DELIVERABLE REPORT

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Project full title: "Engineered nanomaterial mechanisms of interactions with living systems and the environment: a universal framework for safe nanotechnology"

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Goals of the NanoMILE report on experimental protocols for aging reactions

The NanoMILE project aims to establish a fundamental understanding of the mechanisms of manufactured nanomaterial (MNM) interactions with living systems and the environment, across the entire life cycle of MNMs and in a wide range of target species. To establish an understanding of changes in the nature of MNMs as they undergo transformations within products and biological or environmental compartments across their life cycle and critically to feed this information into subsequent research to ensure that these "aged" and transformed MNMs are tested for their biological/environmental role. WP3 of NanoMILE will investigate and quantify the alteration and transformation of MNMs in products and during their use and release into the environment or biota. Exposure to MNMs in occupational, consumer or environmental settings may either be to the original, parent MNMs or to MNMs that have been incorporated into products and subsequently released, either in their original form or in an altered form due to industrial or natural processes. To date, few studies have tried to establish the changes that MNMs undergo when incorporated into, and released from, products. As a result there is major uncertainty as to the state of many MNMs following their release.

WP3 will expose relevant MNMs selected from the libraries of WP2 to different processes, different biophysicochemical conditions, in order to characterize the changes in the MNM, and either deliver altered MNMs or provide detailed protocols on how to induce these alterations, to alternative WPs.

In this report the results from task 3.2. "*Alteration of MNMs*" are reported. In this task a selection of ENM provided by WP2 are exposed to different aging processes that have been identified in task 3.1. The materials that will be studied in this task are ENM from different groups (insoluble, soluble and redox-active ENM). Aging processes that were studied in this task are air exposure and water exposure. This report contains the description of the experimental methods that have been developed, the results obtained using these methods are then contained in Deliverable 3.3 (due month 36).

This report also contains a description of the aging methods used to provide aged materials for the other project partners as specified in the Milestone 6 report.

Summary

In its first part, this report describes the procedures that have been developed by NanoMILE partners to study the aging of selected MNM from the NanoMILE particle library. These are on the one side the aging in air under UV-light and the aging in aqueous suspension. For the aging in air the use of the accelerated weathering testers is described and how the samples are prepared. For the aqueous aging the procedures to age in washing liquids and in waters containing natural organic matter is presented.

In the second part of the deliverable the protocols are described that are used to age the MNM listed in Milestone 6. These aged particles will then be distributed to the NanoMILE partners that requested them. For TiO_2 and ZnO the aging in air is used, in both cases in dry form. For particles that are provided as suspensions it is described how dry particles are obtained. The Ag sulfidation is conducted based on an amalgam of several published procedures using NaS as reactant. For the phosphatization the described procedure is based on a literature reference that has been adapted to the NanoMILE needs. This deliverable contains only the description of the aging procedure, the characterization of the aged materials will be presented in Deliverable 3.3 (due month 36).

Part A: Aging processes

In this part of the report the procedures that have been developed to study the aging of selected MNM from the NanoMILE particle library are described. The results from applying these protocols to the particles and the characterization of the aged particles will be described in Deliverable 3.3, which is due in month 36.

1. Aging in air (UV/solar irradiance) (CEA)

1.1 Accelerated weathering testers

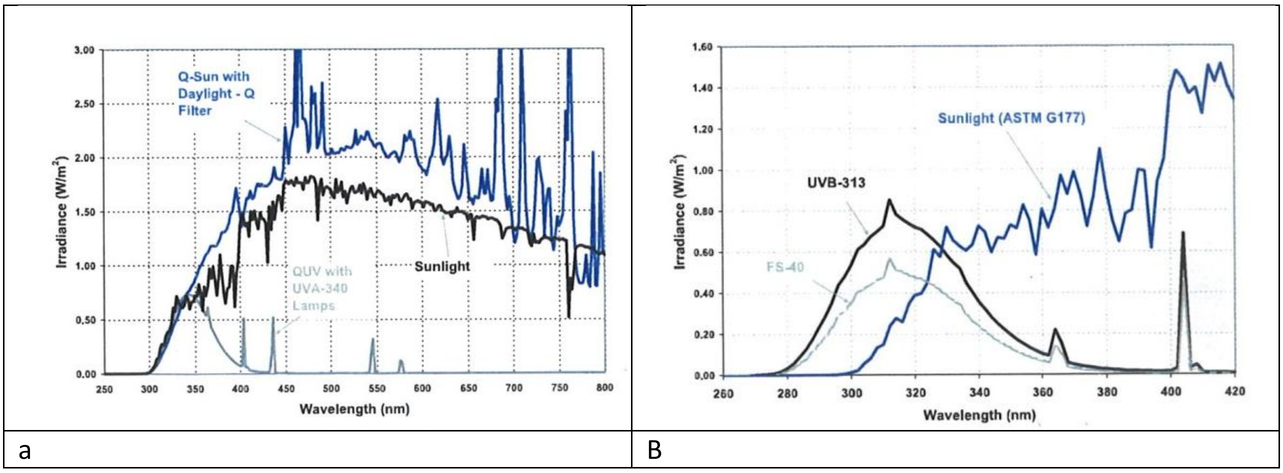
The aging experiments were performed with two different devices, the QSUN (model Xe-3) and the QUV (model QUV/spray) from QLab (Figure 1). These two climatic chambers can reproduce the damage caused by sunlight, rain and dew by exposing the test material to cycles of solar/UV light and moisture at controlled, elevated temperatures and irradiance. Hence, in a few days or weeks, the testers can reproduce the damage that would occur over months or years outdoors. However, the degradation rates are highly material dependent and no single acceleration factor can be applied.

a b

Figure 1: Climatic chambers used for the aging procedures. a: QSUN; b: QUV.

