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OPTIMIZATION AND VALIDATION OF QUANTITATIVE TEM ANALYSIS OF PRISTINE TiO_2 POWDER IN A REGULATORY CONTEXT

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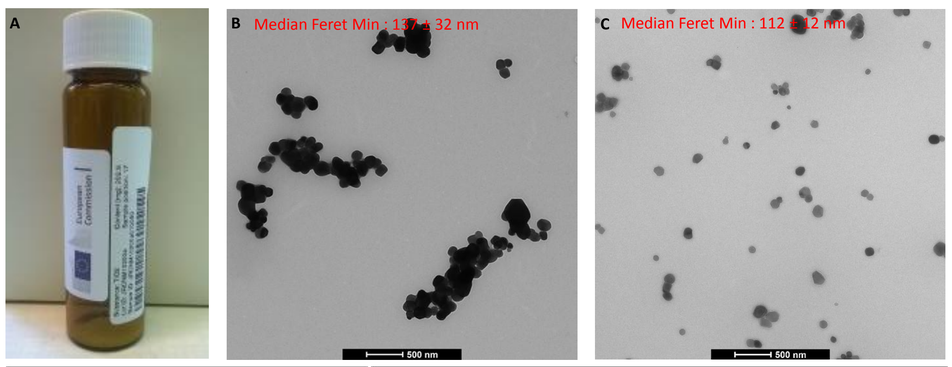
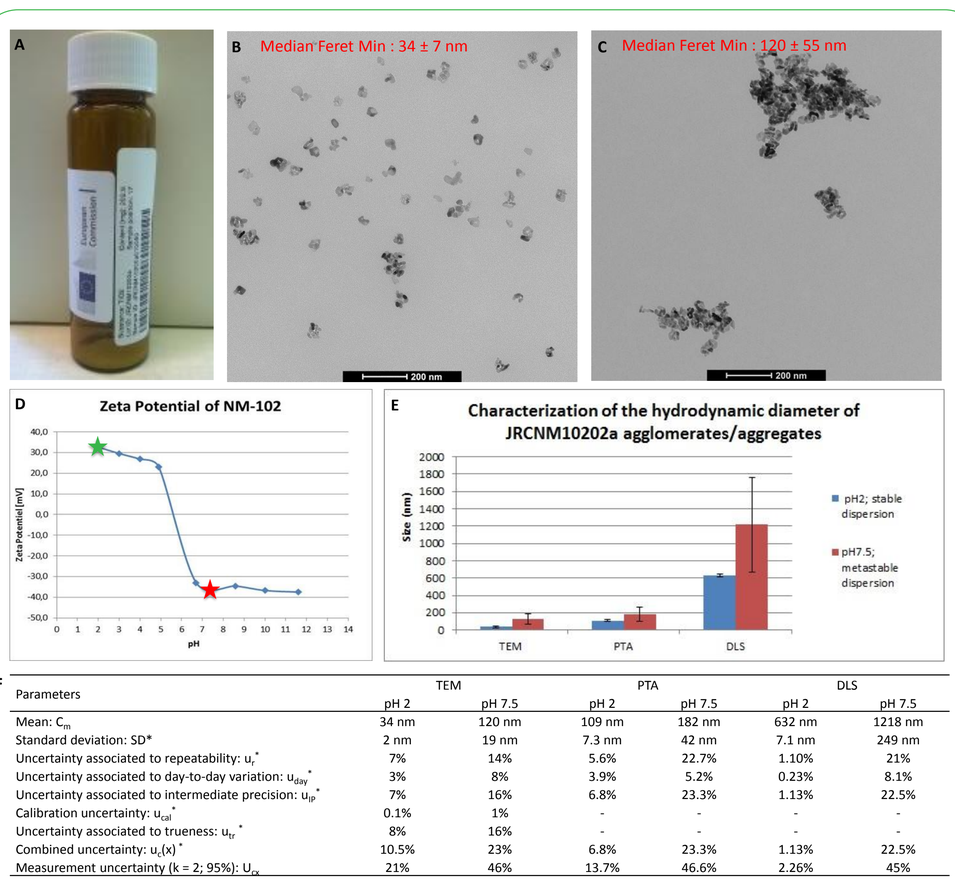
« Comparison of TEM, PTA and DLS measurements and measurement uncertainties, for samples prepared under stable and unstable conditions »



F

Parameters	TEM		PTA		DLS	
	pH 2	pH 7.5	pH 2	pH 7.5	pH 2	pH 7.5
Mean: C_m	352 nm	155nm	201 nm	245 nm	950 nm	280 nm
Standard deviation: SD*	64 nm	12 nm	36 nm	34 nm	433 nm	1.7 nm
Uncertainty associated to repeatability: u_r*	17%	7%	15.4%	8%	41%	0.53%
Uncertainty associated to day-to-day variation: u_{day}*	13%	2%	10.3%	12%	22%	0.3%
Uncertainty associated to intermediate precision: u_{IP}*	21%	7%	18.5%	14.5%	46%	0.61%
Calibration uncertainty: u_{cal}*	1%	1%	-	-	-	-
Uncertainty associated to trueness: u_{tr}*	22%	8%	-	-	-	-
Combined uncertainty: $u_c(x)$*	30.5%	11%	18.5%	14.5%	46.5%	0.61%
Measurement uncertainty (k = 2; 95%): U_{cx}	61%	22%	37%	29%	93%	1.22%

