SOP

Preparing suspensions of nanomaterials in serum-containing medium

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Version
2.0 English

1. Initial Considerations

This document is intended to provide information as to the methods developed and currently used for the preparation of suspensions for biological testing within the NanoGEM project. The described methods are not meant to be comprehensive, but rather should provide the opportunity to prepare suspensions in a manner consistent with that performed within the NanoGEM project. This should allow improved reproducibility of experimental results as well as easier comparison between different studies.
It should be recognized that there may be certain modifications required to give considerations to the diversity of nanoscale material. Therefore, the reader needs to carefully examine the procedures to determine if they are adequate for the selected test material. Please consult the NanoGEM Web site at: http://www.nanogem.de/ to make sure that you have the latest version of this document.

2. Preparing Suspensions for Toxicological Tests

2.1 Preparing the Stock Suspension

The original test suspensions in NanoGEM are delivered with particle concentrations specified as % mass of solid content per total mass of suspension (% weight of NP / total weight). The particle concentration typically is between x = 10 and 40 % [g/g].

The concentration of stock suspensions shall be specified as mass of solid content per total volume of suspension (weight of NP / total volume), and should not exceed 10 mg/ml.

This specification (weight of NP / total volume) allows handling of the diluted dispersions according to established protocols (e.g. SOPs NanoCare).

Dilution procedure:

Before preparing stock suspensions, the original test supensions always have to be shaken and mixed for 2 min using a vortex mixer to ensure a homogeneous distribution of particles.

First, calculate the total weight of particles needed to prepare the desired stock suspension (e.g. a 6 ml stock suspension of 0.01 g/ml would need 0.06 g of NP).
Subsequently, evaluate the weight of original test suspension containing this amount of particles (e.g. 0.06 g NP are contained in 0.6 g of a 10%-test suspension).

The required amount of original test suspension can be weighted in using a 12 ml glass bottle (Wheaton 225535) containing a magnetic stir bar.

Alternatively, for reasons of sterile and easy handling, the required amount of particles can be handled by pipetting a calculated volume* of the original test suspension:

volume of test suspension [ml] = required weight of test suspension [g] / density of delivered suspension [g/ml]

The density of the delivered suspension will be indicated in the specific suspension data sheet, and its relation to the specific density of the nanomaterial is given for reference in the appendix.

After having transferrred the test suspension into the recommended glass bottle, the dilution is performed by adding the test medium up to the intended final volume.

The stock suspension is stirred for 24 hours, lid closed, at 700 rpm and room temperature e.g. on a IKA RO5P bzw. RO10P. These suspensions can be used as-is or after further dilution.

2.2 Preparing the Diluted Working Suspensions

Diluted suspensions are prepared from the stock suspensions (particle concentration specified as % weight of NP / total volume).

Dilution procedure:

Define the dilution factor from the stock suspension to achieve the desired particle concentration. The calculated volume of the stock suspension is pipetted to the - already stirring - volume of test media, together giving the final volume of the dilution.

The diluted working suspensions will be stirred for at least 1 hour, before further use.

2.3 Preparing Suspensions from Powder-Delivered Nanomaterials

For powder-delivered nanomaterials it has been demostrated by further tests that a 24h-procedure for stirring the stock suspension and subsequent 1h-procedure for stirring the diluted working suspension is compatible with the previously used NanoCare protocol (1h + 24h stirring)

Therefore, for reasons of parallel handling of test suspensions and control suspensions (delivered as powder), the NanoCare SOP for preparing suspensions can be adapted to a (24h + 1h) procedure to prepare suspensions from powder-delivered particles in the NanoGEM project.

2.4 Required Materials

Recommended sources for glasses are Wheaton 225535, 12 ml bottles (diameter approx. 22 mm, height 51 mm) delivered with closed cap, via NeoLab Art.-Nr. 9-0222. The respective test medium is used to prepare the stock suspensions. An appropriate magnetic stir bar (15 x 5 mm) is added, and has to be taken into account during determination of weight and volume.

3. Options for Validation of Locally Produced Suspensions

3.1 Photo Documentation

As minimal characterization, the suspensions (stock suspension and dilutions) to be located in a snap-on lid glass are photographed in front of a suitable background, while stirring. This helps to identify the extreme of flocculated preparations or of preparation where the nanomaterial sticks to the walls of the beaker, but it does not allow conclusions on the actual size distribution.