The QSUN is equipped with xenon arc lamps that reproduce the solar spectrum (Figure 2a) while the QUV's fluorescent ultraviolet lamps simulate (and enhance) the short wavelength portion of the spectrum (UVB-313, Figure 2b). UV light is responsible for almost all photodegradation of durable materials exposed outdoors. The UVB-313 lamps maximize acceleration utilizing short-wave UV that is more severe than the UV normally found at the earth's surface. That is why it has also been tested with

TiO_2, a very durable material. However, one should keep in mind that these lamps may produce extreme (and sometimes even unrealistic) degradation results. The conditions used for the NanoMILE aging experiments performed are given in Table 1. These conditions were adapted from guidelines of EN ISO standards (EN ISO, 2013) and are representative for weathering experiments.



a

B

Figure 2: Light spectra provided by a: the QSUN and b: the QUV.

Table 1: Experimental conditions of the NPs aging procedure.

	QSUN	**QUV**
MNM type	TiO_2 as powder or suspension CeO_2 as powder or suspension Ag as suspension	TiO_2 as powder ZnO as powder
Experimental conditions	7 days T = 70°C (black panel), T_{air} = 40°C RH = 50% 1.44W/m^2 (@ 420 nm)	28 days or 42 days T = 70°C (black panel) RH = - (no monitoring available) 0.68W/m^2 (@ 310nm)

1.2 Sample preparation

The MNMs used in this study are listed in Table 2. The particles come either as a powder or as suspensions and can be exposed to solar/UV aging in either form.

Table 2: NMNs provided for aging experiments.

Particle name	**Characteristics**	**supplier**
TiO_2 UNi	Suspension 1.7% TiO_2 Uncoated, 10 nm	Promethean Particles
TiO_2 AA4040	Suspension 2% TiO_2 1% Dispex AA4040 (capping), 10 nm	Promethean Particles
TiO_2 F127	Suspension 1.7% TiO_2 1% Pluronic F127 (capping), 10 nm	Promethean Particles
TiO_2 PVP	Suspension 2% TiO_2 (or 1.05% 1% PolyVinylPyrrolidone (capping), 10nm	Promethean Particles
CeO_2	20nm, spherical, 3.1 % aqueous suspension.	Promethean Particles
CeO_2 NM212	Powder Cubic	Joint Research Center
Ag AL 20nm	Suspension 20 mg/L citrate capping, 20nm	Aldrich
Ag AL 60nm	Suspension 20 mg/L citrate capping, 60nm	Aldrich
Ag AL 100nm	Suspension 20 mg/L citrate capping, 100 nm	Aldrich
Ag UoB Cit	Suspension 40 mg/L citrate capping, 20 nm	University of Birmingham
Ag UoB PEG	Suspension 50 mg/L PEG capping, 20 nm	University of Birmingham
ZnO NM110(1)	Powder uncoated	Joint Research Center
ZnO NM111(1)	Powder Triethoxycaprylylsilane coating	Joint Research Center

The suspensions of TiO_2 were diluted in water at a concentration of 1 g/L (metal base). CeO_2 powder was dispersed in water at a concentration of 1 g/L (metal base) and ultrasonicated for 15 min for de-agglomeration purposes. TiO_2 and CeO_2 suspensions were aged at 500 mg/L (metal base). Ag NPs suspensions were either aged as received (Ag UoB PEG 50mg/L, Ag UoB Cit 40 mg/L, Ag AL 20 nm, 60 nm, 100 nm 20 mg/L) or diluted to 20 mg/L for comparison purposes with Ag AL 20 nm, 60 nm, 100 nm.

For solar/UV exposure of the MNMs in the dry form (powder), droplets of the suspensions were deposited on slides (glass or PTFE) or in polystyrene Petri dishes and placed into a thermal chamber to let the sample dry at 80°C. The substrate with the dry residue was then transferred into the climatic chamber for the aging test process.

Once the aging test was completed, the samples were withdrawn from the chamber. The suspensions were kept as is at 4°C for further characterization using TEM, DLS, Zeta potential determination or FFF-ICPMS. The dry samples were re-dispersed in water using ultrasonication (5-30 min) to bring the particles back into suspension, and were then stored alongside the other suspensions at 4°C until further characterization or shipment to other NanoMILE partners.