Fig 1 Characterization of JRCNM10200a using TEM, PTA and DLS.
Glass vial containing the TiO_2 representative test material as received from JRC (A). Representative images of JRCNM10200a dispersed at pH 2 (B) and at pH 7.5 (C) show, respectively, agglomerated particles, and particles in their most dispersed state, and the median Feret Min diameter of the constituent particles, measured using TEM, with expanded uncertainties (k=2). The red and green stars on the zeta-potential curve (D) correspond to the metastable conditions (shown in B), and to the most dispersed condition (shown in C), respectively. Figure E compares the median ECD of the aggregates/agglomerates measured by TEM and the hydrodynamic diameters measured by PTA and DLS at pH 2 and 7.5. The error bars represent the expanded uncertainties estimated in validation studies summarized in Table (F).



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Parameters	TEM		PTA		DLS	
	pH 2	pH 7.5	pH 2	pH 7.5	pH 2	pH 7.5
Mean: C_m	34 nm	120 nm	109 nm	182 nm	632 nm	1218 nm
Standard deviation: SD*	2 nm	19 nm	7.3 nm	42 nm	7.1 nm	249 nm
Uncertainty associated to repeatability: u_r*	7%	14%	5.6%	22.7%	1.10%	21%
Uncertainty associated to day-to-day variation: u_{day}*	3%	8%	3.9%	5.2%	0.23%	8.1%
Uncertainty associated to intermediate precision: u_{IP}*	7%	16%	6.8%	23.3%	1.13%	22.5%
Calibration uncertainty: u_{cal}*	0.1%	1%	-	-	-	-
Uncertainty associated to trueness: u_{tr}*	8%	16%	-	-	-	-
Combined uncertainty: $u_c(x)$*	10.5%	23%	6.8%	23.3%	1.13%	22.5%
Measurement uncertainty (k = 2; 95%): U_{cx}	21%	46%	13.7%	46.6%	2.26%	45%

Fig 2 Characterization of JRCNM10202a using TEM, PTA and DLS.
Glass vial containing the TiO_2 representative test material as received from JRC (A). Representative images of JRCNM10202a dispersed at pH 2 (B) and at pH 7.5 (C) show, respectively, particles in their most dispersed state, and agglomerated particles, and the median Feret Min diameter of the constituent particles, measured using TEM, with expanded uncertainties (k=2). The green and red stars on the zeta-potential curve (D) correspond to the most dispersed condition (shown in B), and to the metastable conditions (shown in C), respectively. Figure E compares the median ECD of the aggregates/agglomerates, measured by TEM, and the hydrodynamic diameters, measured by PTA and DLS, at pH 2 and 7.5. The error bars represent the expanded uncertainties estimated in validation studies summarized in Table (F).

Methods

- The developed dispersion method, based on the Guiot & Spalla approach, electrosterically stabilizes the (nano)materials, dispersed by sonication, using BSA at a pH determined from the zeta-potential measurement.
- Using a combination of TEM imaging and image analysis, the distribution of the particle properties (size, shape, surface structure) are assessed quantitatively.
- Validation of the TEM methodology by characterizing 3 replicates of the two materials dispersed under both stable and unstable conditions per day within 5 days. For comparison, DLS and PTA validation studies were done in parallel based on the same dispersions.

Results

For both JRC TiO_2 representative test materials :

- Descriptive TEM confirmed that zeta-potential measurement allowed to identify the conditions (pH) where a stable dispersion with a minimal level of agglomeration was observed.
- TEM, DLS and PTA analyses are more precise for stable dispersions.
- Combining EM imaging and image analysis allows measuring the size and shape properties of both the constituent particles and of the AA, PTA and DLS can measure, and overestimate, the AA size.
- The minimal external dimension of the constituent particles was measured most precisely and accurately using TEM in stable dispersions.
- Lower measurement uncertainties show that it is more reliable to characterize the materials under stable conditions.

Conclusions

- Optimized sample preparation conditions are crucial for a precise and accurate characterization of the (nano)materials.
- Quantitative TEM analysis allows to implement the EC definition of nanomaterials, the regulation of Belgian NanoRegistry, EFSA guidance 2018 and Reach annexes on (nano)material characterization, providing precise and accurate measurements.

REFERENCES

- **Guiot, C. and Spalla, O. (2013) Environnemental Science & Technology 1057-1064.**
- **http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm.**

ACKNOWLEDGEMENTS

The representative test material JRCNM10200a was provided by the JRC repository for nanomaterials (JRC, Ispra, Italy). This research was funded by the To²DeNano Brain (BELSPO) project.

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