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Standard Operating Procedure

Title	***PROTOCOL FOR PARTICLE SIZE DETERMINATION OF A GIVEN MNM BY THE CENTRIFUGE LIQUID SEDIMENTATION (CLS) TECHNIQUE***
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PROTOCOL FOR PARTICLE SIZE DETERMINATION OF A GIVEN MNM BY THE CENTRIFUGE LIQUID SEDIMENTATION (CLS) TECHNIQUE

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1 OBJECTIVE AND SCOPE

1.1 Objective

To develop a Standard Operating Procedure (SOP) for Centrifugal Liquid Sedimentation (CLS) on the determination of the particle size distribution (PSD) of MNM.

1.2 Scope

This SOP development for CLS is done in the framework of the NANoREG project WP2, Task 2.4f and Deliverable 2.8. This SOP applies only for the CPS Instruments equipment.

2 ABBREVIATIONS

CLS	: Centrifugal Liquid Sedimentation
EC	: European Commission
Nanomaterial	: A material with at least one dimension in the range of 1 to 100 nm
MNM	: Manufactures Nanomaterial
PSD	: Particle Size Distribution
SOP	: Standard Operating Procedure

3 PRINCIPLE

Centrifugal Liquid Sedimentation (CLS), is a centrifugal method based on the settling rate of particles in a liquid under a centrifugal field. At low Reynolds numbers the particle size in relation to the settling velocity is dictated by Stokes' law. The Stokes diameter D is determined as a function of time t:

$$D = \sqrt{\frac{18\eta \ln\left(R_f / R_0\right)}{\left(\rho_p - \rho_f\right)\omega^2 t}} \qquad \text{(Eq. 1)}$$

where η is the fluid viscosity, R_f the measurement radius, R_0 the starting radius, ρ_p the particle density, ρ_f the fluid density, and ω the rotational speed. All these parameters are constants for a specific measurement.

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4 METHOD

This is an overview of the measurement method: A spinning disk is set at a specific velocity ω. A sucrose gradient is prepared to stabilize the sedimentation. Dodecane is added after the gradient as a buffer layer to prevent streaming, ensuring the injected (nano)particle dispersions a smooth transition into the gradient and laminar flow. A procedure is selected to measure the (nano)particles. A certified calibration standard is used to determine the correct diameter-time relation. Finally, the sample is injected and measured. The concentration of particles in the injected volume should ideally be < 0.25 % (m/v). The measured size range is variable and depends on the speed of the disk and the used gradient, ranging from 5 nm to 40 µm. Other factors, like the difference in particle-fluid density, can affect the minimum measurable size.

5 EQUIPMENT

A. CLS system (from CPS Instruments Inc.)
B. Syringes and needles (Syringes: 1mL Injekt-F sterile for sample and Dodecane injection, BD 3mL syringe for sucrose injection. Needles: BN2015 20 ga for both syringes. A 50 mL syringe and a flexible plastic tube are needed for taking out the sucrose gradient when cleaning the spinning disk.)
C. Deionized water.
D. Sucrose (CAS Number 57-50-1).
E. Dodecane (CAS Number 112-40-3).
F. Calibration standard (Certified PVC microparticles provided by CPS Instruments).
G. Graduated glass beakers.
H. Digital weight balance.
I. Thermometer.
J. Soft tissues (e.g., Tork premium soft tissue).
K. Silica MNM dispersion, diameter: 100 nm.
L. Quality Control, PVC dispersion.

6 SAFETY PRECAUTIONS

6.1 Operator

Always operate and make preparations with a lab coat and gloves. Work on a chemical hood or equivalent ventilation protection environment for any dispersion preparation using a powder MNM (dry) or volatile substances.

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6.2 Instrument

The CLS system must not be used/cleaned with acetone or chlorinated solvents.