2. Aging in water

2.1. Aging in simulated washing liquids (Empa)

2.1.1. Introduction

To date, the predominant focus of environmental health and safety of manufactured nanomaterials (MNMs) has lain in assessing the fate, transport and toxic properties of pristine (i.e. as manufactured) materials (Handy et al., 2008; Klaine et al., 2012). Because environmental systems are dynamic and the surfaces of MNMs are highly reactive, physiochemical changes to engineered or incidental coatings and subsequent reactions will greatly complicate how the particle(s) behave (Lowry et al., 2012). This is also true for MNMs that are released from products, where the same particle may have different transformation by-products depending on its use or purpose (Nowack et al., 2012; Mitrano et al., 2015). Exemplifying this is the case of Ag MNMs used in textiles, whereby depending on material design and external impacts to the textile, MNMs may be released.

Nanomaterial life cycles are strongly dependent on the life-cycle of the nano-enhanced products they are embedded in. Nanomaterials embedded in textiles would transform after exposure to oxidants and detergents during washing. Using a carefully chosen set of liquid and powdered commercially available detergents that span a wide range of different chemistries, washing studies were preformed with one "standard" nanoparticle suspended in wash solution to systematically investigate (changes to) particle size distribution, dissolution, re-precipitation (i.e. "new" particle formation) and complexation of manufactured nanomaterials to particulate matter.

2.1.2. Materials

Ionic and Nanoparticulate Standards

A silver nitrate solution was obtained as 1000 mg/L in 0.5 M HNO_3 (Merck) along with a gold solution of 1000 mg/L in 0.5 M HCl, and diluted accordingly for use as experimental positive controls (Ag only) and instrument calibration solutions, respectively, ranging from 0 to 5 μg/L. Nominal 100 nm metallic Ag MNMs (Sigma Aldrich, stock concentration 20 mg/L) were used for the majority of the work presented here. 60 or 100 nm Au MNMs (NanoComposix, stock concentration 20 mg/L) were used as inert "tracers" to ensure the various solution chemistries of the washing liquids/powders did not change the transport efficiency, ICP-MS sensitivity, or other metrics that may have influenced particle analysis by spICP-MS. In selected experiments, an Au/Ag core/shell particle was used in lieu of the standard particle. Provided by NanoComposix, the Au core was 48.2 nm in diameter with a silver shell of approximately 15 nm for a total diameter of 82.7 nm (particle diameter provided by NanoComposix using TEM). When analyzed by spICP-MS, the total mass of Ag equates to approximately a 70 nm metallic Ag particle, where a decrease in calculated "diameter" can be used as a proxy for dissolution of Ag from the shell of the particles.

Laundry Detergent Solutions

Seven total washing solutions were investigated over the course of this study. Five are "grocery store brand" detergents from a Swiss grocery store and as such are commercially available and intended for use in private homes. Two liquid detergents ("color" and "all purpose") and three powder detergents ("color", "all purpose", and "oxi") were chosen. Additionally, two detergents (one liquid, one powder) were acquired from a company producing detergents for industrial-scale laundering, e.g. for hospitals, nursing homes, restaurants, etc. (labeled industrial detergent hereafter). The detergents are distinguished by the presence/absence of oxidizer, the presence /absence of particulate matter and the pH value.

All detergents were used at 4 g/l concentration diluted in DI water, or in a few cases tap water. The pH was measured in all solutions using a Metrohm 827 pH meter. All but one of the washing liquids had a pH value between 8.9 and 11.5, except for the commercial liquid detergent measuring 3.1 (Table 1).

Chloride is an important ion that leads to the precipitation of AgCl and so was measured in each wash solution by ion chromatography of the raw (settled) solution. A Metrohm 733 ion chromatograph equipped with a MetroSep A Supp 5 column was used. None of the washing liquids or powders specified any chloride salt was added, but in previous studies had been present as an impurity (Mitrano et al., 2014b). The concentrations here were in the range from 0.5 to 5 mg/l, except for the commercial powder detergent, which was nearly 12 mg/L (Table 3). In the single experiment conducted at the Colorado School of Mines in Golden, CO, USA, tap water was collected from the laboratory, which contains 1 mg/L residual free chlorine, to make the wash solutions.

Table 3: Measured pH and chloride levels of the washing solutions.

Washing Solution Name	**pH**	**Chloride (mg/L)**
Grocery Store Liquid Color	9.3	0.5
Grocery Store All Purpose	9.2	0.5
Grocery Store Powder Color	11.4	4.4
Grocery Store Powder All Purpose	10.1	3.3
Grocery Store Powder Oxi	10.9	4.5
Industrial Powder	8.9	11.9
Industrial Liquid	3.1	1.2

2.1.3. Methods

Simulated Washing Procedures

In an abbreviated version of washing procedures from previously published literature (Geranio et al., 2009; Mitrano et al., 2014b), simulated washing experiments were conducted where each detergent was made up in 18 m-ohm DI water (except where noted) at 4 g/L and the washing medium was kept at a constant temperature of 40 ± 2 °C, controlled by a thermostat. Each replicate was made in 15 mL polypropylene tubes (sample volume of 10 mL) with regular end over end agitation throughout the 45 minute wash cycle. Initial particle concentration in the wash solutions was 80 μg/L for MNMs (Ag and Au) and 250 μg/L for Ag^+ (added as $AgNO_3$), which was further diluted to 100 ng/L for spICP-MS analysis. The concentration of MNMs in solution was chosen as a balance between representing the realistic concentration of Ag which is released from washing a silver-enhanced textile in one wash from our previous studies (Geranio et al., 2009; Mitrano et al., 2014b), and being conservative with the amount of material needing to be purchased for the study. The higher Ag^+ concentration was a more realistic total Ag release level, which could influence the formation of particulate matter and/or the amount of Ag^+ that could adhere to any remaining undissolved solids in the washing solutions.

