Evaluation of health risks of technical nanoparticles – the contribution of characterization

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Introduction

According to ISO/TC 229, nanomaterials represent structures whose units are usually smaller than 100 nm. In order to recognize size-related risks of these new materials early, toxicological studies in-vitro and in-vivo have to be conducted at nanoparticles. A relevant assessment for the interpretation of toxicological examinations requires the comprehensive chemical-physical characterization of the used powders (marked in orange in figure 1).
Nanomaterials are dispersed and exposed to cells in in-vitro tests. A standard operation procedure for suspension preparation is needed, which may be used for different kinds of powders. In order to quantify the changes of the particle properties in physiological media, the agglomeration behaviour of the particles as well as the interactions with proteins, which are a main element of body fluids, have to be evaluated (marked in blue in figure 1).

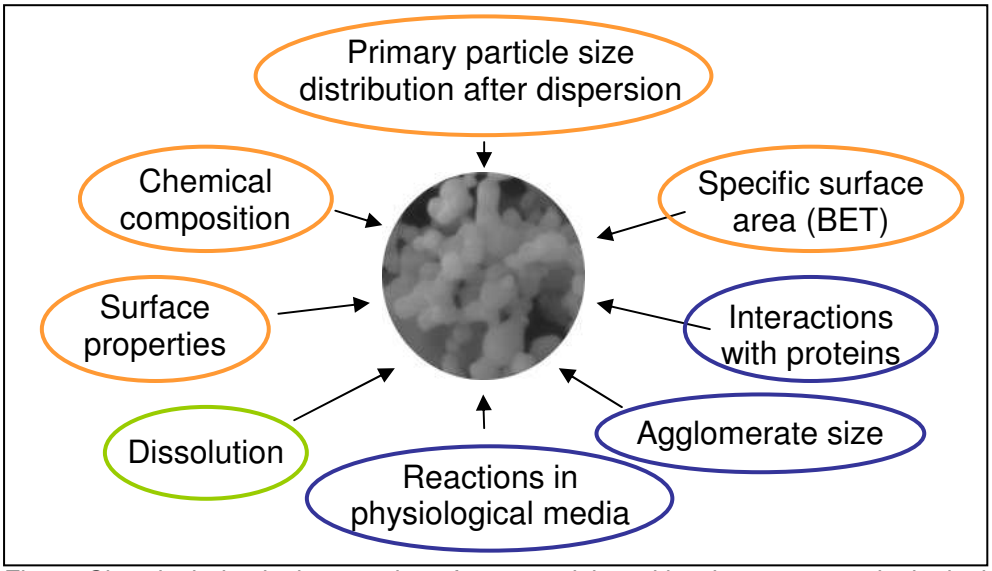

Fig. 1: Chemical-physical properties of nanoparticles with relevance to toxicological examinations

Materials and methods

Two commercially available nanoparticles were selected and used without any modification. TiO_2 P25 is produced by Evonik. A TEM image at data sheet shows an aggregated structure; the specific surface area adds up to 50 ± 15 m²/g and the primary particle size averages out 21 nm. P25 consists of anatase and rutil. WC_L particles were manufactured via a carbothermal route. Their primary particle size was measured between 100 and 200 nm. XRD analysis showed a WC phase only. No relevant impurities were found in both samples.
Investigations of particle and agglomerate sizes were done by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ld.). According to ISO 22412 harmonic intensity-weighted arithmetic average particle diameter x_{DLS} was analyzed. The zeta potential was studied by measuring the electrophoretic mobility of particles in the suspension followed by calculation using the Smoluchowski equation. Specific surface area (BET) was measured using an

accelerated surface area and porosimetry analyzer ASAP 2010 (Micromeritics GmbH).

Results and discussion

Powder characterization

The analysis of both powders indicates great differences. While SEM image of the TiO_2 powder shows a narrow primary particle size distribution and a high state of aggregation the particle size distribution of WC is broad, and the primary particles are separable (figure 2). Specific surface areas of 56 m²/g for TiO_2 and 6,9 m²/g for WC were measured corresponding to primary particle sizes x_{BET} of 27 nm and 56 nm, respectively.

