A Safer-by-design Concept for Flame-generated Engineered Nanomaterials

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ABSTRACT

Here, we present a safer formulation concept for flame-generated engineered nanomaterials (ENMs) based on a one-step, in flight SiO₂ encapsulation process, which was recently introduced by the authors as a means for a scalable manufacturing of SiO₂ coated ENMs. Core-shell particles exhibit the surface properties of their amorphous SiO₂ shell while maintaining specific functional properties of their core material. The versatility of the SiO₂-coating process is demonstrated by applying it to four ENMs (CeO₂, ZnO, Fe₂O₃, Ag) marked by their prevalence in consumer products as well as their range in toxicity. We present the coating fundamentals and the effects of the SiO₂-coating on core material structure, composition and morphology. Finally, the biological interactions of SiO₂-coated vs. uncoated ENMs are evaluated using both cellular bioassays and in in-vivo animal inhalation experiments, providing valuable evidence for reduced toxicity for the SiO₂-coated ENMs. Results indicate that the proposed ‘safer by design’ concept bears great promise for scaled-up application in industry in order to reduce the toxicological profile of ENMs for certain applications.

Keywords: ENM, nanotoxicology, safer by design ENMs, FSP, core-shell nanoparticles, SiO₂ coating.

1 INTRODUCTION

The global nanotechnology industry reached over 1.5 trillion USD last year and has become a major economic force in the 21st century [1]. Engineered nanomaterials (ENMs) are by far the largest segment of the nanotechnology market, accounting for 80% of all revenues [2]. Global ENM production rates are expected to increase by more than 20 times within the next 15 years [3]. Meanwhile, the number of consumer products containing ENMs is growing at a similarly exponential pace. Preliminary evidence demonstrates the potential for ENMs to cause adverse biological effects [4].

While significant research has been directed toward understanding nano-bio interactions as well as fundamental rules of nanotoxicology, research toward devising safer ENM formulation concepts that can readily be adopted by the nanotechnology industry is very sparse. Here, we present “safer formulation concept” for one of the largest ENM families by volume of production [5], flame generated nanomaterials. Flame spray pyrolysis is the preferred routes for scalable ENM synthesis as they do not create liquid by-products, offer easier particle collection, include fewer process steps, can yield up to kg/hr and result in high purity materials with unique morphologies including the synthesis of metastable phases [6, 7]. In this study, a recently developed, flame spray pyrolysis based ENM synthesis platform (Harvard VENGES) [8, 9] was modified to allow for the in-flight coating of ENMs with a nano-thin layer of amorphous SiO₂. Amorphous SiO₂ is considered a biologically and environmentally inert material, often used as a negative control material in in-vitro ENM screening assays [10, 11]. Such nanothin SiO₂ is therefore ideal to shield otherwise potentially toxic core-materials from any interactions with environmental and biological media. The “core-shell” ENMs exhibit the surface properties of their shell while preserving certain important bulk properties (i.e. optical, magnetic properties) of their core materials [12-14].

Herein we demonstrate demonstrate the versatility and applicability of the coating process on a comprehensive panel of and industry-relevant nanopanel of ENMs (CeO₂, Fe₂O₃, ZnO, Ag) marked by their range in toxicity as well as their prevalence in consumer products. We look on the effect of the SiO₂-coating on core material structure, composition and morphology. The coating efficiency is also assessed with XPS and Chemisorption. We also examine the colloidal properties of the coated particles in water and cell media. To validate the safer-by-design concept we provide in-vitro and in-vivo toxicological evidence.

2 RESULTS AND DISCUSSION

2.1 SiO₂ Encapsulation Strategy

Figures 1 illustrate the proposed approach and underlying theory to the one-step nanoparticle synthesis, and in-flight SiO₂-encapsulation. Core-ENMs are synthesized by the flame spray pyrolysis (FSP) of a highly
combustable solution with an organometallic compound precursor dissolved in high enthalpy solvents. The freshly formed core-ENMs are convected to an in-line SiO2 coating reactor, where they are encapsulated with a nanothin amorphous SiO2 layer by the swirl-injection of HMDSO-laden N2. The key to successful hermetic coatings is to reduce the temperature in the coating region as to inhibit further core particle growth, maintaining however, sufficient temperatures for the conversion of HMDSO to SiO2-coatings (heterogenous nucleation). The gas phase HMDSO conversion reaction to SiO2 results from the high temperatures generated by the combustion of the high enthalpy solvents used in the core particle formation. Under optimal supersaturation conditions, defined by the temperature profile in the reactor’s coating zone as well as the HMDSO injection mass, the SiO2 vapor condenses onto the surface of the core ENMs creating the desired nanothin coating layer of SiO2 (heterogeneous nucleation).

**Figure 1**: Approach to SiO2-coated ENM synthesis.

Varying molar flow rate ratio of the amount of HMDSO vapor that is injected to the one of the core material (\(\dot{n}_{\text{HMDSO}}/\dot{n}_{\text{core}}\)) enables precise control over the SiO2-coating thickness.15, 16, 18

Figure 2 shows transmission microscope (TEM) images of uncoated and SiO2-coated CeO2, Fe2O3, Ag and ZnO synthesized by the aforementioned process. It is evident that the coating is uniform and very precisely encapsulating the generated particles. The SiO2 layer (2-5 nm) is shown to encapsulate entire agglomerates rather than individual particles. The TEM is also confirming the structure of the synthesized materials before and after the coating as the maintain the same characteristic shapes of the core particles (CeO2: polyhedral, Fe2O3: hexagonal/spherical, Ag: spherical, ZnO: rod-like) are preserved by the SiO2-coating.

