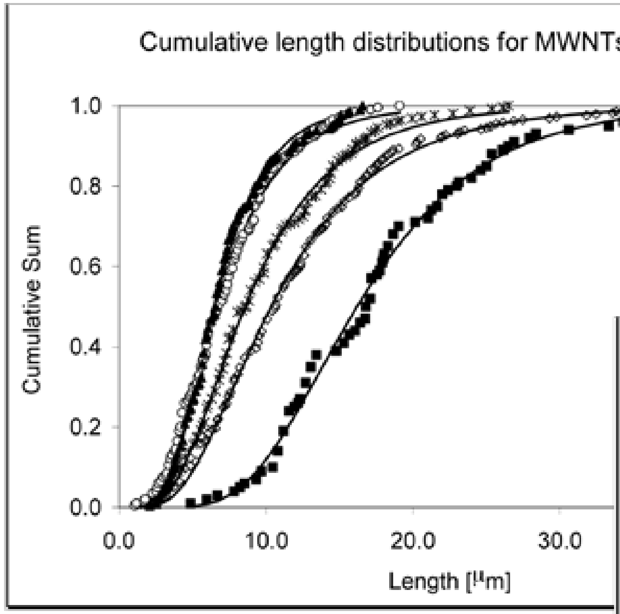


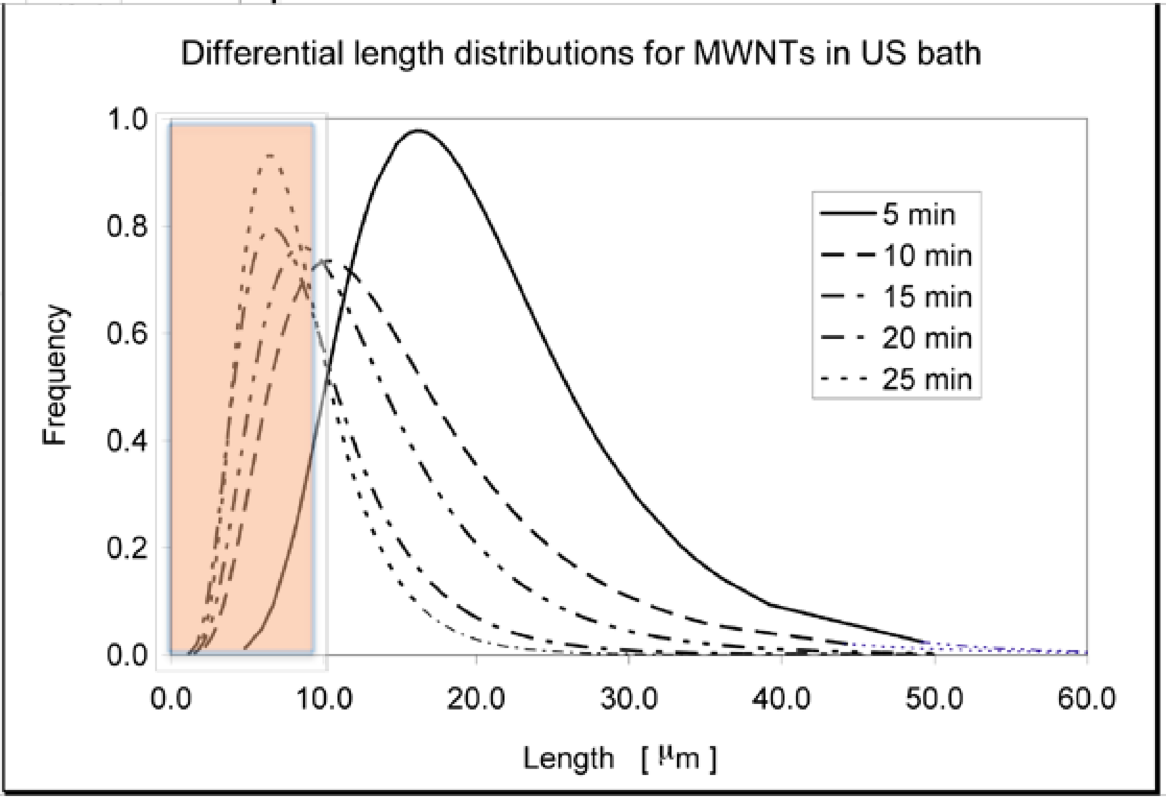
Ceria. Primary particle size: ~5nm;
Shape: platelet;
HRTEM and electron diffraction pattern:
High crystallinity

TEM shows primary particle size, but is not definitive about aggregation/agglomeration.

Particle size distribution: MWNTs

Ultrasonication is often used to disperse carbon nanotubes in liquids: it also fractures the tubes, changing their length distribution.





Above: cumulative frequency MWNT length distributions of 100+ individual tubes. Lognormal models shown as solid lines

Right: differential frequency distributions. Fraction of material less than 10 mm in length in orange rectangle.

15μm cutoff for material to be engulfed by macrophages.

Particle size distribution: TiO_2 aggregates (Degussa P25)

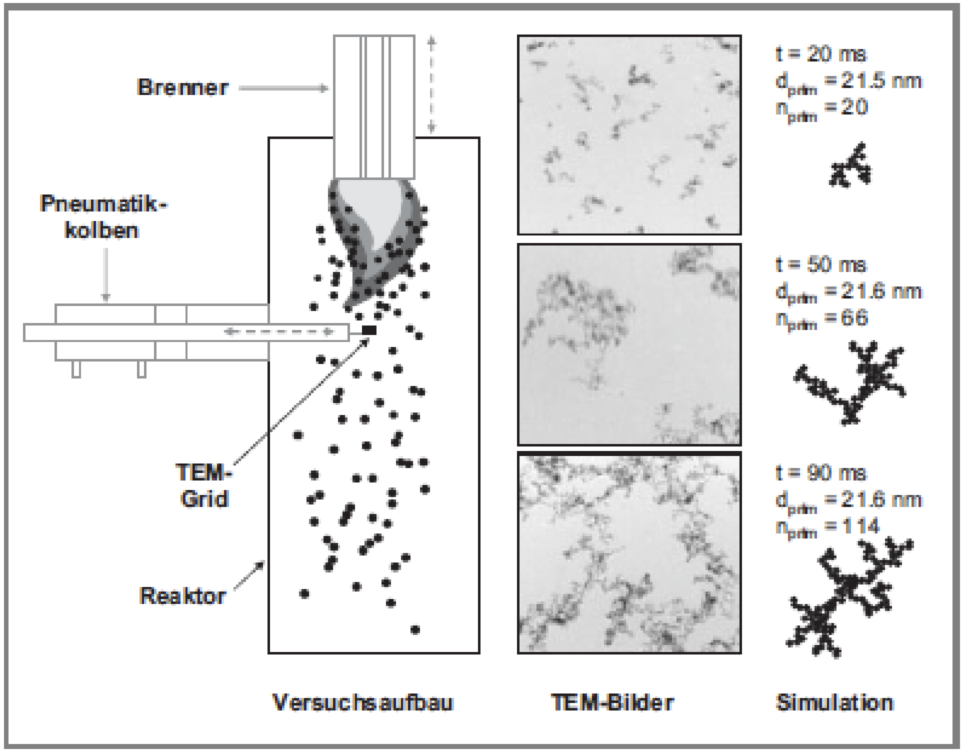


Abbildung 3. Schema des an einen Flammenreaktor angebauten TEM-Grid-Samplingsystems, TEM-Bilder und entsprechende Simulationsergebnisse.

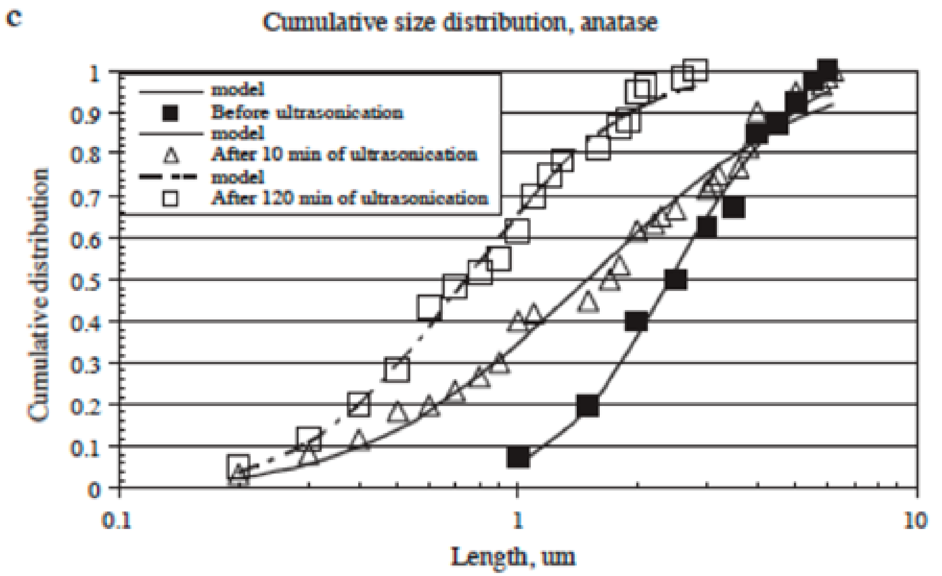
Mechanical handling of nanoparticle powders often fractures fused aggregates, changing the dose of a specific particle size. Determining the fraction of the sample that can penetrate biological structures is a challenge.