In each washing solution, total Ag measurements in addition to ultrafiltration (VIVASPIN centrifugal filters, 10 kDa cutoff) Ag analysis of the samples were made by ICP-MS, with samples acidified to 2% HNO_3. Additionally, aliquots were taken for spICP-MS analysis before and after the wash cycle. In studies designed to represent extended aging of the particles after the washing cycle, the same particle suspension was analyzed on day 1, day 2, and day 5, with samples stored in the dark in the interim.

Filtration Studies

In an effort to vet the spICP-MS technique over another standard practice of serial filtration with subsequent total metals analysis to determine size fractions, we used 60 nm Au MNMs at the same concentration and volume as in the simulated washing tests (80 μg/L; 10 mL), diluted appropriately for spICP-MS, and determined the amount of Au caught by the filters. We used Au MNMs as opposed to Ag to ensure we were only testing physical mechanisms opposed to possible chemical changes that may additionally change Ag suspensions in these solutions (e.g. dissolution, surface changes, etc.). The tests were completed in a variety of solutions including DI water, liquid and powder detergents to investigate if solution chemistry or form plays a significant role in the efficacy of filtration. Finally, we also tested if larger sample volumes would increase the proportion of particles passing the nominal size of the filter in relation to our previous washing studies, where 70 mL of solution were passed through one filter. Particle size fractionation in the wash solution was accomplished through serial 0.45 μm and 0.1 μm filtrations (cellulose nitrate filters, Sartorius Stedim). The Au MNM profile was determined by spICP-MS for the unfiltered, 0.45 μm and 0.1 μm filtrates with the same dilution factors, which aimed to measure the unfiltered sample at 50 ng/L.

Single particle (sp)ICP-MS Measurements

Particle concentrations and size before and after laundering was measured using single particle ICP-MS as described according to published methods (Laborda et al., 2011; Mitrano et al., 2012; Mitrano et al., 2014a). The fundamental assumption behind spICP-MS is that under sufficiently short dwell time and low particle number concentration, a pulse will represent a single particle event. The number of pulses can be directly correlated to the n umber concentration of particles (particle number per volume) and the intensity of the pulse (i.e. height) can be related to the particle size through particle mass, by making assumptions about particle geometry. Converting pulse height to particle diameter hinges on the calculation of an efficiency factor, that can be measured using a standard, well-characterized metal nanoparticle. In this current study, the Ag and Au content and spICP-MS measurements of the majority of experiments was performed on an Agilent ICP-MS. Here, 5 ms dwell times were used to capture ion plume events as pulses with a reduced settling time of 100 ms seconds. When the Perkin Elmer NexION 300Q ICP-MS, equipped with a Type-C Miramist nebulizer and baffled cyclonic spray chamber was used, and a dwell time of 3 ms was chosen with settling time of 100 ms. In both cases, operating conditions were optimized to produce maximum ^{107}Ag intensity and a data collection of 120 s was used. Instrument calibration utilized a blank and four dissolved Ag solutions made in 2% HNO_3 (0-1 μg/L), with data collected in spICP-MS mode. 100 nm Au NPs (and associated dissolved Au calibration curve ranging in concentrations from 0 -1 μg/L) was to determine transport efficiency on a daily basis. To monitor instrumental drift over time, a single 100 ng/L Ag dissolved calibration check standard was analyzed in spICP-MS mode after approximately every ten MNM samples. If drift in the standard signal was detected, the particle sizing equation was adjusted accordingly for the decrease in sensitivity.

Scanning Transmission Electron Microscopy (STEM) and EDX Analysis

Pristine nanomaterials were drop deposited onto carbon coated Cu TEM grids. Washing solution(s) that contained nanomaterials were centrifuged under gentle conditions (500 rpm for 4 min) to settle large undissolved solids from the washing powder. The supernatant was transferred to new vials where particles were deposited directly onto TEM grids by centrifugation using a swinging bucket rotor, with rotation speed of 4500 for 2 hours. Under the applied conditions, deposition of metallic Ag particles (density 10.1 g/cm^3) >10 nm would be completely deposited on the grid. All samples used for microscopic analysis were processed within 24 hours of preparation.

Particle images were obtained *via* scanning transmission electron microscopy (STEM) combined with EDX for element detection using a JOEL JEM 220 fs operated at 200 kV. The nominal spot size of the STEM probe was 0.7 nm using a beam convergence angle of 10.8 mrad. High-angle annular dark-field STEM micrographs were recorded using an inner detector angle of 100 mrad, while the bright-field STEM images were recorded with a detector angle of approximately 15 mrad. EDX spectra of individual particles were recorded either by positioning the electron probe on a selected particle or by scanning the electron probe on a small frame centered on the particle.