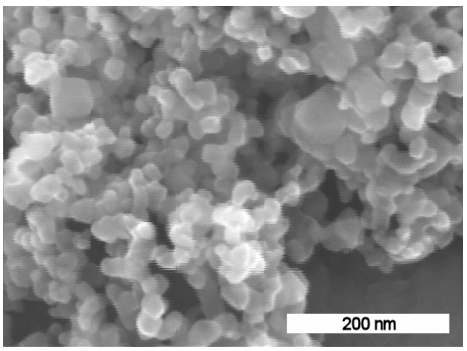

Fig. 2A: SEM image of TiO_2 P25

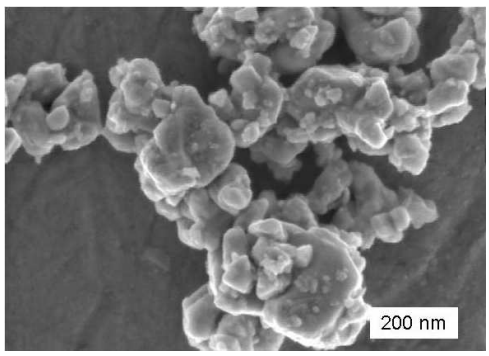

Fig. 2B: SEM image of WC_L

Development and characterisation of suspensions for in-vitro investigations

Beside the knowledge of powder characteristic data the properties of nanomaterials in water are necessarily to analyze prior to toxicological investigations. Stock solutions of high reproducibility and electrostatic stability need to be developed for each kind of nanomaterial separately. For this, zeta potential measurements are utilized to quantify the effect of pH or different non-toxic dispersant aids. An electrostatic stable suspension is determined by a high absolute value of zeta potential (> 30 mV).

Figure 3 shows the different behaviour of TiO_2 and WC in 1 mM NaCl solution. Due to oxidation at the particle's surface WC is negatively charged within the whole pH range. Titanium dioxide particles are positively charged in acid media, whereas in the alkaline region the hydroxide ions adsorb and the surface is negatively charged. In order to prepare stable solutions WC will be dispersed in water, while TiO_2 needs to be suspended in an acid solution.

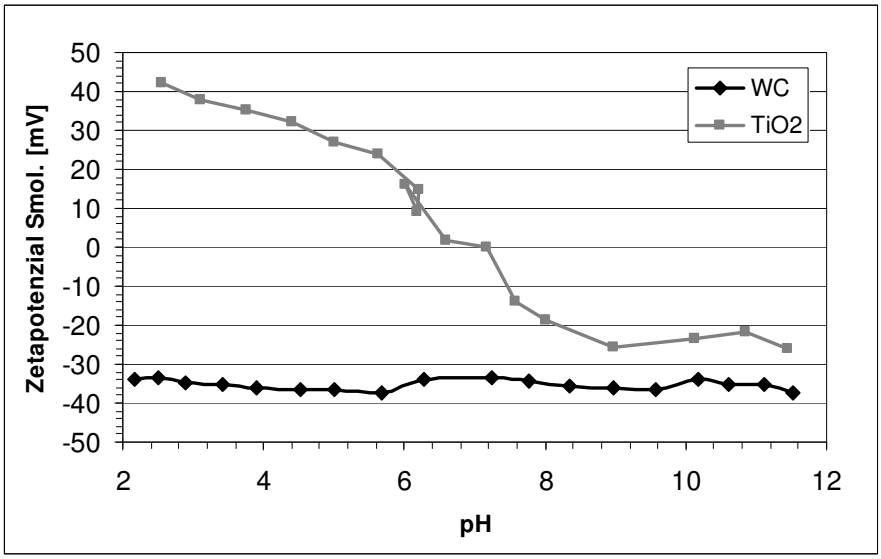

Fig 3: Zeta potential against pH for TiO_2 and WC in 1 mM NaCl

Energy input by ultrasound is used for deagglomeration for both particles. This procedure leads to suspensions, which contain "smallest dispersable units" of a powder and which are characterized by x_{DLS} and might be exposed to cultural media. x_{DLS} of 170 nm were calculated for TiO_2, whilst WC was dispersable in units of 115 nm in size. These results correspond with SEM pictures in figure 1. Although the primary particle size of titanium dioxide is much smaller compared to WC these strong bounded aggregates are destroyable by milling only. WC shows single structures; therefore the difference between x_{BET} and x_{DLS}, which represents the state of aggregation, is smaller than for TiO_2.

Particles in physiological media

The stable and homogenized suspensions are added to phosphate buffer saline (PBS) with or without bovine serum albumin (BSA) as physiological media in a ratio of 10 Vol% to 90 Vol%. Figure 4 shows the surface charge properties of TiO_2 and WC in dependence of the BSA presence.