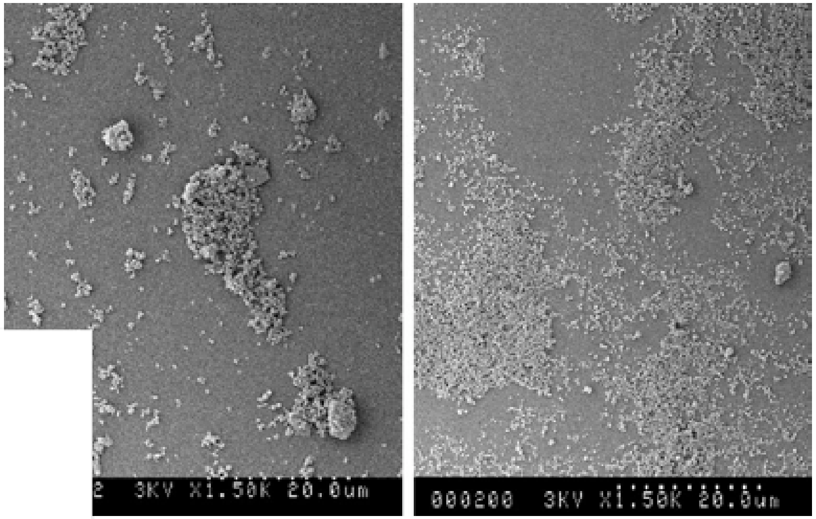
Correlation between simulations and actual product demonstrate fundamental understanding of the synthesis process, which can lead to better process control and higher product uniformity.

A. Gutsch, J. Averdung, H. Muhlenweg, Chemie Ingenieur Technik, 77(9), 1377-1392 (2005). Flame synthesis of metal oxide nanoparticles – ZnO, CeO_2, ZrO_2, ITO, …

Particle size distribution: TiO_2 aggregates (Degussa P25)

PSD challenge: ~ 3 orders of magnitude between smallest primary particle and largest aggregate. Aggregate porosity is difficult to measure, so a number- or volume-based distribution is difficult to compute.





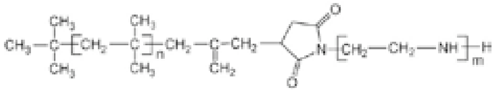
What is the dose of a specific size range?

Mandzy et al., Breakage of TiO2 aggregates in electrostatically stabilized aqueous dispersions, *Powder Technology,* **160,** 121 (2005).

Dispersant agglomeration:

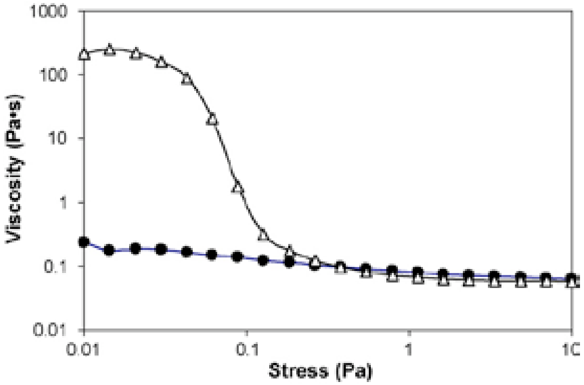
MWNTs in poly(a-olefin), PIBSI di-block copolymer dispersant

PIBSI. Poly(isobutylene succinimide)

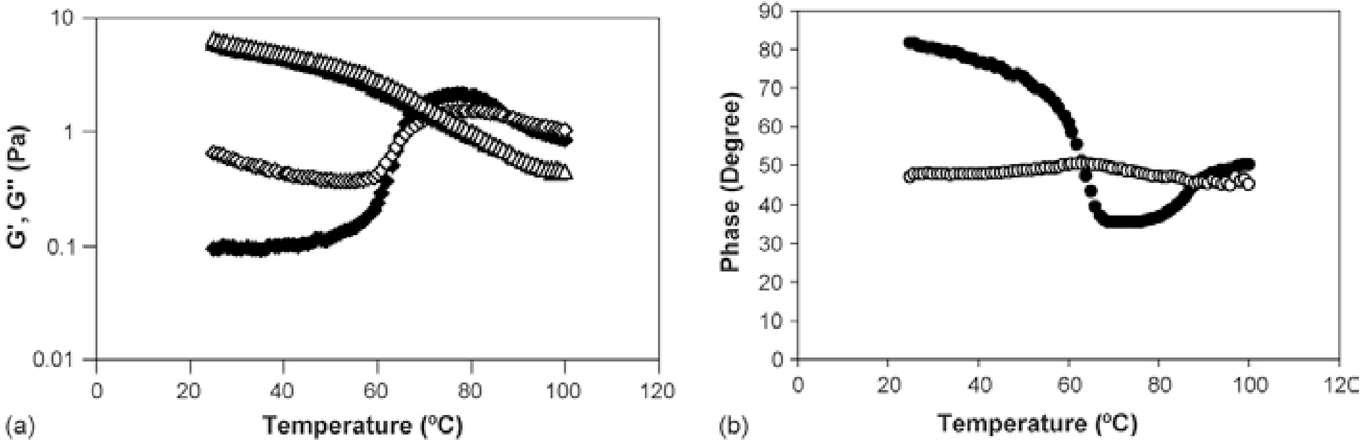


Temperature effects on the rheological properties of carbon nanotube-in-oil dispersions, Yang et al., *Colloids Surf., A*, **298**(3) 216 (2007).

Short chain PIBSI does not disperse CNTs. Short chain (550 Mw) PIBSI D: long chain (1000 Mw) PIBSI ¢



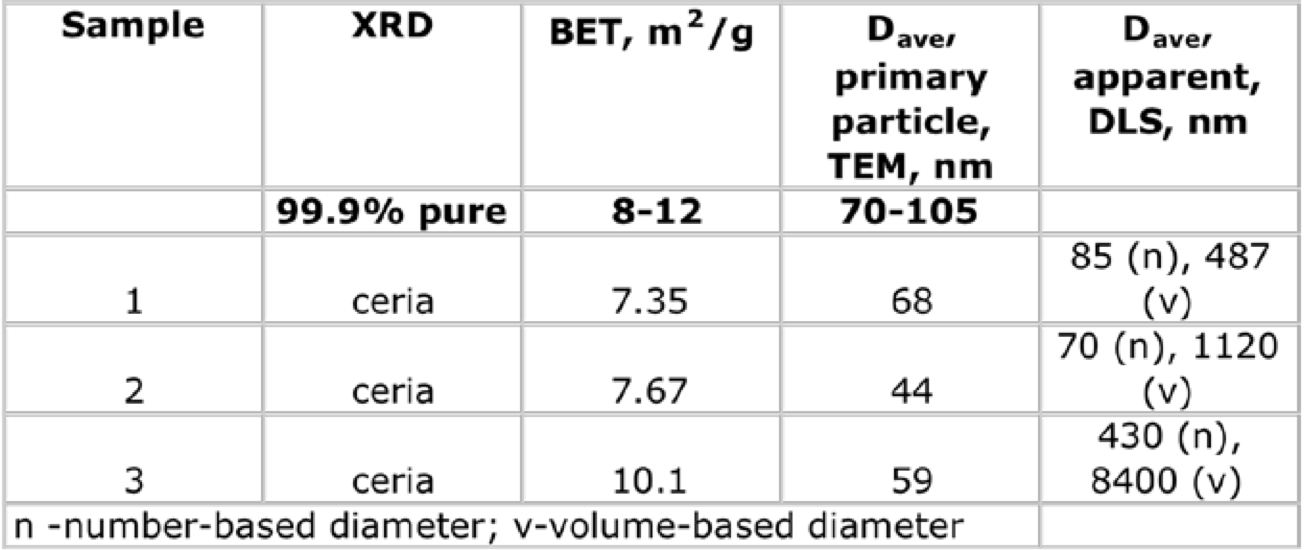
Bulk property measurement, rheology, that reflects dispersion of nanoparticles.



Long chain PIBSI agglomerates at T> 60° C per phase angle vs. T curves. Short chain (550 Mw) PIBSI D: long chain (1000 Mw) PIBSI ¢

Lot-to-lot variability

- 3 commercial samples of CeO_2 – 3 lots of one catalog #
- Manufacturer: $D_{ave,primary}$~ 70-105 nm; surface area 8-12 m^2/g



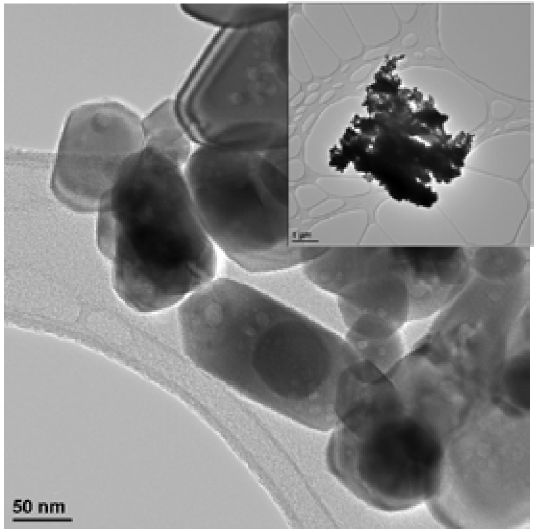
Sample	XRD	BET, m^2/g	D_{ave}, primary particle, TEM, nm	D_{ave}, apparent, DLS, nm
	99.9% pure	**8-12**	**70-105**	
1	ceria	7.35	68	85 (n), 487 (v)
2	ceria	7.67	44	70 (n), 1120 (v)
3	ceria	10.1	59	430 (n), 8400 (v)
n -number-based diameter; v-volume-based diameter				

For unimodal, spheroid samples, surface area increases as D_{ave} decreases. The PSDs of these primary particles are multimodal.