Sequential Aging: UV Ageing followed by simulated washing

Particles were acquired from the CEA following aging protocols described in section 1.2. Sequential particle aging was performed where the previously UV aged particles were suspended in one of two types of laundry detergent: grocery detergent color or grocery detergent oxi. Subsequent simulated washing procedures were performed as described in Section 3.1 with TEM analysis of the particles and spICP-MS analysis of these solutions as described in Section 3.4.

2.2. CeO_2 ageing with NOM (UoGen)

2.2.1. Introduction

ENMs undergo different types of transformations and aging processes when they are released into the environment. Natural processes such as dilution, transformation caused by the presence of dissolved electrolytes, suspended matter etc. lead to the changes of nanomaterial properties. One of the components that dramatically influence the fate of nanoparticles is natural organic matter (NOM). In this set of aging experiments, we used fulvic acids (FAs) as a surrogate for NOM and we investigated the fate and behavior of CeO_2 nanoparticles coated with FAs complexes over time.

2.2.2. Materials

Engineered nanoparticles

Uncoated CeO_2 NMNs as a powder from the JRC Nanomaterials repository NM-212 were used. The primary particle size is equal to 28.4 ± 10.4 nm and specific surface area 27.8 ± 1.5 m^2/g (XRD and BET methods from Singh et al., 2014).

NOM

The Suwannee River Fulvic acids (Standard II, 2S101F) as a powder were purchased from International Humic Substance Society, USA.

Chemicals

Ultrapure water (Milli Q water, Millipore, Switzerland, with R > 18MOhm, TOC < 2ppb). HCl and NaOH (Merck, Germany), Titrisol®, concentration 1M, 0.1M, 0.01M, were used for pH adjustment. NaCl, powder 99.5%, (Acros Organics, Switzerland).

2.2.3. Methods

Scanning electron microscope (SEM) images

Uncoated and FAs coated CeO_2 MNMs were imaged with a JEOL JSM-7001FA scanning electron microscope (SEM). For each samples 10 µL of MNMs suspension was placed on one aluminum stub covered with 5×5 mm silica wafer Agar Scientific (G3390) and wrapped with 3 – 5 nm of gold or platinum coating.

Dynamic light scattering (DLS)

Zeta potential and z-average hydrodynamic diameter were measured using laser Doppler technique and dynamic light scattering method respectively using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK). The autocorrelation function accumulated ten runs for each samples for five parallel measurements with time delay of 5 seconds. The samples temperature was 298°K. First, electrophoretic mobility was measured and then Smoluchowsky approximation (Gregory, 2005) was used to calculate zeta potential.

Nanoparticle tracking analysis (NTA)

Visualization and distribution of particles by size were investigated applying nanoparticle tracking analysis (NTA) with a NanoSight LM14 instrument and NTA 2.3 Analytical Software (NanoSight Ltd, UK). The NTA LM14 consists of a nanoparticle viewing unit with an integrated blue laser illumination (wave length of 405 nm), CMOS high sensitivity camera and integrated temperature control unit. The device analyses the particle paths under Brownian motion and determines the average distance moved by each particle in *x* and *y* direction. This value allows obtaining the diffusion coefficient and using the Stokes-Einstein equation to calculate the sphere-equivalent, hydrodynamic diameter (Carr et al., 2006; Hole et al., 2013).

2.2.4. Procedures

Preparation of CeO_2 MNMs suspension

First, we prepared the initial CeO_2 suspension at 1 g/L and then we diluted it to obtain the suspension with appropriate concentration (50 mg/L) for further experiments. The pH of suspension was adjusted to acidic conditions and corresponded to pH 3.0 ± 0.1, which avoided nanoparticle aggregation during the storage and dilution process.

50 mg of CeO_2 powder was weighed in a 50 ml tube. The powder was hydrated with previously prepared ultrapure acid water in order to create a thick paste. Next, the tube was filled with ultrapure acid water to 50 ml and the suspension was mixed. The prepared suspension was sonicated with a microtip. The microtip was positioned in a way where it was in the suspension, but did not touch the bottom of the sample tube. During this process, the tube was put into a beaker filled with cool water to avoid sample overheating. The parameters that were used during the sonication process are presented in Table 4.

Table 4. Parameters of sonication process

Parameter	Value
Power of ultrasonic processor	130 Watt
Resonance frequency of probe	20 kHz
Amplitude	75 %
Sonication time	15 min

The sample was allowed to settle over the course of one hour and then diluted suspensions (50 mg/L) were prepared from the aged stock (1 g/L). Dilutions were made using ultrapure acid or basic water depending on the desired pH of suspension. Ionic strength was adjusted with diluted NaCl solution to obtain final concentration of NaCl 10^{-3} M. pH was adjusted by adding diluted HCl and NaOH. To develop this protocol we used the dispersion protocol provided by JRC and different sources of literature (Singh et al., 2011; Rasmussen et al., 2013; Taurozzi et al., 2013; Singh et al., 2014).