### 2.2 Assessing SiO2 Coating Efficiency

X-Ray Photoelectron Spectroscopy (XPS) is a highly sensitive and quantitative method for evaluating atomic percentage on surfaces. As it is penetrating only a few nanometers of the surface it is ideal for evaluating the coating quality of the ENMs. Figure 3 shows the XPS survey spectra for the representative case of CeO2 with varied SiO2 wt%. As the amount of injected HMDSO in the coating reactor increases the Ce electron peaks intensity is gradually decreasing to the point that completely disappears at 20 wt% SiO2, where the spectra of the coated CeO2 matches that of the pure SiO2. This is an indication of a fully coated of the CeO2 particles. Quantitatively this is also confirmed by calculating the surface (~5nm deep) atomic concentration ratio, \((\text{Ce}/(\text{Ce}+\text{Si}))\), which was <1% indicating that there is no evident ceria peak. This results were also confirmed with chemisorption (data not shown). All other materials in the nanopanel exhibit similar behavior.

**Figure 2**: TEM images of uncoated (A-D) and SiO2-coated (E-H) CeO2, Fe2O3, Ag, and ZnO. SiO2-coating appears as lighter contrast contour around stronger contrast core particles.

### 2.3 ENM-Cell Interactions

The role of ENM surface coatings on ENM-cell interactions was assessed with three relevant cell lines: human primary monocyte-derived macrophages, PMA-matured THP-1 macrophages and human alveolar basal epithelial A549 cells. The cells were exposed for 24 hours the coated and
uncoated ENMs, following a well established dosing and dosimetry protocol, over a dose range, as described in the method section below.

Figure 3: XPS survey scans for CeO$_2$, SiO$_2$-coated CeO$_2$ (9, 15, 20 wt% SiO$_2$), and SiO$_2$ with relevant photoelectron peaks (diamonds).

The ENM cytotoxicity was assessed using a variety of standardised assays such as LDH, MTT and live/dead screening assay. Figure 4 illustrates collectively the cellular toxicity data between ZnO (d$_{XRD}$ = 23.2 nm) and SiO$_2$-coated ZnO (47 wt% SiO$_2$) (d$_{XRD}$ = 24.6 nm), as compared to SiO$_2$ (d$_{BET}$ = 18.6 nm). As expected the ZnO nanoparticles exhibited a dose response curve with increasing toxicity as doses rise above 50 µg/ml. On the other hand the SiO$_2$ coated ZnO ENMs indicated minimal toxicity at the same level as those from pure SiO$_2$ which is regarded as non-toxic at these doses in the literature [15]. The results are similar for the rest of the materials showcasing the safety of the coating technique. In addition an animal (rodent) inhalation study for the case of ceria was performed [REF]. The in-vivo results for the case of uncoated CeO$_2$ demonstrate signs of lung injury and inflammation as indicated by the high numbers of PMNs and LDH measured in the recovered bronchoalveolar lavage fluid. (figure 5). In contrast the coated ceria resulted to no sign of lung injury and inflammation.

3 CONCLUSIONS

In conclusion, we showcase the effectiveness of a novel safer formulation concept for flame-generated ENMs by applying it to a comprehensive and industry-relevant nanopanel. We present a valuable technique for quantitatively assessing the coating efficiency of the SiO$_2$-encapsulation process (XPS). Finally, we provide valuable toxicological evidence for the safety of this novel formulation. The described concept bears great promise for large-scale industrial application as a means of effectively inhibiting nanoparticle toxicity.

Figure 4: Cytotoxicity of ZnO and SiO$_2$-coated ZnO (A549, MTT/LDH assays).

Figure 5: Alveolar macrophages (AM) (A), polymorphonuclear neutrophils (PMNs) (B), albumin (C), lactate dehydrogenase (LDH) (D) levels in bronchoalveolar lavage (BAL) of rat sacrificed 1 day post-exposure.

4 METHODS

CeO$_2$, Fe$_2$O$_3$, ZnO and Ag core particles were synthesized by the Flame Spray Pyrolysis (FSP) of precursor solutions containing organometallic compounds (Cerium(III) ethylhexanoate, Iron(III) acetylacetone, Zinc naphthenate, and Silver acetate, 0.5 M, respectively). Optimal synthesis parameters determined by previous
computational [16] and experimental work [17] were applied to the SiO2-coatings synthesis.

X-ray Diffraction (XRD) patterns were obtained using a Scintag XDS2000 powder diffractometer (Cu Ka (λ = 0.154 nm), -40 kV, 40 mA, stepsize = 0.02°). The crystal size was determined by applying the Sherrer Shape Equation to the Gaussian fit of the major diffraction peak. ENMs were deposited onto lacey carbon grids for TEM imaging (TEM: Libra 120). Highly surface sensitive X-Ray Photoelectron Spectroscopy (XPS) (ESCA SSX-100, X-ray source: monochromatic Al Kα, 10 kV was used to assess SiO2 coating efficiency [30, 31]. All XPS spectra were calibrated using the C1s hydrocarbon contamination peak (BE: 284.6 eV). Analysis was done with CASA XPS software. ENMs were dispersed and delivered to the cells as described in [18]. Human alveolar basal epithelial A549, cultured in F-12/K supplemented with 3% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 10 mM HEPES, were exposed to 0 – 100 µg/ml ENMs for 24 h. Cellular metabolic activity and cytotoxicity were measured via MTT assay and LDH assay respectively according to the standard protocols. Cytotoxicity was evaluated by dual live/dead staining and fluorescence microscopy. The inhalation study was preformed as is described in detail by Demokritou et al. [19].

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