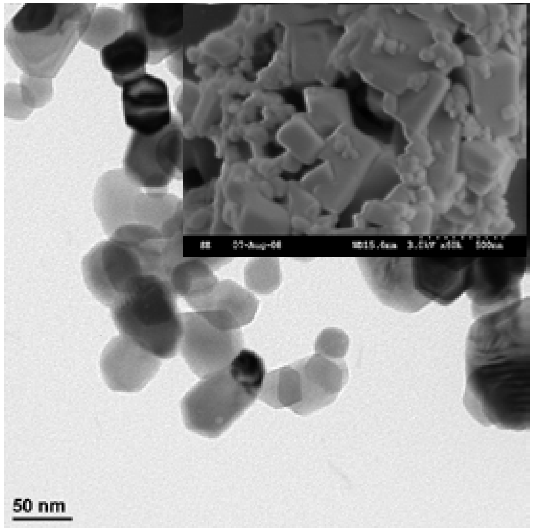
Data obtained under contract PR-NC-08-10414 for Kevin Dreher, USEPA, ORD, NHEERL, Research Triangle Park, NC

Primary particles & aggregates

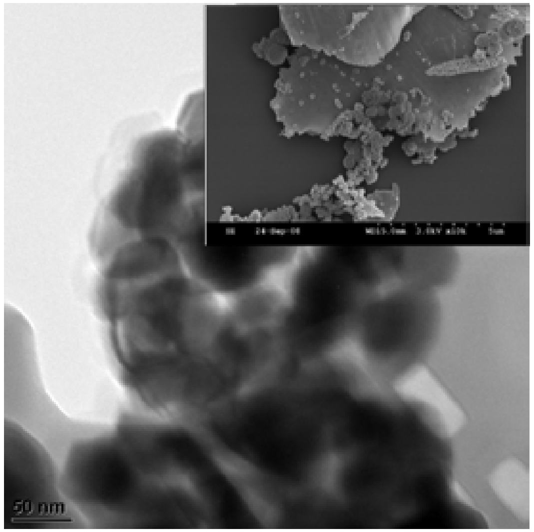
Sample 1 ($D_{p\ ave}$ 68 nm)



Sample 2 ($D_{p\ ave}$ 44 nm)



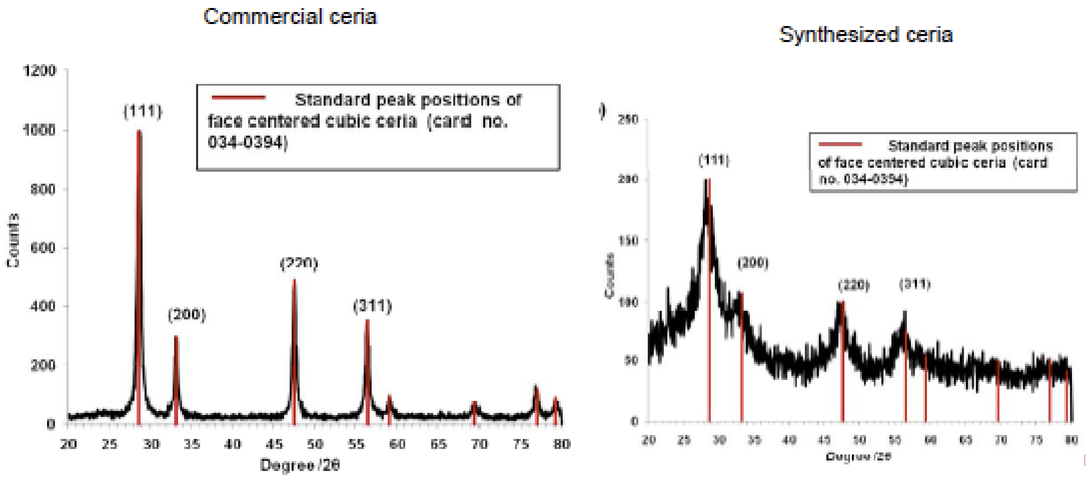
Sample 3 ($D_{p\ ave}$ 59 nm)



Aggregates seem to have different morphologies.

Data obtained under contract PR-NC-08-10414 for Kevin Dreher, USEPA, ORD, NHEERL, Research Triangle Park, NC

XRD – x-ray diffraction patterns from commercial ceria nanoparticles (~30 nm) and synthesized ceria (~5 nm), showing the presence of the same crystal planes, but broader peaks for the smaller nanoparticles.



Synthesis & use establish critical physico-chemical properties

Example –fly ash from electrostatic precipitator (the ‘starting’ nanomaterial for environmental studies)

COMPOSITION

Association of the sites of heavy metals with nanoscale carbon in a Kentucky electrostatic precipitator fly ash, Hower, J.C., Graham, U.M., Dozier, A., Tseng, M.T., Khari, R.A.,, *Environ. Sci. Technol*.**, 42**, 8471 (2008). HRTEM-STEM-EELS.
Characterization of fly ash from Kentucky power plants, Hower, J.C., Robertson, J.D., Thomas, G. A., Wong, A. S., Schram, W.H., Graham, U.S., Rathbone, R. F., Robl, T. L.,, *Fuel*, **75** (4), 403 (1996).PIXE.

Combustion processes generate 'nanoproducts' + micron-sized particles

A combination of high-resolution transmission electron microscopy, scanning transmission electron microscopy, and electron energy-loss spectroscopy (HRTEM-STEM-EELS) was used to study fly ashes produced from the combustion of an eastern Kentucky coal at a southeastern-Kentucky wallfired pulverized coal utility boiler.

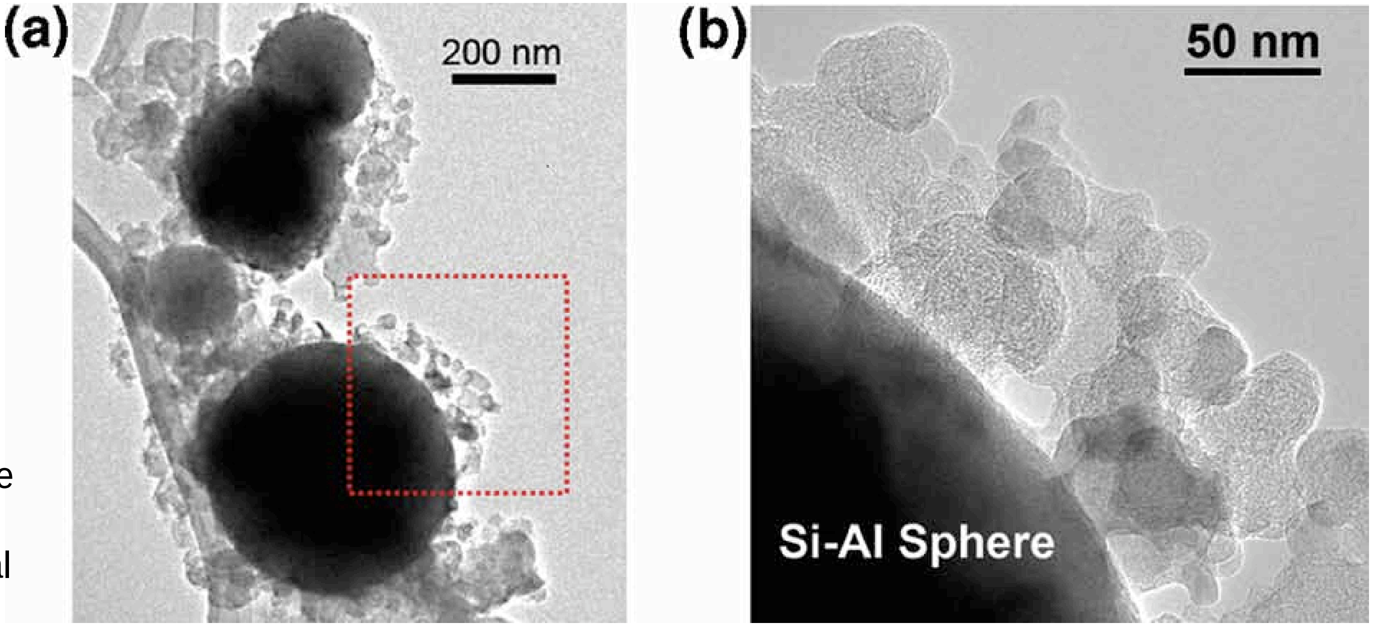
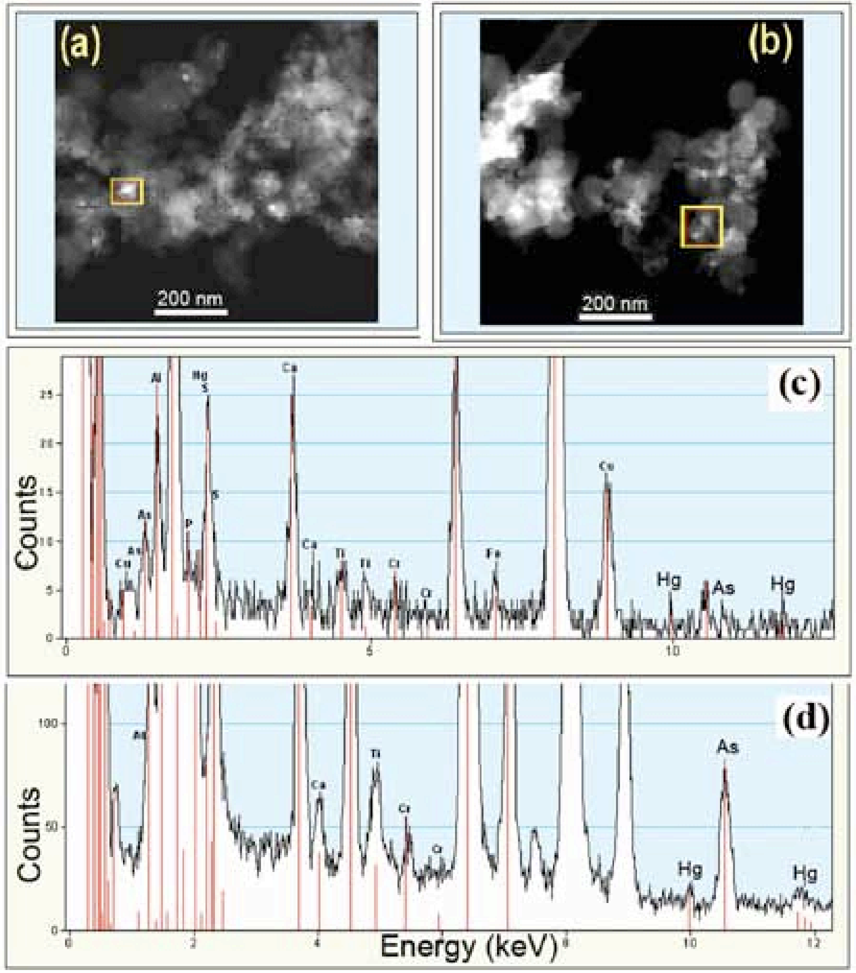


FIGURE 1. (a) HRTEM image of nanometer-sized C deposits intergrown with Si-Al glassy fly ash particles (dark spheres); (b) insert showing agglomerated nanocarbons with soot-like appearance coating fly ash sphere in a porous shell or nanocoating.