The sample was stored in the fridge at a temperature of approximately 4 °C. Thereafter, samples were sonicated in a water bath prior to use.

Preparation of FAs solution

1 g/L of Suwannee River FAs solution was prepared and diluted to 100 mg/L. This solution was the stock solution from which we made our further dilutions. The stock was made by weighing 10 mg of FAs powder in a 10 ml flask and filled with ultrapure water. The sample was allowed to settle over the course of one hour, then diluted suspensions were prepared. All solutions were maintained in a dark, cold place with temperature ranging between 0 and 4 °C.

2.2.5. Aging procedure

Time stability

The stability of FAs-CeO_2 complexes were investigated during a time period up to 7 weeks. The final concentration and component characteristics of the solution was 50 mg/L of CeO_2 NPs and 2 mg/L of FAs with a final suspension pH equal to 3.0 ± 0.1. The pH of the suspension was controlled each week during all experiments and measurements of zeta potential and z-average hydrodynamic diameters.

pH variation stability

For experiments of stability versus pH, a 50 mg/L suspension of CeO_2 with 2 mg/L FAs was prepared at pH 3.0 ± 0.1. After 2 hours of equilibration we sequentially increased the suspension pH from 3.0 to 10.0 at 0.5 intervals.

Dilution impact

The influence of suspension stability versus decrease of varying component concentration was investigated at two different pH values that corresponded to pH 3.0 ± 0.1 and pH 8.0 ± 0.1. First, a suspension with component concentrations of 100 mg/L CeO_2 NPs and 4 mg/L FAs was prepared. The solution was then diluted by factors of 2, 4, 10, and 20 times. The corresponding concentrations are provided in Table 5.

Table 5. Component concentration in dilution experiments

Component	**Component concentration, mg/L**				
Dilution factor	1:1	1:2	1:4	1:10	1:20
CeO_2	100	50	25	10	5
Fas	4	2	1	0.4	0.2

Influence of the increase of ionic strength on stability

We evaluated the stability of nanoparticles coated with FAs with increasing ionic strength (NaCl as electrolyte). The experiment was conducted at pH 8.0 ± 0.1, corresponding to environmentally relevant conditions. We gradually increased the concentration of NaCl added to the FAs-CeO_2 suspensions. The corresponding values of NaCl concentrations used were: 0.05, 0.1, 0.15, 0.175, 0.2, 0.225, 0.25, 0.3, 0.35, 0.4, 0.5, 0.6 and 0.75 M. The concentration of CeO_2 NPs and FAs was the same for all samples and corresponded to 50 mg/L and 2 mg/L respectively. Over 60 min, the z-average hydrodynamic diameter was measured by DLS method at time intervals of 30 seconds.

It should be noted that the order components were added to the solution was important to successfully disperse the NPs and avoid aggregation. First, the relevant volume of FAs was added into a tube and then was diluted with ultrapure water. Next, we adjusted the pH and added the appropriate concentration of CeO_2 NPs. The last step was addition of the relevant concentrations of NaCl. Measurement of z-average hydrodynamic diameter was done directly after mixing all components.

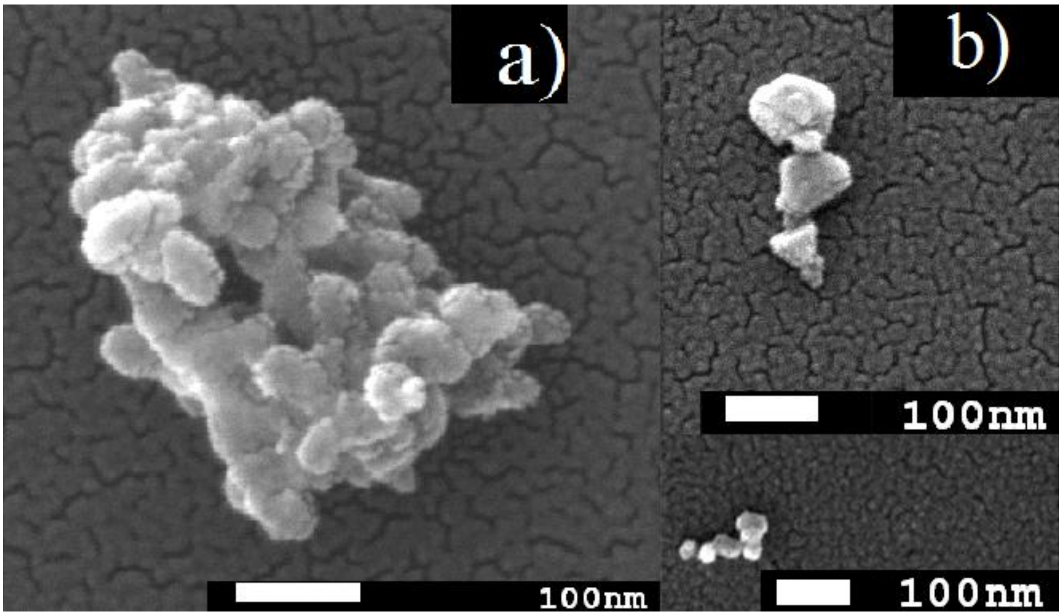


Figure 3: SEM images of cerium (IV) oxide NPs coated with FAs (a) and pristine (b) particles.