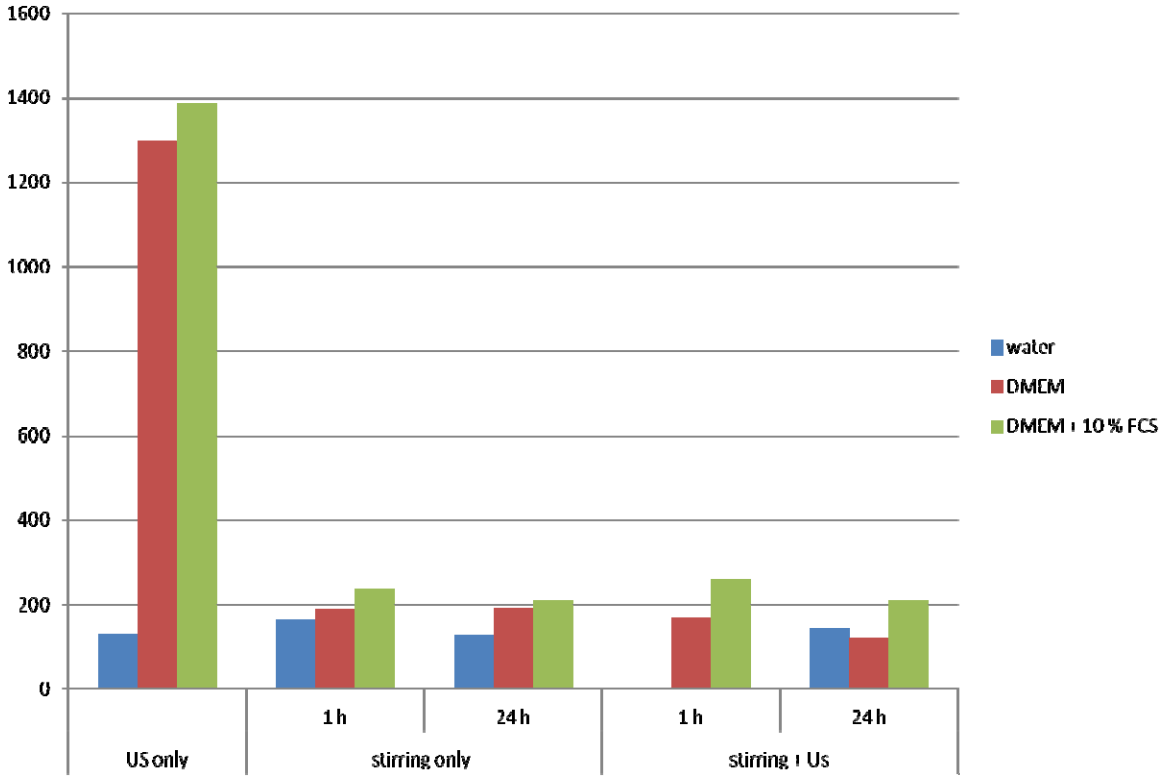
3.2 Characterization of State of Agglomeration

NanoGEM recommends complementary methods for characterization: Single-particle-based (here: PTA), scattering-based (here: DLS), fractionation-based (here: AUC).

Appendix: Benchmark results

The following results as benchmarks were obtained with the above described protocol, starting from the material NanoGEM_SiO2_naked_2010.10.18 dispersed in DMEM + 10% FCS.

Particle Tracking Analysis (PTA, NanoSight)