STEM for composition analysis

FIGURE 5. STEM images of C-rich nanoclusters with Fe-rich inclusions; with the respective spectra for the points in a and b shown in c and d, respectively. Note the presence of As and Hg in the spectrum. Both As and Hg show stronger, more highly defined signals than the points shown on Figure S1, but Se does not occur at significant levels in these fields.



The STEM EELS analyses can be used to determine the composition within nanoparticle aggregates. In this case Hg is associated with the nanocarbon, and arsenic, Se, Pb, Co, and traces of Ti and Ba are in Fe-rich particles within the nanocarbon deposits. Other methods can be used to identify trace components throughout the sample.

valence state – Ce^{3+}/Ce^{4+} on NP surface

surface charge – z-potential, ceria dispersions in water

Functionalization – citrate ion stabilization

reactivity – ‘Tier one’

SURFACE PROPERTIES

Valence state: Ce^{3+}/Ce^{4+}

EELS of ceria nanoparticles.

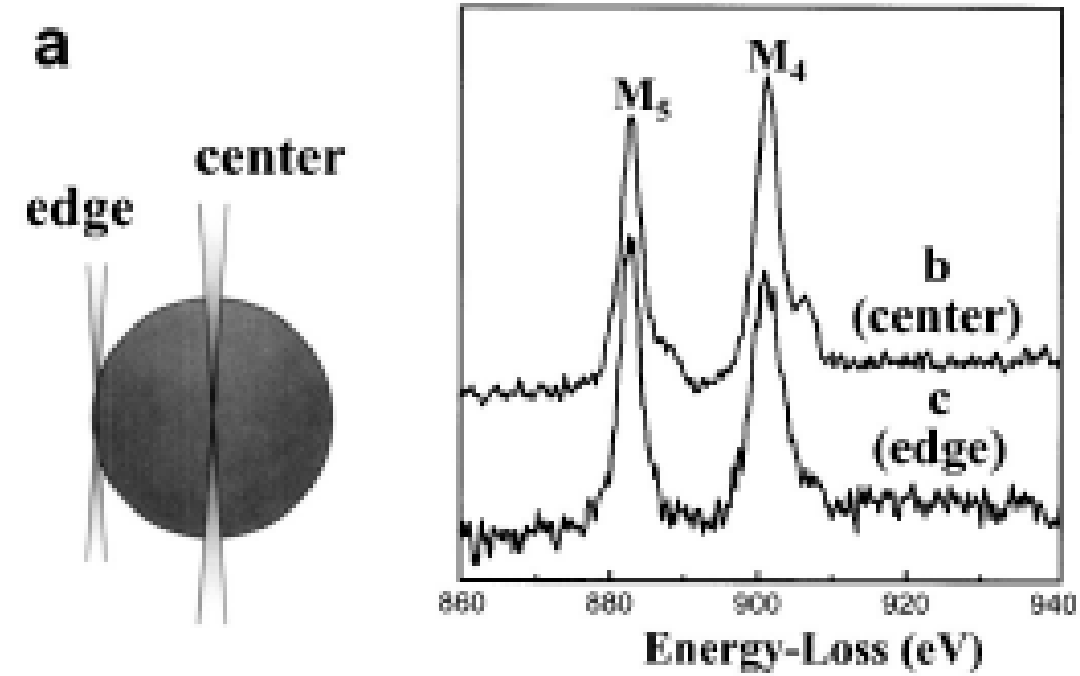
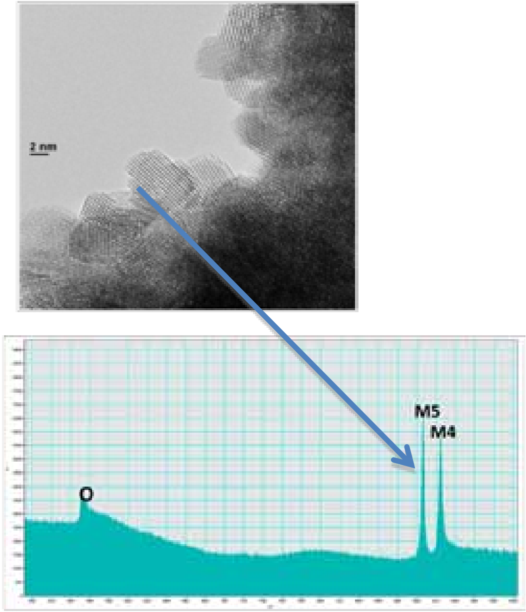


FIG. 5. (a) Experimental setup to measure the difference in the M_5/M_4 ratios between the surface and interior of a particle. The signal will mostly come from the surface when the beam is focused at the edge, while it will mainly come from the interior when the beam is focused at the center. EELS spectra obtained from the center (b) and edge (c) of a particle with $d = 15$ nm.

Oxidation state and lattice expansion of CeO2-*x nanoparticles as a function of particle size,* Lijun Wu, H. J. Wiesmann, A. R. Moodenbaugh, R. F. Klie, Yimei Zhu, D. O. Welch, and M. Suenaga, *Phys. Rev. B,* 69, 125415 (2004)



Reported EELS of ceria

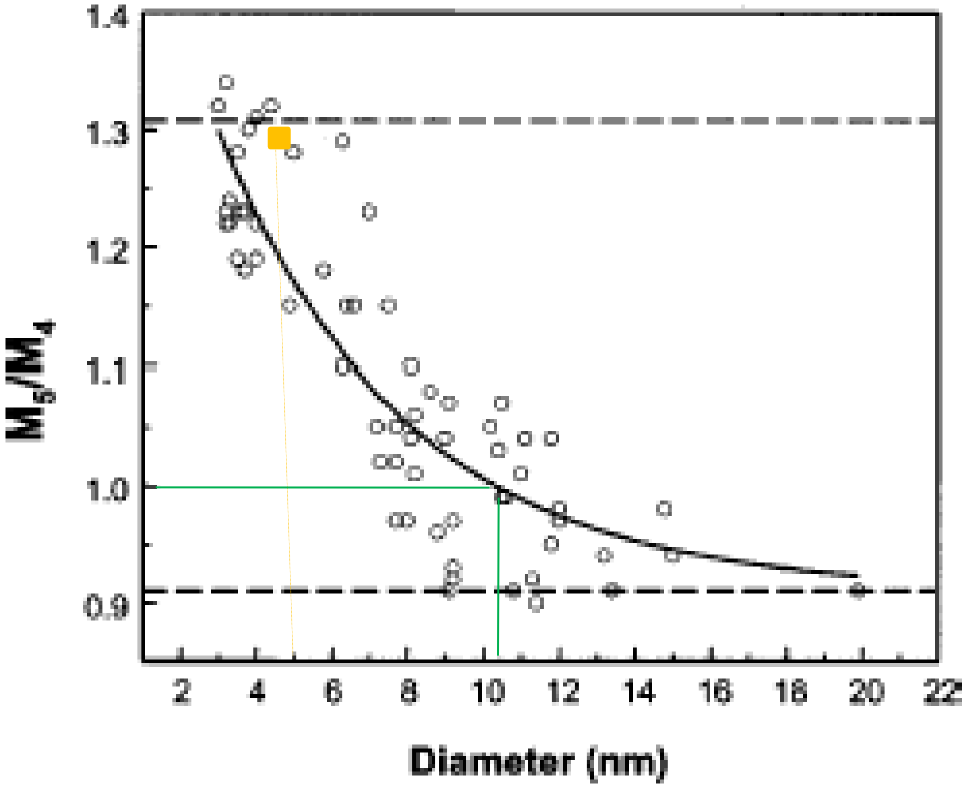


FIG. 7. Dependence of the M_5/M_4 ratios on the particle size of CeO_{2-x} nanoparticles. A fitted curve based on an exponential function is represented by the solid line.

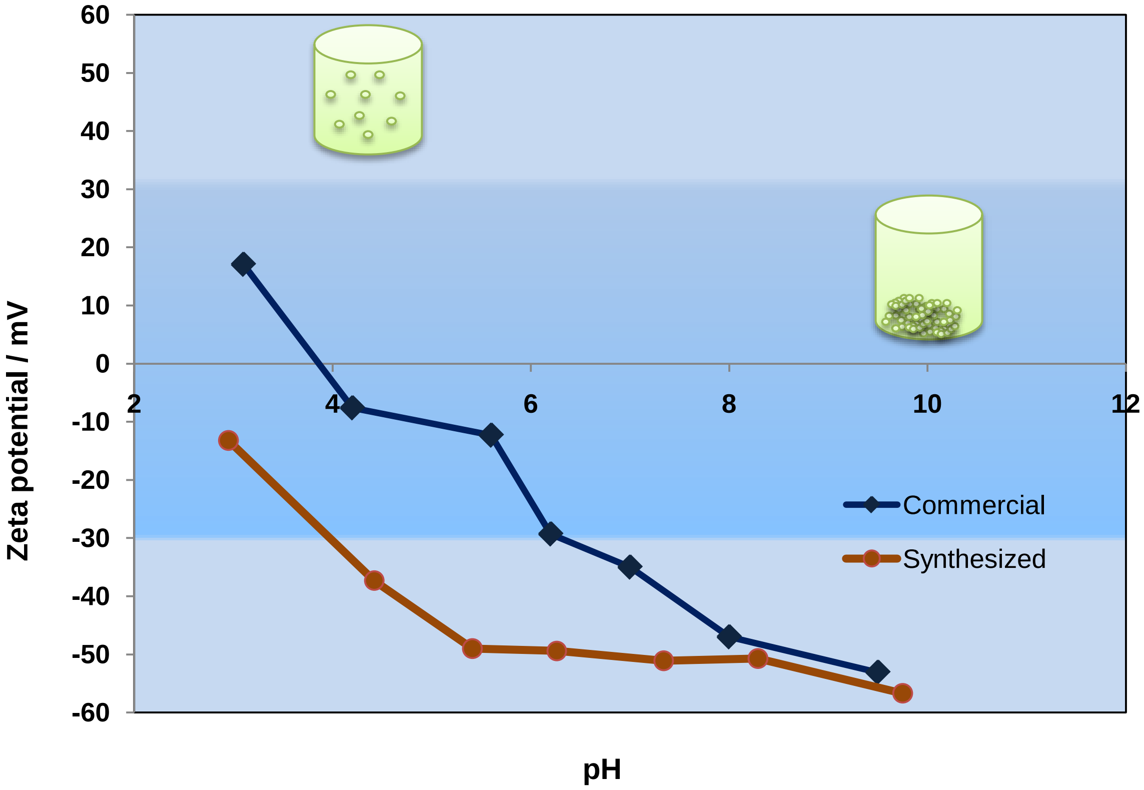
$Ce^{3+}/Ce4^{+}$ ratio is also observable in the UV-visible range. The 230 – 260 nm spectral range corresponds to the Ce^{3+} concentration, while the absorbance in the 300-400 nm range related to the concentration of Ce^{4+}.

