PATROLS
Advanced Tools for NanoSafety Testing

Robust oral dissolution testing

Challenge of oral uptake

Dissolution of ingested nanomaterials (ENM) under physiological conditions is essential to predict the uptake. Ingested ENM pass through at least three clearly differentiated compartments: mouth (saliva), stomach, intestine. Reactive processes in the relatively aggressive compartments may induce transformation of the crystallinity, surface chemistry, particle size and state of agglomeration, to name the descriptors that are most important next to the dissolution rate. Although it is regarded as gold standard, in in-vivo study alone cannot decide if any particles that became systemically available are a *fraction* of the pristine material, or a *transformation* thereof.

Implementation of a robust alternative method

Several implementations of cascaded GIT dissolution testing have been published, and two OECD projects are developing guidance and guidelines. Both target a cascaded incubation in saliva, stomach and intestine simulants. However, the robustness of the methodology depends on the exact composition of simulant fluids, the most appropriate methods of sampling, of separation of ions from remaining particles, and analytics of both fractions.

Scientific reasoning

We assessed the relevance of the enzymes in DIN19738 media via their impact a) on ion dissolution and b) on particle transformation. Enzymes have a relevant, but unspecific effect on the kinetic as they can act as ions scavengers. The formation of enyzme-ion complexes shifts the equilibrium to dissolution, e.g. in the case of $BaSO_4$ NM220, where a 2.4-fold higher dissolved content was observed within the enzyme containing media by ICPMS. On the primary particle transformation and agglomeration though, enzymes only have limited effects. However, enzymes influence the sampling by clogging the filters and negatively affect the homogeneity during sampling.

Recommendations for OECD guidance development

Apart from the indispensible ion analysis by ICPMS, not many particle analysis methods are meaningful. TEM is unique on morphology changes, and also widely available, but particles should be considered as transforming only if the XRD diffractogram shows the emergence of new peaks or the loss of peaks compared to the pristine materials. Methods that require concentrations far above the expected daily intake (XRD, SAXS), or far below (spICPMS) are not preferred.

This project has received €12.7M in funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 760813

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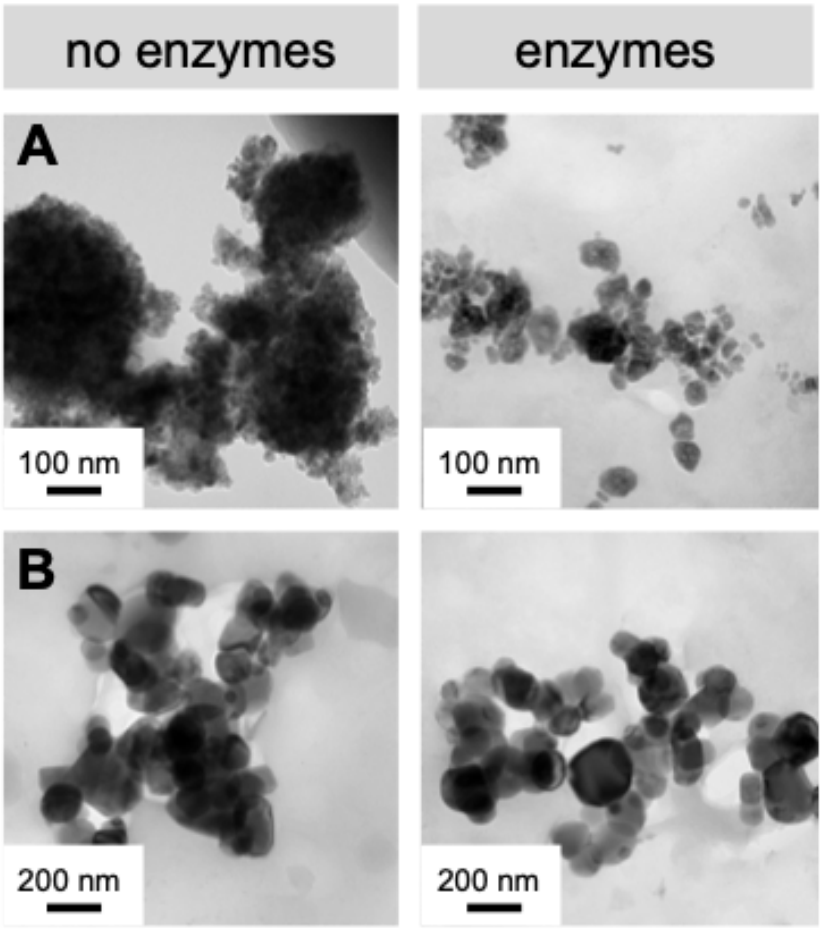

TEM analysis after cascaded gastro-intestinal aging: enzymes promote the dissolution of partially soluble materials such as $BaSO_4$NM220 (A), but do not transform the crystallinity, size or shape of materials such as TiO_2 E171 (B), that are insoluble in either the more “robust” enzyme-free medium or in the more “realistic” enzyme-containing medium.

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