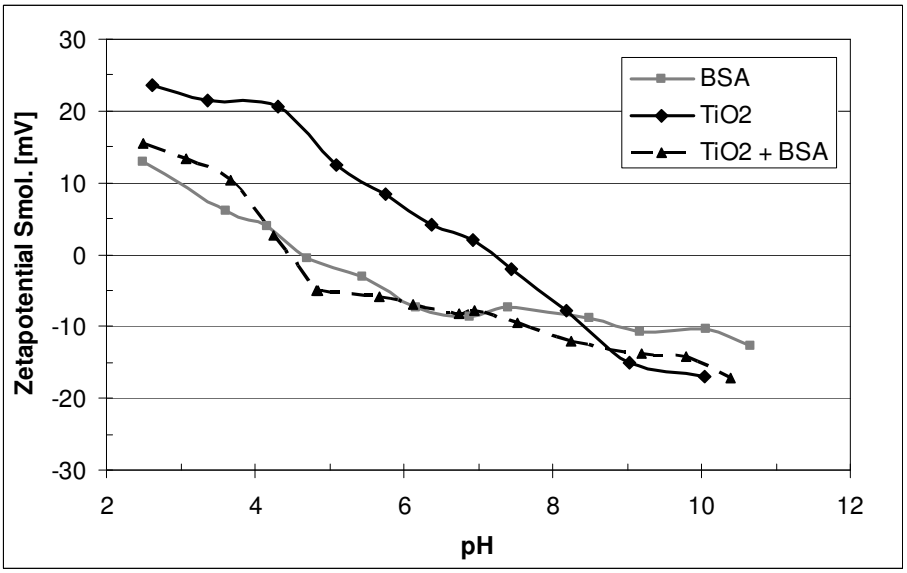

Fig. 4A: Zeta potential against pH for TiO_2 in physiological media

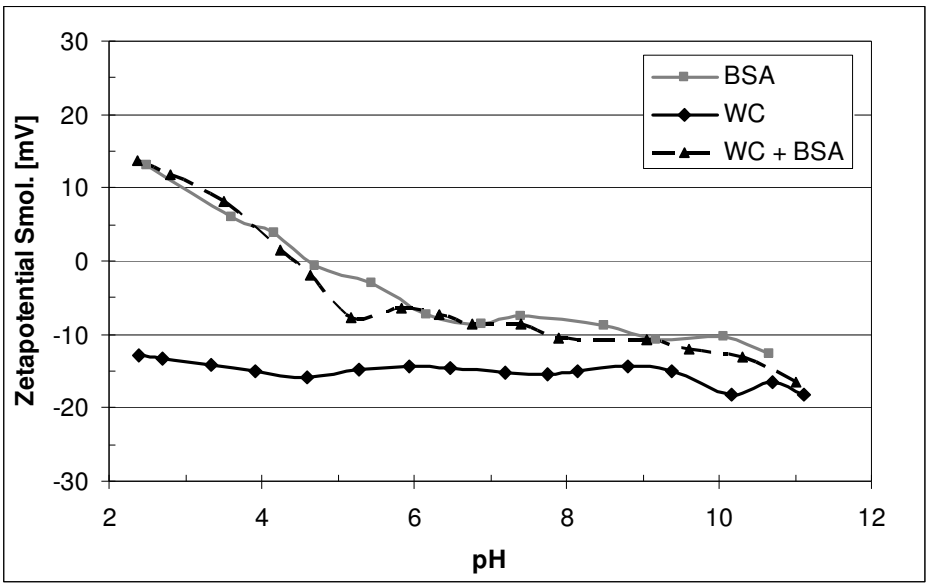

Fig. 4B: Zeta potential against pH for WC in physiological media

Both figures show that the particles act in physiological media w/o BSA very similarly compared to their behaviour in non-physiological media. The protein BSA itself is mainly positively charged in acid media and mainly negatively charged in alkaline media. Although the nanomaterials show different properties in non-physiological solutions at both´ surfaces BSA adsorbs and finally determines the surface charge properties. The protein reacts as a “coating”. This effect is important for toxicological tests: Cells may accept nanomaterials as biological media and even interact with them.

Conclusions

In order to interpret toxicological tests with technical nanoparticles the material needs to be investigated. Three steps are necessary:
(1) The original powder must be characterized using parameters like primary particle size distribution, specific surface area, particle form and crystallinity.
(2) An electrostatic stable suspension – acting as a stock solution – must be developed prior to in-vitro or in-vivo investigations. Because toxicological effects might be determined by surface (reactions with cells) or by aggregate size (transportation into cells limits the effects) both investigations are necessary.
(3) The properties of nanoparticles in physiological media must be investigated because agglomeration effects or the adsorption of biological material determine the particle’s behaviour.

Acknowledgements

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