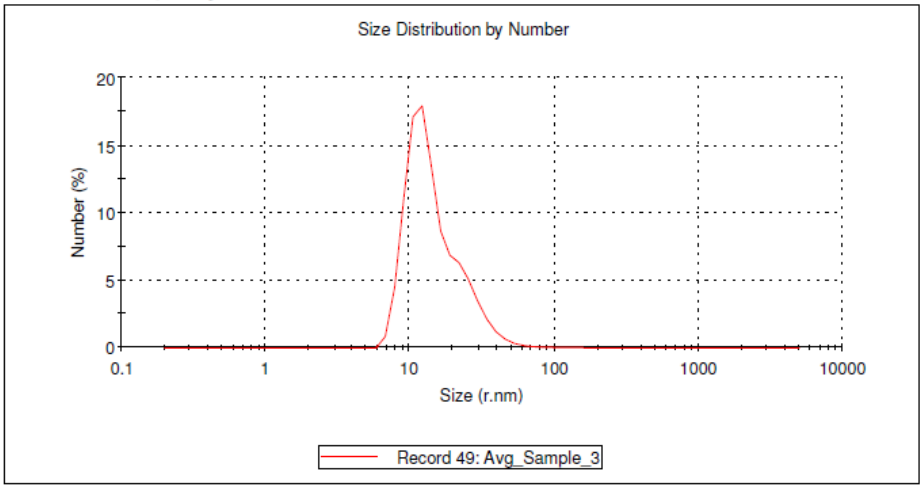


Dynamic Light Scattering, DLS.

Presentation by particle number after Mie-correction of scattering intensities (suppressing agglomerates) (The polydispersity index PDI is larger than 0.2; this indicates that the shape of the distribution cannot be trusted)

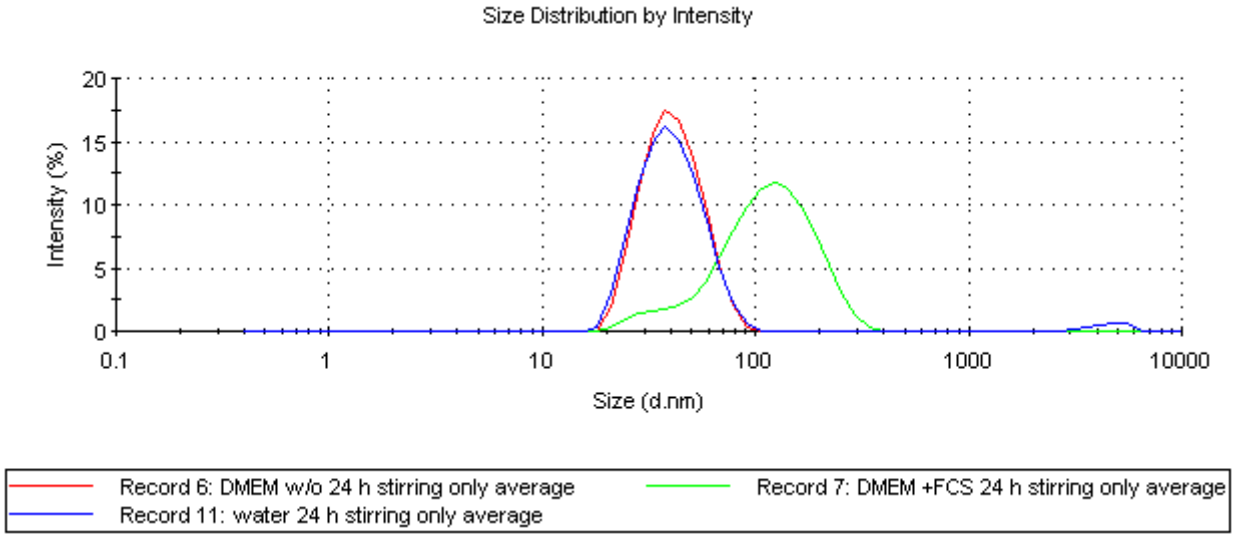
		Diam. (nm)	% Number	Width (nm)
Z-Average (r.nm): 67.22	Peak 1:	15.74	100.0	8.871
PdI: 0.364	Peak 2:	0.000	0.0	0.000
Intercept: 0.972	Peak 3:	0.000	0.0	0.000

