Sample preparation is critical both for substances and products

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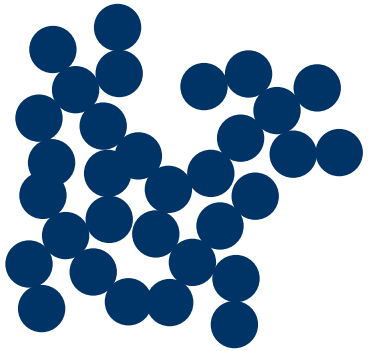
Overall objectives of the work package

- Development of dispersion protocols for powders (substances)
- Development of sample preparation protocols for complex matrices (products)
- Development of techniques and protocols for NM deposition on EM sample carriers

Dispersion protocols

Substances



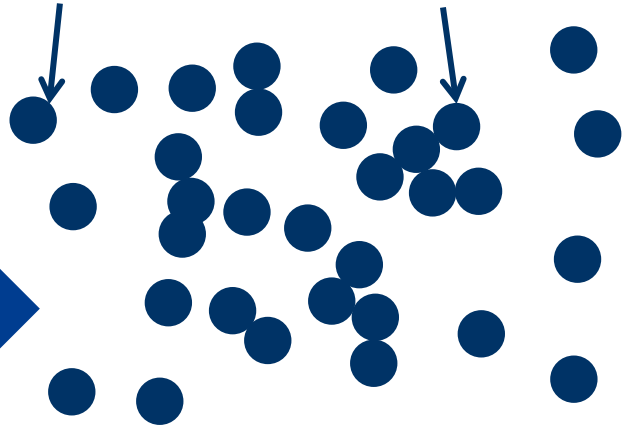
Powder agglomerates

Dry powder

Disintegration

Maximum of primary particles

Minimum of agglomerates/ aggregates



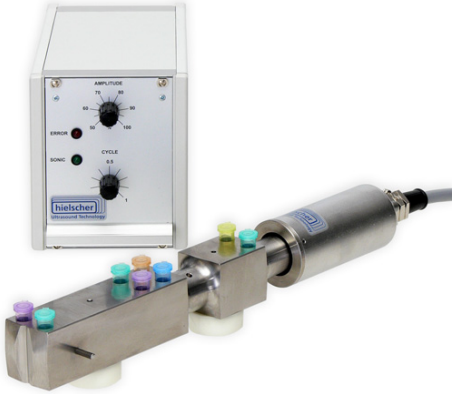
Liquid dispersion

DLS, PTA, CLS, AUC, FFF, spICP-MS, TEM…

Dispersion equipment

Bath sonicator

Probe sonicator



Vial sonicator
(vial tweeter)