Part B. Protocols for aging NanoMILE particles

In this section the protocols are described that are used to age the MNM listed in Milestone 6. These aged particles will then be distributed to the NanoMILE partners that requested them. This deliverable contains only the description of the aging procedure, the characterization of the aged materials will be presented in Deliverable 3.3 (due month 36).
These MNMs were chosen (see Milestone MS5 “Selection of MNMs for ageing studies with reference to needs of WPs 5-8“) so that they include MNMs from the different groups that were selected in WP2 and different aging processes: CeO_2, Ag, TiO_2 and ZnO. The aging reactions were based on D3.1 “Report on environmental transformation reactions” where important aging reactions for the NanoMILE particles were reviewed. These particles were chosen because they are either known to undergo transformation reactions or were identified in D 3.1 as relevant from a life-cycle point of view.

Table 6: List of aging reactions for the selected MNM

MNM	**Aging reaction**	**Form delivered**	**Comments**
CeO_2	Phosphatization	Solution	$CePO_4$ is much more stable than CeO_2 and is therefore formed in presence of dissolved phosphate, e.g. in soils or wastewater
Ag	Sulfidation	Solution	Sulfidation is the most important transformation reaction for Ag, many reference papers are available
TiO_2	UV exposure in air	Powder	The UV light will not affect the TiO_2 but the organic coating on the surface
ZnO	UV exposure in air	Powder	The UV light will not affect the ZnO but the organic coating on the surface

3.1. Aging in air: TiO_2 and ZnO (CEA)

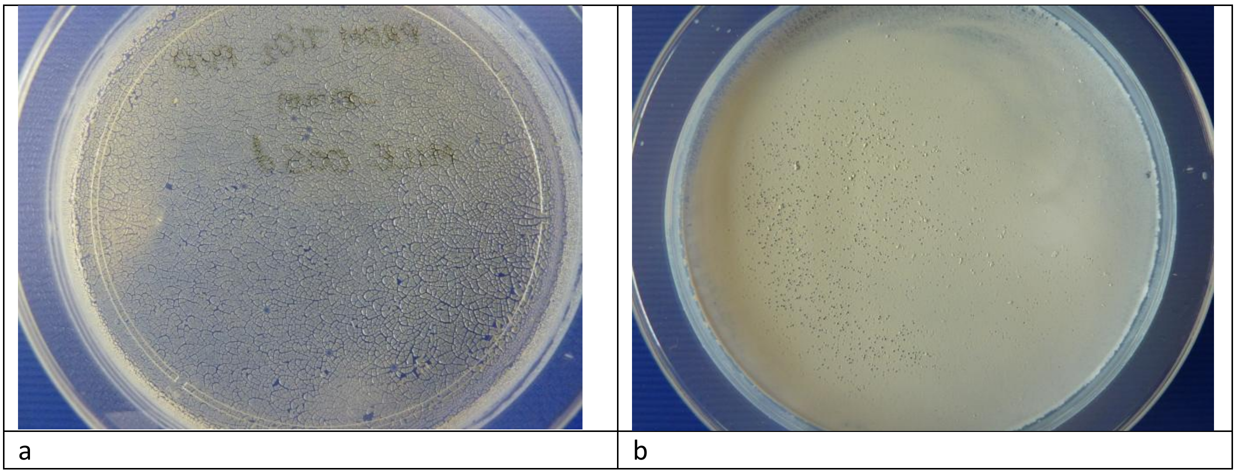
UV exposure in air was chosen as the aging process for TiO_2 and ZnO MNMs to be delivered to the partners in WP 4-9. Table 7 summarizes the conditions of the aging protocols applied to each type of NP.

Table 7: Particles to be aged for other partners.

Particle name	Amount	Batch
TiO_2 UNi	10mL 1.7%	121113i
TiO_2 AA4040	10mL 2%	121113h
TiO_2 F127	10mL 1.7%	121113h
TiO_2 PVP	10mL 2% 210mL 1.05%	121113i MILE005d
ZnO NM110	2g (powder)	0589b, 0801b, 1301a
ZnO NM111	1g (powder)	2062a, 2854d, 2939b

The MNMs that were received in powder form (ZnO) were dispersed in ultra-pure water (NM110) or in ethanol (NM111) at a concentration of 20 g/L (metal basis). TiO_2 suspensions were used as received, without dilution (5 to 10 g/L).

Aliquots of the suspensions (30 mL for TiO_2 and 15 mL for ZnO) were sampled and deposited in polystyrene Petri dishes (Figure 4), in order to form a uniform layer. The dishes were then placed into a thermal chamber to let the samples dry at 80°C. Finally, the residues were transferred into the QUV (Figure 5).



a b

Figure 4: Sample preparation prior to UV exposure in the QUV climatic tester. Dried sample of a: TiO_2 PVP and b: ZnO NM110.

Figure 5: Position of the samples in the QUV tester.

The aging process was run for 42 days according to the experimental conditions described in Table 1 for the QUV tester. Once the aging test came to completion, the samples were withdrawn from the chamber. Depending on the partner request, samples were either re-dispersed in water using ultrasonication (5-30 min) or left in the powder form.