Result quality Good

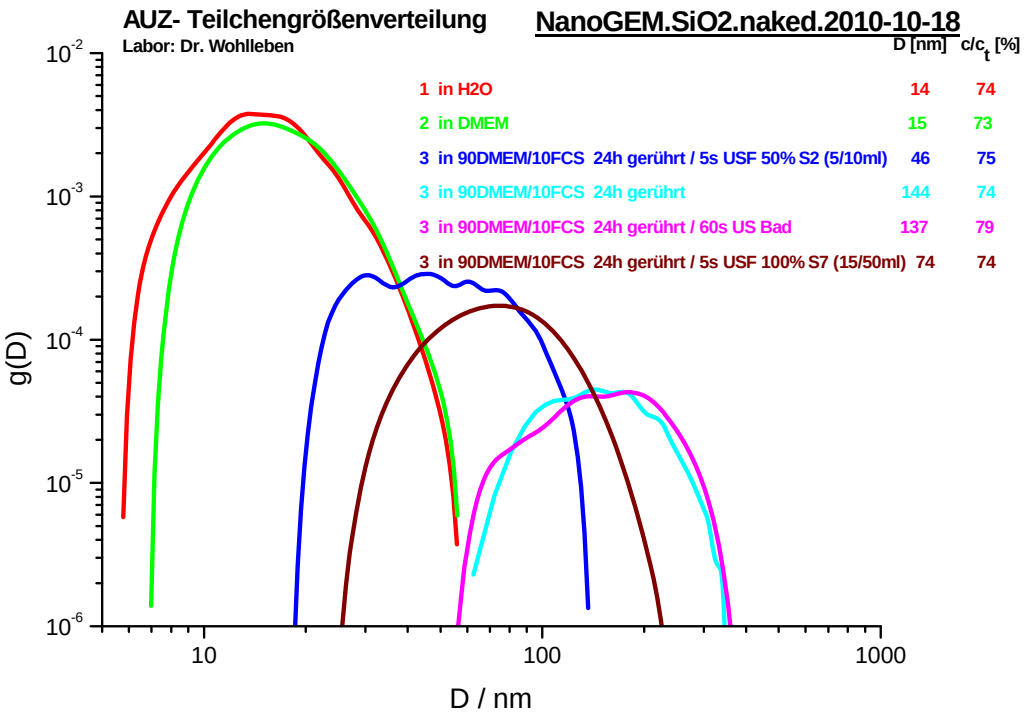


Dynamic Light Scattering, DLS

Presentation by scattering intensity (less theory involved, but suppresses dispersed primary particles)



Analytical Ultracentrifugation, AUC
(Beckman XLI)
Presentation as mass distribution (directly from interference detector).



Further test where performed to verify that there the current protocol (24h + 1h stirring, focused on suspension-delivered nanomaterials) is compatible with the previously used NanoCare protocol (1h + 24h stirring, focused on powder-delivered nanomaterials). The experiments were performed in DMEM / 10% FCS with the four reference nanomaterials from NanoCare, and quantified by AUC. There are differences between the dispersion results form the two SOPs, but these remain on the qualitative level. The mean diameters of the agglomerates are up for two, and down for the two other materials. The dispersed fraction increases for the 24h+1h protocol (nanoGEM) significantly for the TiO2 – which is especially dificult to disperse.

(Results table on next page)

We thus recommend to use identical stirring times 24h + 1h for materials irrespective of their form of delivery as suspension or as powder.

TiO2			
Rühren der			
Stammkonzentration	Messkonzentration	D_{50} [nm]	c/c_t [%]
1h	24h	1470	27
24h	1h	1770	45

ZnO			
Rühren der			
Stammkonzentration	Messkonzentration	D_{50} [nm]	c/c_t [%]
1h	24h	2600	60
24h	1h	2140	40

BaS04			
Rühren der			
Stammkonzentration	Messkonzentration	D_{50} [nm]	c/c_t [%]
1h	24h	1220	105
24h	1h	850	62

Böhmit-1			
Rühren der			
Stammkonzentration	Messkonzentration	D_{50} [nm]	c/c_t [%]
1h	24h	571	58
24h	1h	827	53

Appendix: Formulas

The density of the delivered suspension ρ is related to the specific density of the nanomaterial ρ_{NP} by the weight concentration x:

$$\rho(x) = \frac{1}{\frac{1-x}{\rho_{H2O}} + \frac{x}{\rho_{NP}}}$$

Alternatively, one can transform the weight concentration x indicated on the data sheets of the delivered suspensions into a weight / volume concentration y:

$$y^{-1} = \rho_{NP}{}^{-1} - \rho_{H2O}{}^{-1} + (x \cdot \rho_{H2O})^{-1} \cong \rho_{NP}{}^{-1} - 1 + x^{-1}$$

Specific results for the nanoGEM naterials as delivered are included below.

Core	SiO2		ZrO2	Ag
density [g/cm3]	2,3	2,3	5,8	10
x [g/g]	40%	20%	10%	25%
y [mg/ml]	517	225	109	323