The role of cerium redox state in the SOD mimetic activity of nanoceria, Eric G. Heckert a, Ajay S. Karakoti , Sudipta Seal, William T. Self , Biomaterials 29 (2008) 2705–2709

Particles with diameters larger than 11 nm should have the ratio of M_5 / M_4 less than 1.

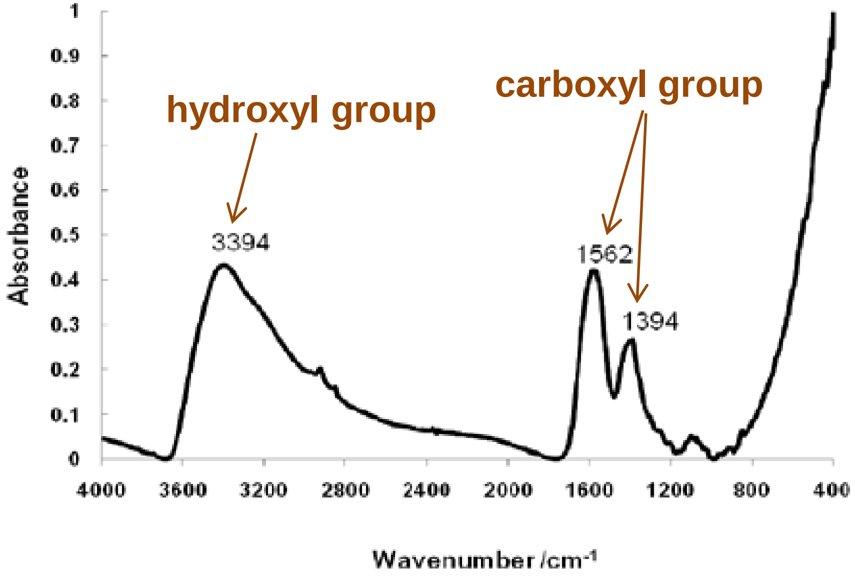
Surface charge. z-potential. Ceria

|ζ|> 30 mV: criterion for electrostatic stabilization of solutes in water



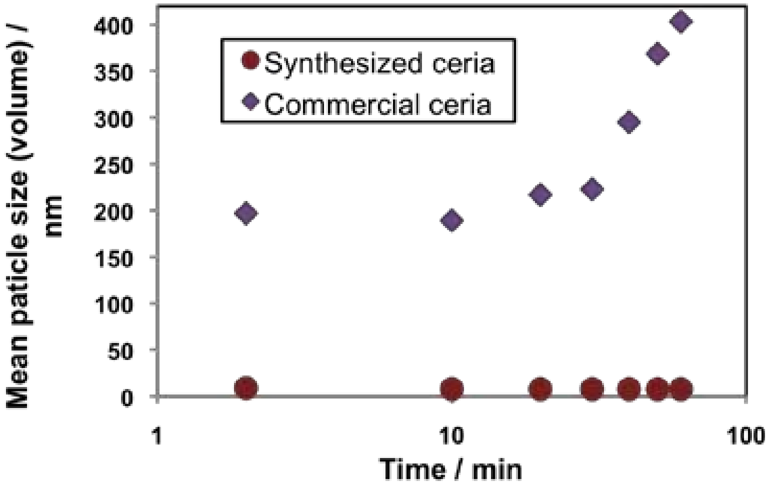
	pH (as supplied or produced)	Zeta potential (mV)	Stability of dispersion
Commercial	**4.2**	**- 8**	**bad**
Synthesized	**8.5**	**- 51**	**good**

Functionalization: acid stabilizing metal oxide



Citric acid on synthesized ceria NP surface. ATR-FTIR on powder sample.

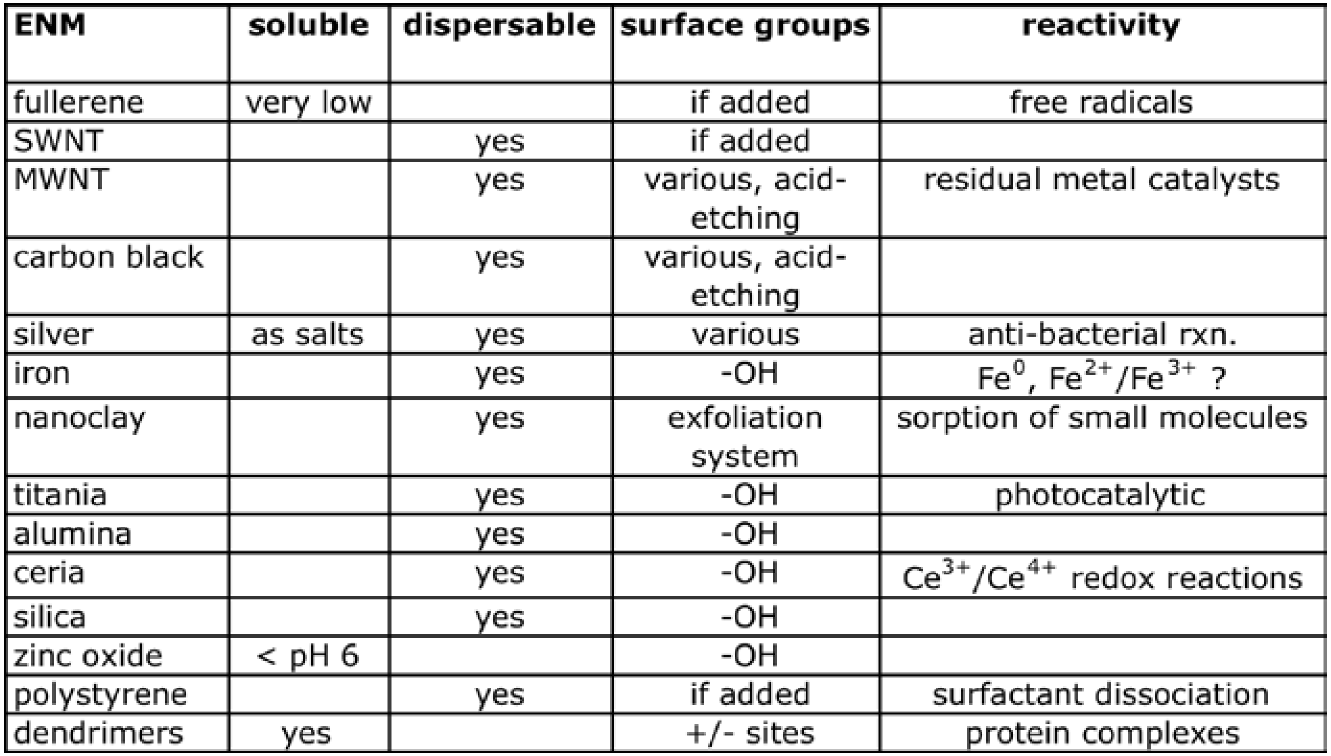
Little effect of temperature on the stability of ceria w/citric acid @ T= 37 °C. Commercial product slowly agglomerates @ T=37 °C.



Reactivity

Materials Science

- BIOMATERIALS
- Composites
- MAGNETIC MATERIALS
- METALS
- Electronic, optical, photonic
- Superconducting materials
- POLYMERS
- Catalysts
- Nanomaterials



ENM	soluble	dispersable	surface groups	reactivity
fullerene	very low		if added	free radicals
SWNT		yes	if added	
MWNT		yes	various, acid-etching	residual metal catalysts
carbon black		yes	various, acid-etching	
silver	as salts	yes	various	anti-bacterial rxn.
iron		yes	-OH	Fe^0, Fe^{2+}/Fe^{3+} ?
nanoclay		yes	exfoliation system	sorption of small molecules
titania		yes	-OH	photocatalytic
alumina		yes	-OH	
ceria		yes	-OH	Ce^{3+}/Ce^{4+} redox reactions
silica		yes	-OH	
zinc oxide	< pH 6		-OH	
polystyrene		yes	if added	surfactant dissociation
dendrimers	yes		+/- sites	protein complexes

A Tier-one list of reactivities for OECD ENMs. The synthesis pathway, manufacturing systems, and end-use applications help establish whether dispersing aids are used, which chemical groups are on the ENM surfaces, residual catalysts, and the chemical reactivity.