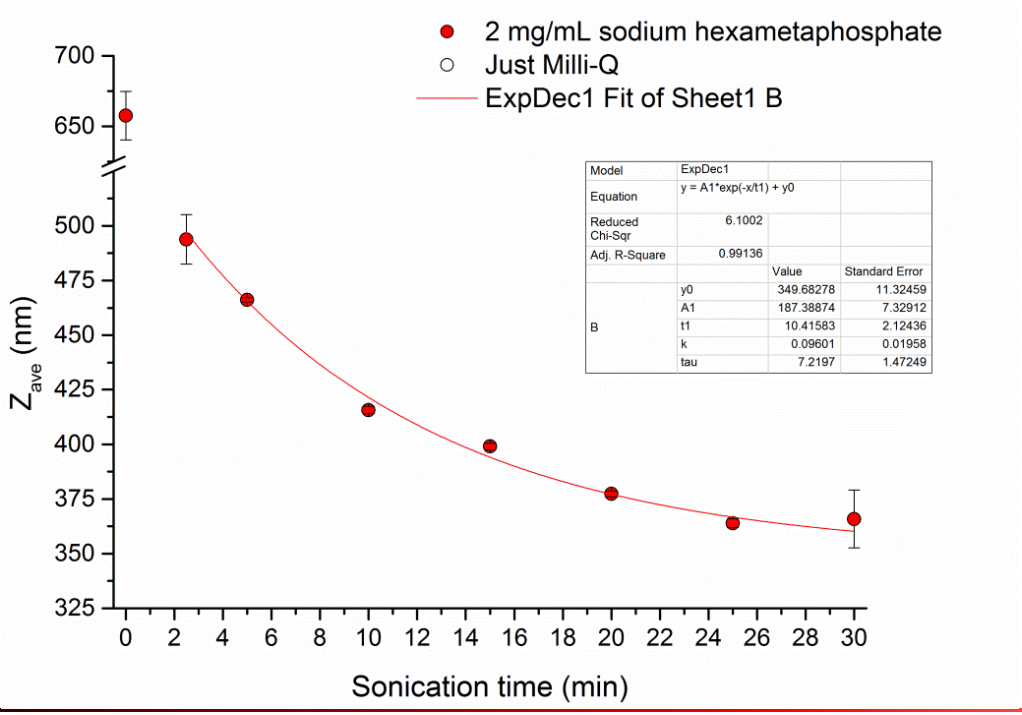
Disperser/
homogenizer

Vortexer

Examples from: www.bandelin.com / www.ika.com / www.hielscher.com

Sonication

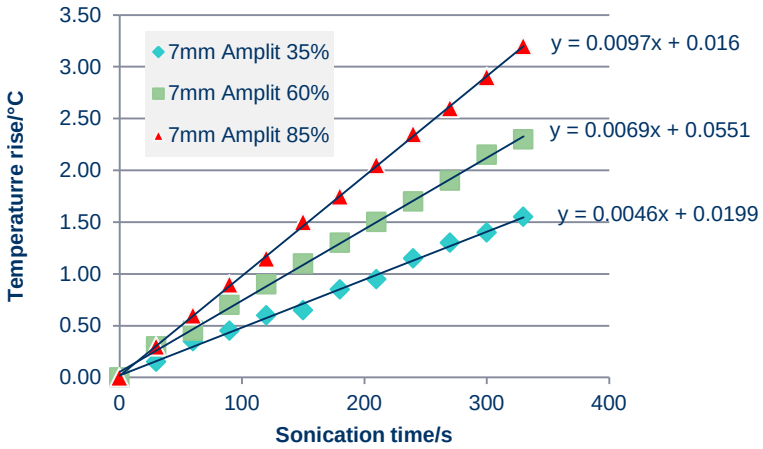
- Optimum sonication time / power based on evaluation by tier 1 method (DLS, CLS, or LD)



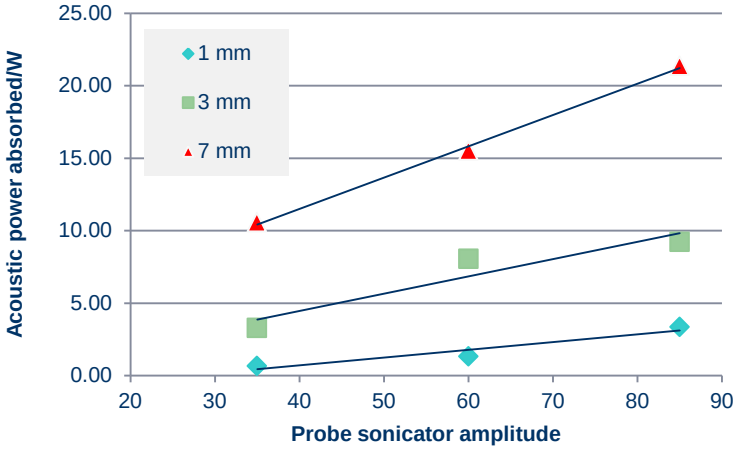
Towards harmonization of sonication by calorimetric calibration

Set-up for probe calibration using thermocouple

Sonicate and monitor the water temperature (T) as a function of time (t)



Use $\Delta T/\Delta t$ to determine the power absorbed at different amplitude/power settings and probe diameters



Variability due to sonicator instrumentation minimised (P_{ac} provided in SOPs) → Inter-laboratory transferability

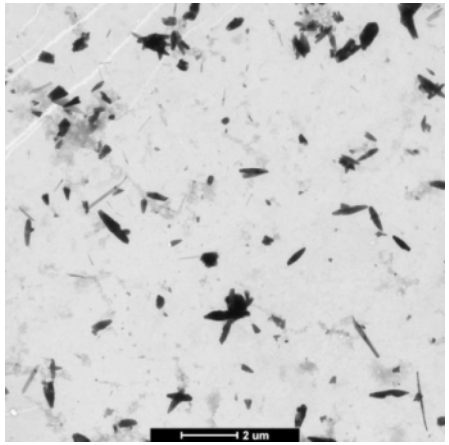
Taurozzi et al., Nanotoxicology, 2011; 5(4); 711-729

collaboration with NANOREG

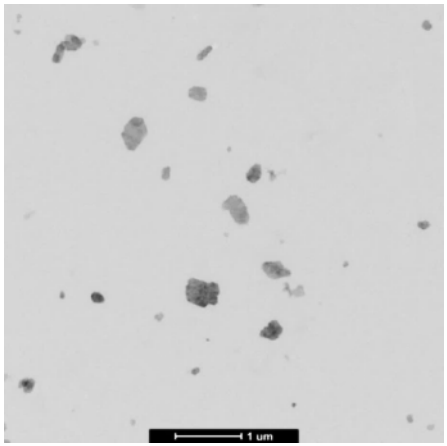
Dispersion protocols

11 SOPs developed (include: required acoustic power $\mathbf{P_{ac}}$ for sonication; size distribution from DLS, CLS, or LD; qualitative and quantitative evaluation by TEM)