3.2. Ag MNM Sulfidation (Empa)

Suflidized Ag MNMs were provided to other NanoMILE partners and prepared according to an amalgam of several previously published methods (Levard et al., 2011; Liu et al., 2011; Reinsch et al., 2012; Devi et al., 2015; Starnes et al., 2015). The Ag-MNMs (NM300K, size 20 nm and stock concentration 1 mg/mL) were sulfidized using a sodium sulfide (Na_2S) solution of approximately 10^{-3} M in a 0.1 M $NaNO_3$ electrolyte. Stock Ag particles were diluted with the sulfide mixture to their final concentration, where the ratio of Ag to S was fixed to produce the maximum amount of Ag_2S, where the HS^-/Ag ratio was prepared at a ratio of minimally 2 (Reinsch et al., 2012). The aim was to achieve complete sulfidation of the silver by an excess of sulfide over Ag. After the mixture was allowed 48 hours to react under gentle shaking (20 rpm), the solution was washed by ultracentrifugation three times with DI water to remove excess sulfide and were stored and shipped in DI water. The final silver content/concentration was analyzed by ICP-MS. Sulfidation of the particles was verified by confirming the particles were Ag/S with TEM and EDX analysis. Light-blocking PE plastic containers were used throughout the experiment and storage to minimize photocatalytic reactions of the silver. Partners were provided with suspensions of Ag/S material and analysis on the (new) particle distribution and chemistry.

3.3. Aging of CeO_2 MNMs with phosphate (UoB)

The used protocol was based on trials and modifications of a published method (Zhang et al., 2012). For aging of the NanoMILE ceria particles, it was decided that the particles would be added to a solution of 5 mM of KH_2PO_4, citric acid and ascorbic acid, 5 times the concentration used in the original publication to induce a faster and more complete transformation of the CeO_2 MNMs into $CePO_4$ by providing a molar excess of phosphate over Ce and thus improving the kinetics of transformation. The particles used for ageing were Promethean CeO_2 (see Table 2).

The final concentrations of CeO_2 MNMs in the suspensions were 496 (used for TEM, UV-vis and zeta potential) and 6200 mg/L (used for XRD). After 7 and 21 days of static incubation, the lesser concentrated CeO_2 sample (496 mg/L) was suitable to be used for TEM observations (JEOL 1200X) while the more concentrated (6200 mg/L) suspensions were dried in a 50°C oven for three days and measured by XRD, using a Powder Diffractometer (Bruker D8 Autosampler), before delivered to other partners for further work. The analyses established that transformation of ceria to a phosphate phase was complete within the analytical detection limits of the characterization methods.

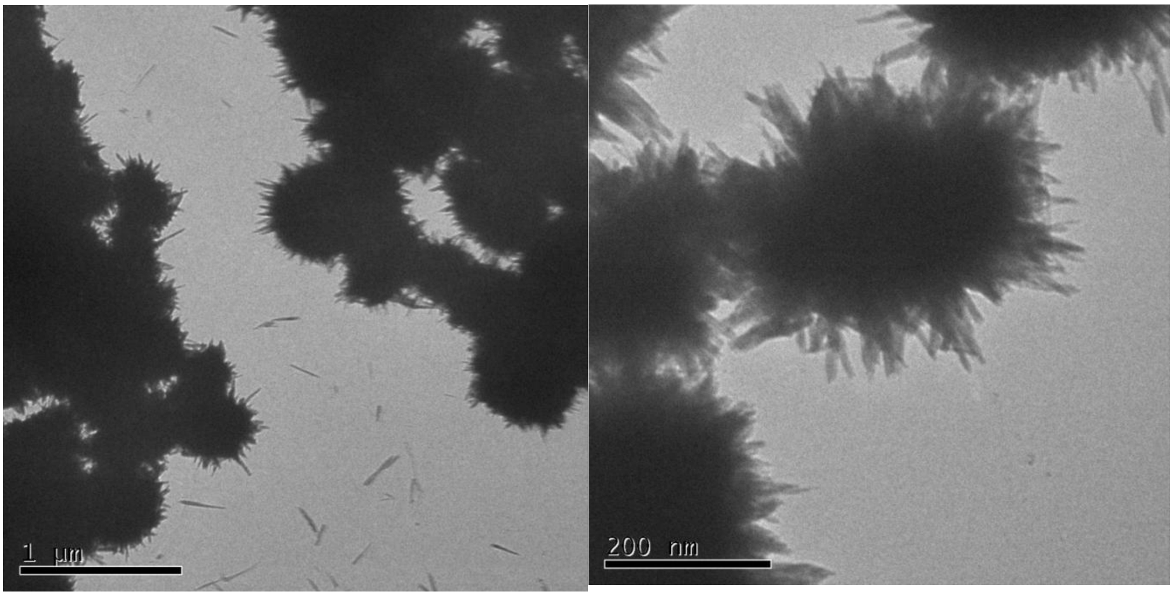


Figure 6. TEM images obtained for the $CePO_4$ nanoparticles following aging of CeO_2.

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