Ions – metal oxide nanoparticles in saline, buffers, T

Protein sorption – alumina, titania

coupling agents - ZnO

Redox reactions - ceria

Surfactants – Au nanoparticles with CTAB

Steric stabilizers – proteins sorbed on Au nanoparticles

Other – dispersants, surface energy

AQUEOUS DISPERSIONS

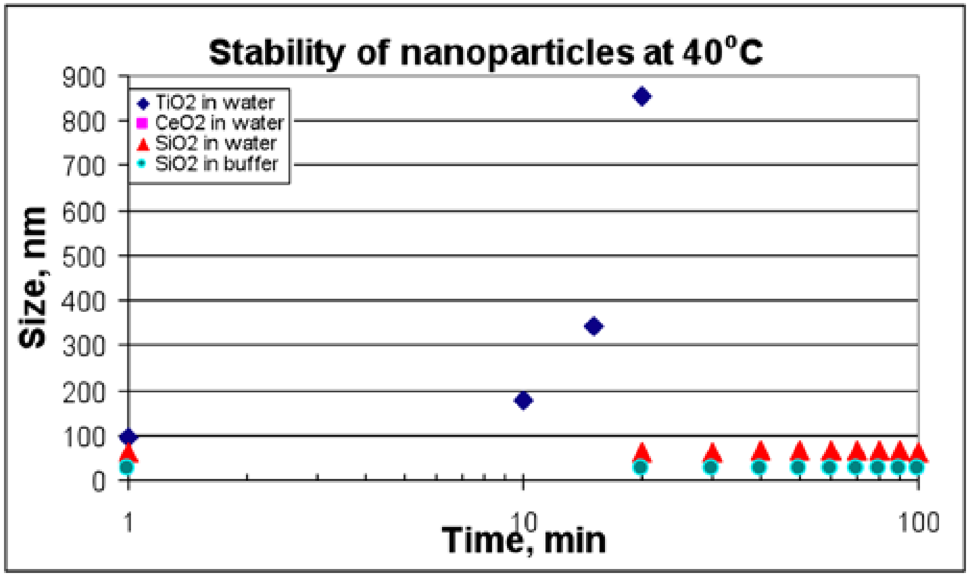
Ions:

0.9% NaCl, temperature

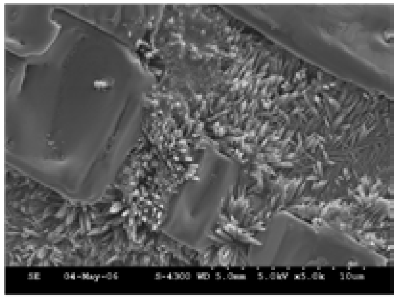
		Volume-based PSDs, nm		
Sample	**parameter**	**no additive**	**0.9 wt% NaCl**	
			20 min.	**24 hr.**
in-lab ceria	D_{ave}	8.5	9.1	8.4
	90 % range	5.7 - 12.0	6.0 - 12.9	5.6 - 12.1
commercial	D_{ave}	127	1730	precipitated
	90 % range	70.9 - 205	678 - 3450	N/A

Stability in 0.9 wt% saline

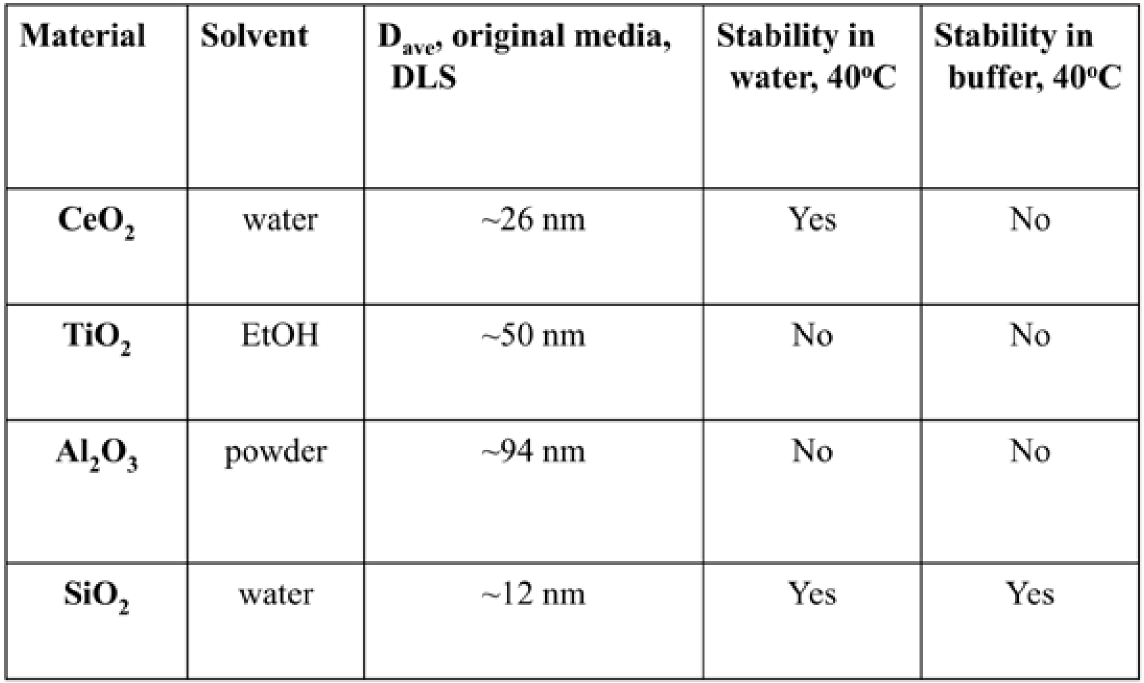
Effects of T on dispersion stability



Dispersion stabilities: water, Krebs-Henseleit buffer



- Commercial ceria in water, stabilized with acid
- Titania in ethanol, stabilized with acid
- Commercial alumina in water
- Commercial silica in water, silane coupling agent?
- All had average PS < 100 nm
- All suspensions were 2 wt %



Material	Solvent	D_{ave}, original media, DLS	Stability in water, 40°C	Stability in buffer, 40°C
CeO_2	water	~26 nm	Yes	No
TiO_2	EtOH	~50 nm	No	No
Al_2O_3	powder	~94 nm	No	No
SiO_2	water	~12 nm	Yes	Yes

- NPs can precipitate with salts often present in the body, and can act as seed crystals
- In the absence of precipitation with divalent salts, NPs in water solution can agglomerate slowly when raised to body temperature
- Agglomeration is likely related to changes in the electrostatic stabilization with T

REDOX reactions

NANOPARTICLES IN CELLS: CE^{3+}/CE^{4+} WITH PH, H_2O_2

$Ce^{3+}/Ce(IV)$-O_2: oxidation

$$Ce^{3+} + 4H_2O \longrightarrow Ce(OH)_4 + 4H^+ + e^-$$

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

$$4Ce^{3+} + O_2 + 14H_2O \longrightarrow 4Ce(OH)_4 + 12H^+$$

$$\log\frac{[Ce(OH)_4]}{[Ce^{3+}]} = 3pH - 12.81 + \frac{1}{4}\log p_{O_2}$$

$Ce^{3+}/Ce(IV)$ - H_2O_2: reduction, $0.7 < pH < 2.1$

$$\log\frac{[Ce^{3+}]}{[Ce(OH)^{3+}]} = 16.95 + \frac{1}{2}\times\log[H_2O_2] - \frac{1}{2}\times\log p_{O_2}$$

$Ce^{3+}/Ce(IV)$- H_2O_2: oxidation

$$2Ce^{3+} + H_2O_2 + xH_2O \longrightarrow 2Ce(OH)_{1+x/2}^{(3-x/2)+} + xH^+$$

$$\log\frac{[Ce(OH)^{3+}]}{[Ce^{3+}]} = 1.319 + \frac{1}{2}\times\log[H_2O_2]$$

$$\log\frac{[Ce(OH)_2^{2+}]}{[Ce^{3+}]} = pH + 0.60 + \frac{1}{2}\times\log[H_2O_2]$$

$$\log\frac{[Ce(OH)_4]}{[Ce^{3+}]} = 3pH - 3.567 + \frac{1}{2}\times\log[H_2O_2]$$

$$\log\frac{[CeO_{2(precip)}]}{[Ce^{3+}]} = 3pH + 1.961 + \frac{1}{2}\times\log[H_2O_2]$$

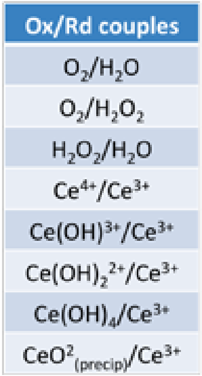
Catalysis of other reactions: neat, +oxidizing agents, + reducing agents

Electrochemical potential of cerium vs. pH; air or hydrogen peroxide

Application – possibility of in-cell dissolution at non-physiological pH

Electrochemical deposition of CeO_2 anti-corrosion coating on aluminum to replace hexavalent chromium systems, Yu, P., The phase stability of cerium species in aqueous systems,II. Ce(III/IV)-H2O-H2O2/O2 Systems., *J. Electrochem. Soc.*, **153** (1), C74 (2006).