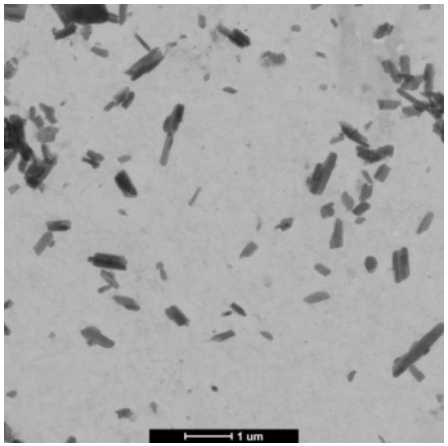
$CaCO_3$ (fine)



Kaolin



Pigment (opaque)



→ Most materials could be dispersed to primary particles and small aggregates/agglomerates (2-10 primary particles)

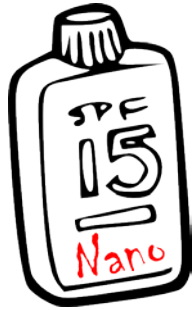
Main points regarding the developed dispersion SOPs

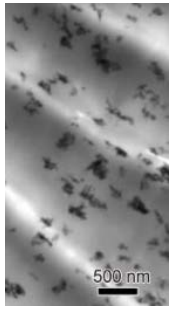
- For maximum compatibility and safety all are aqueous based with/without dispersant/stabilizers
- Methods verified only for volumes of 2-10 mL
- Sonication power densities required are typically around 1-4 WmL^{-1} (corresponding to energy densities of 0.4 – 6.4 kJ/mL)
- ENM concentration ranges are typically 0.1-10 $mgml^{-1}$
- Sonication times typically 10-30min and all less than 60 min
- Colloidal stability checked and verified for a minimum of 30minutes (NanoSteel and Zeolite not stable again sedimentation)

Sample preparation protocols for products

Methods for 3 products developed

- TiO_2 NPs in sunscreen
  - Dilution* → spICP-MS
  - Centrifugation + washing → EM
  - Dilution* + sonication → FFF
- Al_2O_3 NPs in toothpaste
  - Dilution* → spICP-MS
  - Chemical oxidation + dilution* → FFF
- Fe_2O_3 NPs in polyethylene
  - Cryo ultramicrotomy + straightening → EM





*use of dispersing agent

Sample preparation protocols for EM

Steps to be considered:

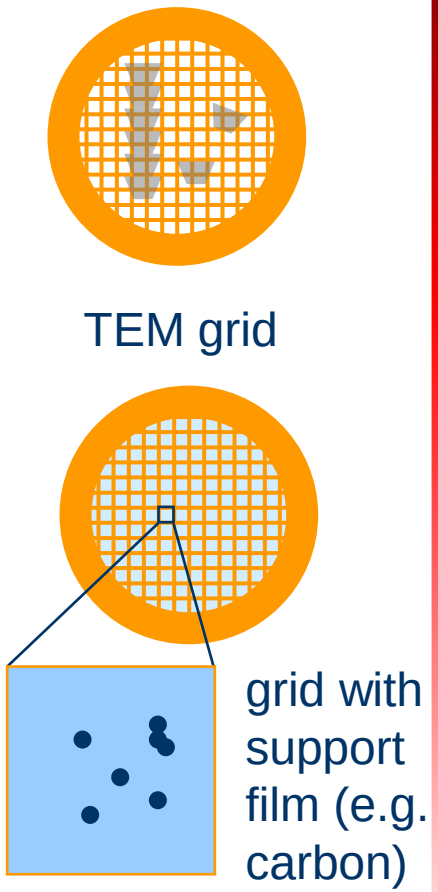
a) Pre-treatment of sample carriers

- glow discharging
- high vacuum baking
- coating with Poly-L-Lysine (PLL)
- coating with Alcian blue

b) NM deposition on EM sample carriers

- drop deposition
- electrospray ionization
- centrifugation

c) Sample storage and transportation



Summary / conclusions

- Dispersion protocols
  - Material- (and technique-) dependent
  - Transfer of sonication power levels between labs based on P_{ac} obtained by calorimetry promising
  - Similar results of vial tweeter and probe sonicator
- Sample preparation for NM in products:
  - Remains challenging
  - Needs to be developed for each NM / matrix combination
- Sample preparation for EM:
  - Requires special attention
  - Can have additional requirements regarding dispersions

Thank you WP2!

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