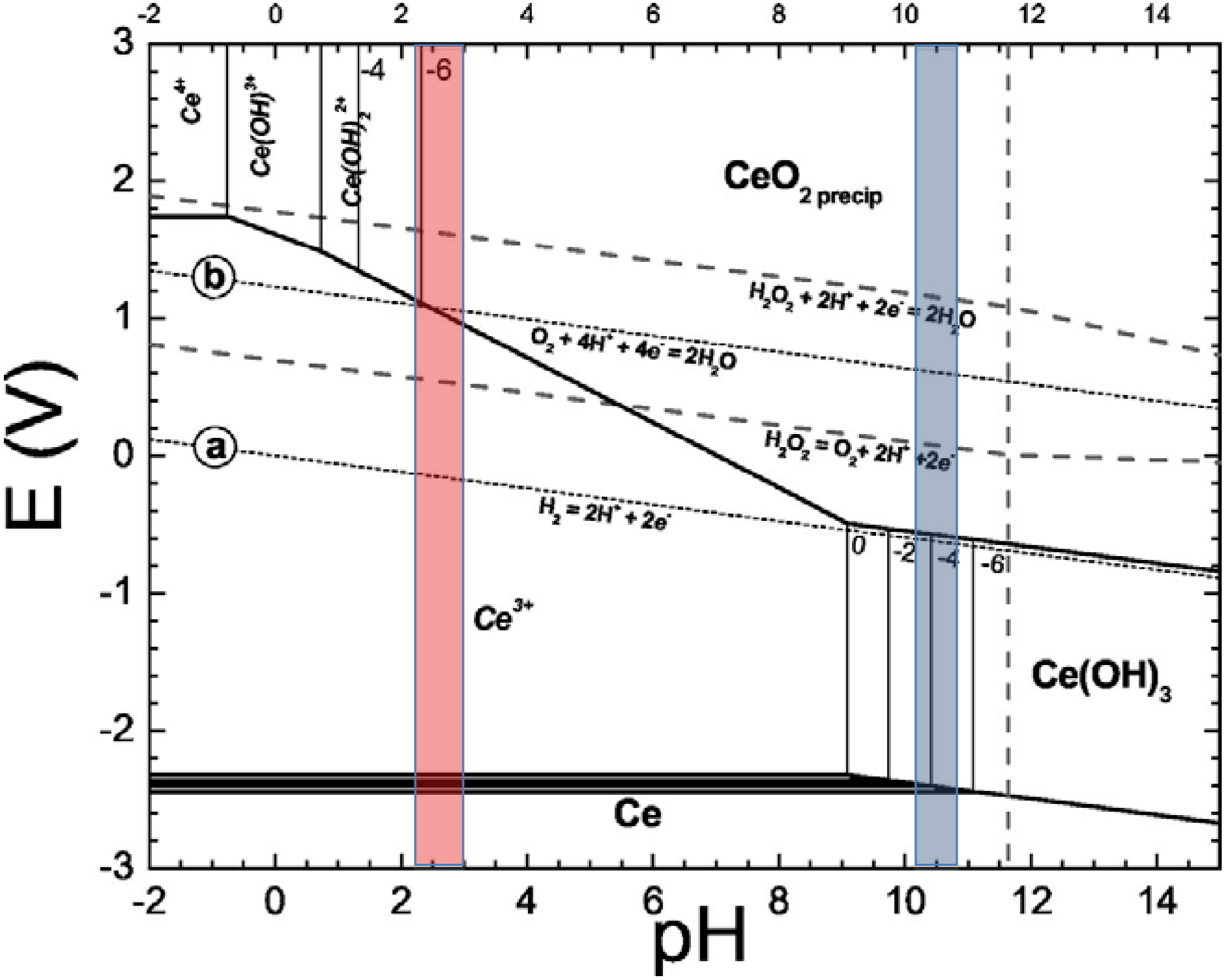
- **H_2O_2 can oxidize or reduce Ce based on pH**
- **Oxidation of Ce3+ to Ce4+ may be done by H_2O_2 or O**



Ox/Rd couples
O_2/H_2O
O_2/H_2O_2
H_2O_2/H_2O
Ce^{4+}/Ce^{3+}
$Ce(OH)^{3+}/Ce^{3+}$
$Ce(OH)_2^{2+}/Ce^{3+}$
$Ce(OH)_4/Ce^{3+}$
$CeO^2_{(precip)}/Ce^{3+}$

Simplified Pourbaix diagram
Ce(III)/Ce(IV)-H_2O-O_2-H_2O_2

Small ceria NPs have high levels of Ce^{3+} on their surfaces – can dissolution be catalyzed? Some subcell structures can be acidic/oxidizing (pink) or basic/reducing (blue). Do NPs get to these structures, and can they participate in oxidation or reduction electrochemistry?



Protein sorption:

Binary protein mixture on a-alumina

a-alumina – D_{50} = 116 nm

calcined to remove acid groups; ionic strength < 1 mM KCl; 1000 ng protein/cm^2 NP; 16 hr sorption [<5 min.]; isoelectric point (a-Al_2O_3) = 9.1; BSA = 5.2 [+ charge]; lysozyme = 9.9 [- charge]; proteins mask the surface

Research needs: kinetics; links between z-potential, NP surface energy and NP partitioning to solid surfaces; what is the reactivity of sterically protected NP surfaces? Positively charged metal oxides (TiO_2) show similar effects.

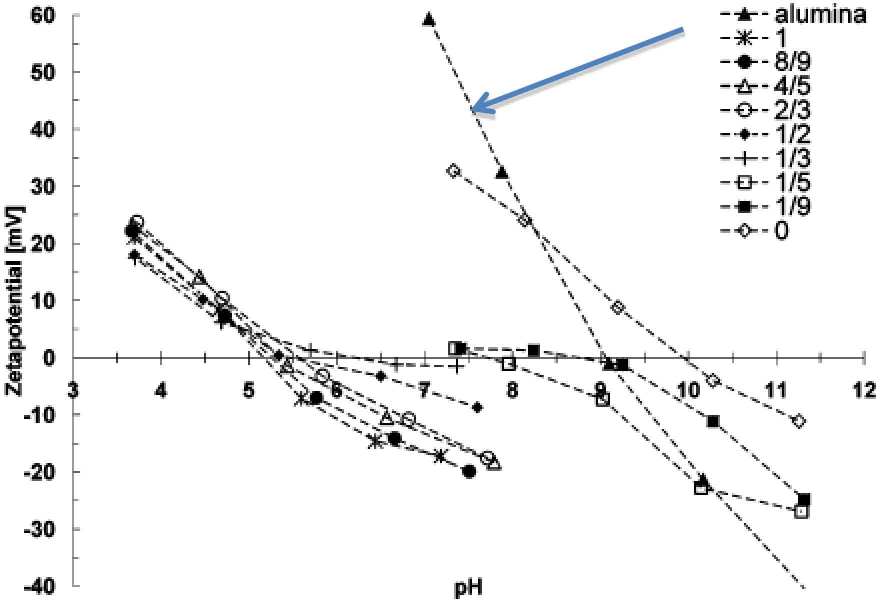
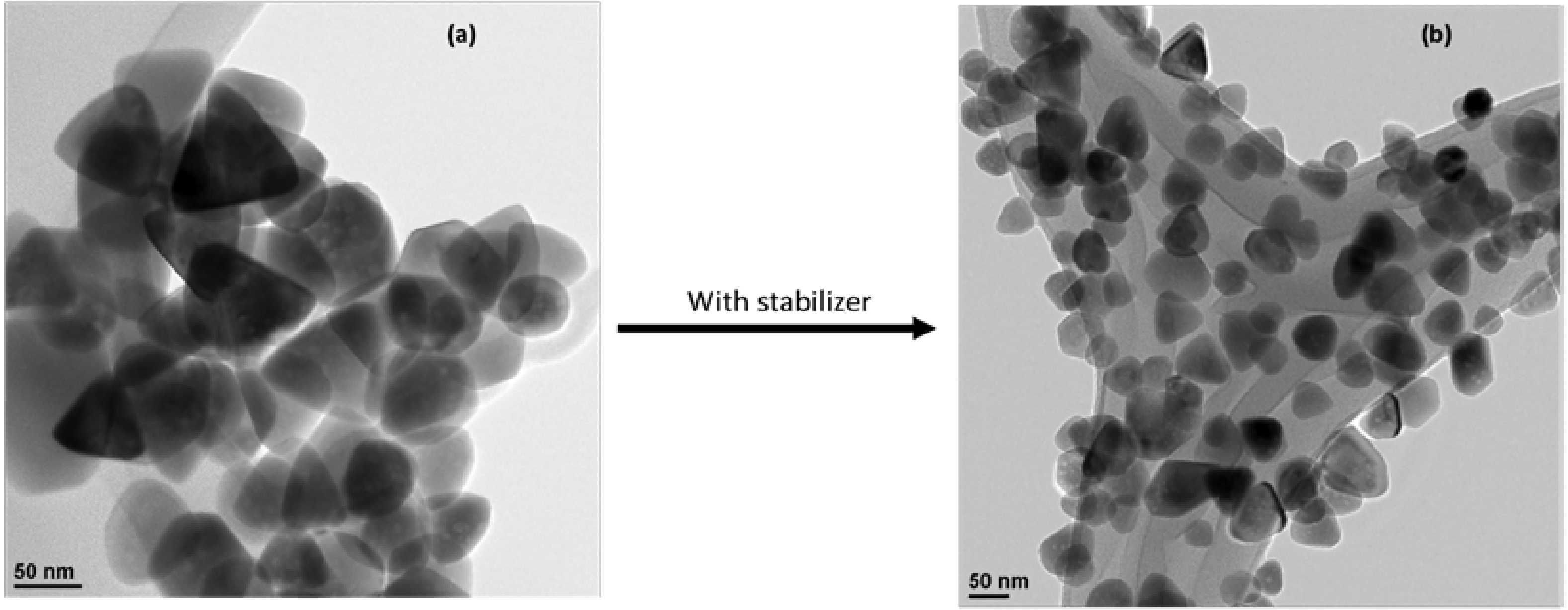


Figure 1. Zeta potential measurements of 2 vol % alumina suspensions with and without adsorbed protein mixtures of different BSA to LSZ mole fractions after 16 h. The total added protein amount was 0.5321 g, which equals 1000 ng/cm^2 normalized to the alumina surface area.

Prediction method for the isoelectric point of binary protein mixtures of BSA and Lysozyme adsorbed on colloidal titania and alumina particles, K. Rezwan, L.P.Meier, L. J. Gauckler,, *Langmuir*, 2005, **21**, 3493-3497.

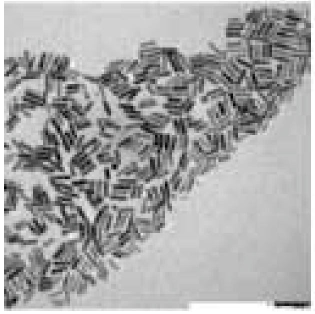
Coupling agents:
ZnO



Zinc oxide nanoparticles have –OH groups on their surfaces and are relatively hydrophilic (LHS shows as-synthesized ZnO nanoparticles agglomerating on a lacey carbon grid (TEM)). A coupling agent (RHS) alters the surface energy so the nanoparticles selectively adhere to the carbon surface rather than to each other.

Some nanoparticle products are treated by the manufacturer to improve their dispersion in specific solvent systems.

Surfactants: CTAB-capped Au nanorods



Gold nanorods were synthesized using CTAB (cetyltrimethyl ammonium bromide) which directed NP growth in a preferred axial direction. Tests with human colon carcinoma cells (HT-29) showed that apparent toxicity was due to free CTAB in solution (desorbing from the nanorods).

Serum proteins from the media sorbed to the nanorods, giving them a uniform charge. One possible mechanism for receptor-mediated endocytosis may be cellular recognition of the proteins sorbed to the nanorods.

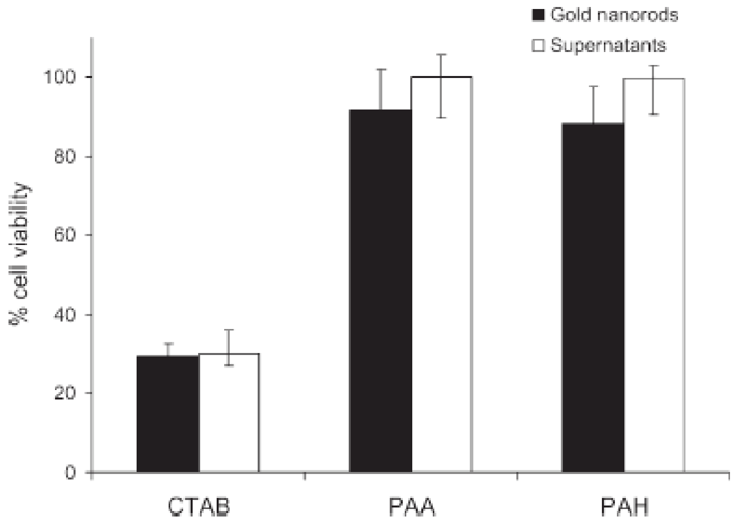


Figure 4. Viability of HT-29 cells exposed to 0.4 nM of CTAB-, PAA-, and PAH-coated gold nanorod solutions (filled bars) and their supernatants (open bars) for four days. Aspect ratios of all gold nanorods were 4.1. Error bars represent one standard deviation.

Surfactants stabilize nanoparticles, but can dissociate *in vitro* or *in vivo*. Toxicity effects may be due to the surfactant, not the nanoparticle.

Overcoating the CTAB/Au nanorods with positively (PAH) or negatively (PAA) charged polymers improved cell viability.

Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects, Alkilany, A.A., Nagaria, P. K., Hexel, C. R., Shaw, T. J., Murphy, C. J. Wyatt, M. D., *Small,* **5**(6), 701 (2009).

Steric stabilization: protein sorption on Au nanorods

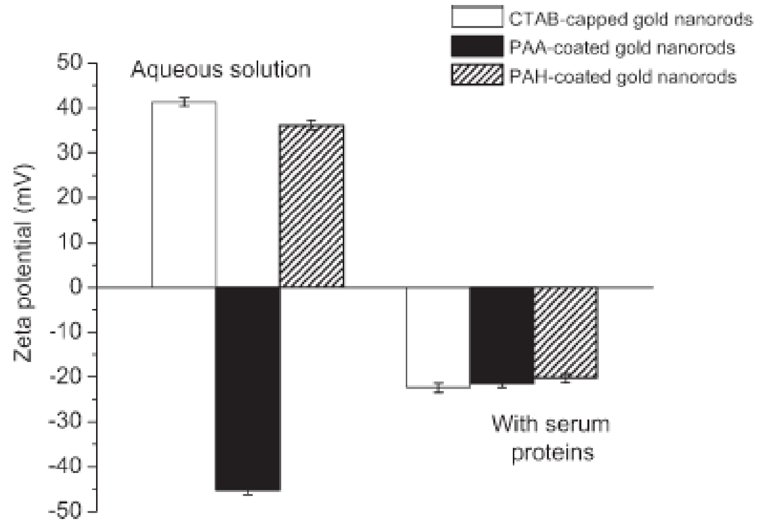


Figure 7. Effective surface charge (zeta potential) of gold nanorods coated with CTAB, PAA, and PAH in water before exposure to growth media and after exposure to growth media with serum proteins (containing 10% bovine serum albumin). All gold nanorods were centrifuged after 30 minutes of exposure and re-suspended in deionized water before measurements were obtained. Aspect ratio for all gold nanorods was 4.1. Error bars represent one standard deviation.

Poly(acrylic acid) [-] and poly(allylamine) hydrochloride [+] were coated on Au nanorods stabilized by CTAB. Surface charge reached a steady state 5 minutes after exposure to growth media with serum proteins (10% BSA). BSA has an isoelectric point of 4.6, and exhibits a negative charge at physiological pH. BSA is known to sorb to both negatively and positively charged surfaces.

Sorbed proteins can provide steric stabilization since |z| < 30. Note: PAA and PAH provide electrostatic stabilization since each produces surfaces with |z|>30.

Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects, Alkilany, A.A., Nagaria, P. K., Hexel, C. R., Shaw, T. J., Murphy, C. J. Wyatt, M. D., *Small,* **5**(6), 701 (2009).

Instrumentation, metrology and analytical methods

Nanomaterials and human health

RESEARCH ‘GAP’ ANALYSIS

Instrumentation, metrology ‘gaps’

NSTC, NNI Strategy for nanotechnology-related environmental, health and safety research, 2008, p.18

Prioritized EHS research needs	Specific tasks
1. Detect ENMs in biological matrices, the environment, the workplace	Suite of analytical methods can address many immediate questions
2. Relate modification to ENM physico-chemical properties	Partitioning of ENMs to hydrophilic and hydrophobic fluids and surfaces
3. Develop standardized assessments of PSDs, shape, structure, surface area	Automated, rapid analysis of PSD and composition is needed
4. Certified reference materials for physico-chemical characterization of ENMs	Standard samples with known surface treatments
5. Characterization methods for ENM spatio-chemical composition, purity, heterogeneity	Suite of analytical methods can address many immediate questions

Human health research ‘gaps’

NSTC, NNI Strategy for nanotechnology-related environmental, health and safety research, 2008, p. 24

Prioritized EHS research needs	Specific tasks
1. Understand absorption and transport of ENMs throughout the body	Suite of analytical methods to find NPs in tissues, cells
2. Quantify & characterize ENMs exposure, ENMs in biological matrices	As-fed dose by size/shape; NP stability in media, T and other environmental conditions; surface reactivity
3. *In vitro* and *in vivo* assays/models to predict *in vivo* human responses	
4. Relate ENM properties to inhalation, ingestion and dermal uptake, assess body burden	Dose-response for inhalation, dermal exposures; known NP stability, surface chemistry
5. Interactions between the body and EMNs at the molecular, cellular, and tissue levels	NP partitioning to fluids and surfaces; long-term reactivity;

After a clean jump over the ‘characterization’, obstacle, ...

THANK YOU FOR YOUR ATTENTION

…, you'll be ready to look ahead for the next fence.

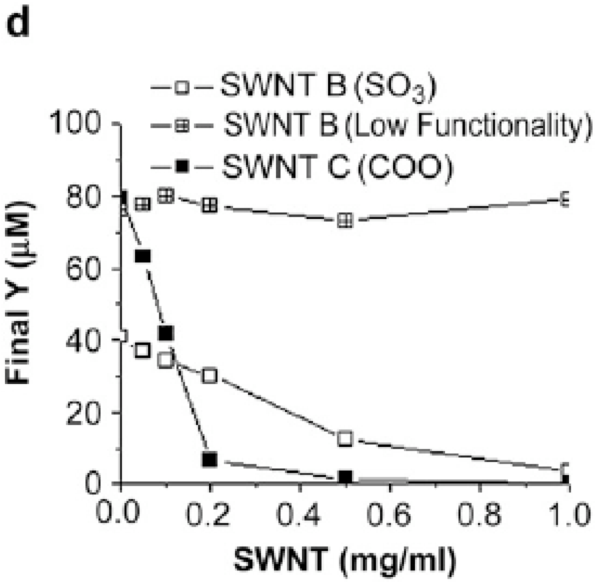
ANY QUESTIONS?

My thanks to:

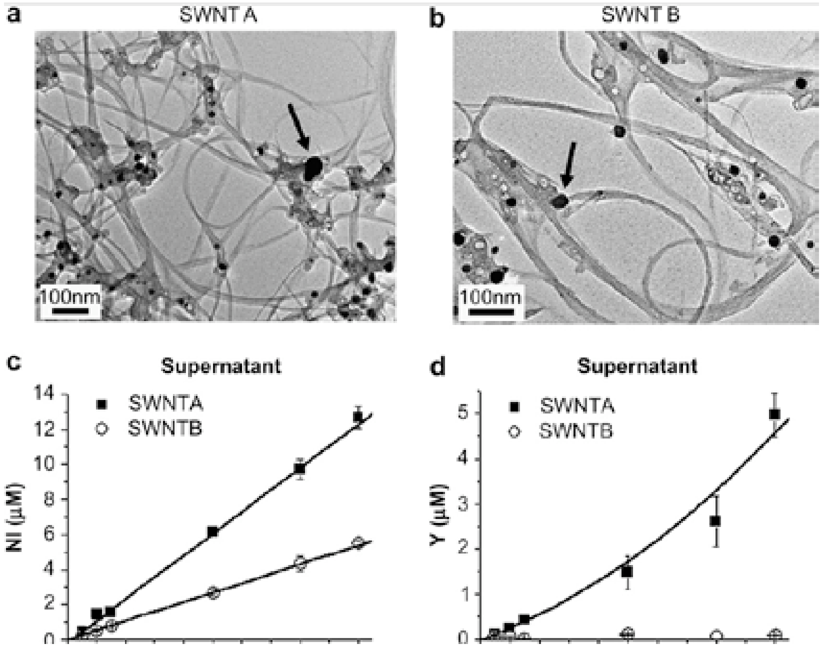
D.A. Butterfield, U. Graham, M. Tseng, J. Unrine, R. Yokel.

V. Kanniah, G.C. Laine, N. Mandzy, B.H. Wang, P. Wu.

Inhibition by trace catalyst levels



The inhibition of neuronal calcium ion channels by trace levels of yttrium released from carbon nanotubes, I.M. Jakubek, S. Marangoudakis, J. Raingo, X.Y. Liu, E. Lipscombe, R. H. Hurt, *Biomaterials*, **30**, 6351 (2009).



SWNTs can be used to transmit electrical stimulation to cultured neurons. In this case, inhibition of calcium ion channels was linked to trace amounts of yttrium leached from the SWNTs