7 STANDARD OPERATING PROCEDURE

7.1 Preparing the sucrose solution

8 % and 24 % (m/m) sucrose solutions will be prepared. Two graduated glass beakers properly identified as 8 % sucrose and 24 % sucrose are required. Preparation:

1. 8 % sucrose: Place the 8 % sucrose beaker on the microbalance, making sure it is offset at zero with the beaker. Then add 2 g of sucrose to the beaker, followed by deionized water until a weight of 25 g is obtained.
2. 24 % sucrose: Place the 24 % sucrose beaker on the microbalance, making sure it is offset at zero with the beaker. Then add 6 g of sucrose to the beaker, followed by deionized water until a weight of 25 g is obtained.

Agitate each beaker gently until all the sucrose is dissolved.

6.2 Starting the CLS equipment, injection of gradient and Dodecane

The equipment should be ON, indicated by the red light ON in the right side of the front panel. If the laser has been off, turn it on (black button on the back of the instrument) and the red light ON (right side of the front panel) should appear. No measurement should be performed until the laser has been ON for at least 1 hour.

Place the plastic cap (attached to the cap screwdriver) into the disk, make sure it is well pressed to the disk. The cap has a small hole marker which should be aligned with a similar hole marker made on the disk. Ensure the disk is clean, if not use a soft tissue and water or ethanol to clean the transparent side of the disk (front and back). Be careful not to touch the laser diode. Close the CPS lid.

In the computer run the CPSV95 executable file (or the name of the executable file that controls the CPS equipment). In the Set Point Control section select the option 'Manual' and set the rotation speed to 18000 rpm.

The sucrose gradient is injected with a series of sucrose mixtures:

Table 1. Sucrose mixtures (24 and 8 %) and order for building the sucrose gradient in the disk.

Order	Sucrose 24 %	Sucrose 8 %	Total volume
	mL		

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1	1.6	0	1.6
2	1.4	0.2	1.6
3	1.2	0.4	1.6
4	1.0	0.6	1.6
5	0.8	0.8	1.6
6	0.6	1.0	1.6
7	0.4	1.2	1.6
8	0.2	1.4	1.6
9	0	1.6	1.6

Use a BD 3mL syringe to inject the sucrose mixtures. With each mixture in the syringe, shake the syringe horizontally at least 10 times to ensure a proper mixture. As a first step, inject the first gradient volume (no. 1) to the disk, then press START to begin the disk rotation. Afterwards, when the disk reaches the set rotation speed (18000 rpm) inject the subsequent gradient volumes (no. 2-9). The sucrose gradient is built by injecting progressively to the disk (i.e. starting with 1.6 mL from 24 % sucrose). Each time a total volume of 1.6 mL is injected.

Using a new BD 3mL syringe inject 0.5 mL of Dodecane. Allow 30 minutes for the Dodecane and gradient to stabilize.

6.3 Defining a sample procedure

A procedure is needed for each type of particle being measured. It contains the physical parameters of the particle, the calibration to be used and the gradient. To define a procedure click on the button 'Procedure Definition' from the main menu, opening a window as shown in Figure 1. For silica particles the procedure parameters are described in Table 2. Note that the maximum and minimum diameters have to be adjusted to measure the particles within the selected range. The physical sample parameters (density, refraction index and absorption) are values taken from the literature (bulk material) or provided by the material supplier.

Table 2. Parameters to be input for Silica measurement (example). * Calibration Standard Parameters should correspond to the calibration standard used at each lab.

Procedure	SiO_2
Sample Parameters	
Maximum diameter (µm)	10.0
Minimum diameter (µm)	0.03
Particle density (g/mL)	2.31
Particle refraction index	1.46
Particle absorption (K)	0.001

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Non-sphericity factor	1
Calibration Standard Parameters	
Peak diameter (□m)	0.226 *
Half Height Peak Width (µm)	0.1 *
Particle density (g/mL)	1.385 *
Fluid Parameters	
Fluid density (g/mL)	1.064
Fluid refractive index	1.349
Fluid viscosity (cps)	1.3
Presentation parameters	
Display Mode	Weight
Display Curves	Differential
Y-axis Scaling/Normalization	Height
Show Grids	Horizontal and Vertical
Peak Detection Sensitivity	05 Height 10 Window
Display Operating Data	No
Distribution Table	None
X-axis Scale	Log

In 'New Name' enter the name 'Silica MNM'. Click on 'Save and Exit'.

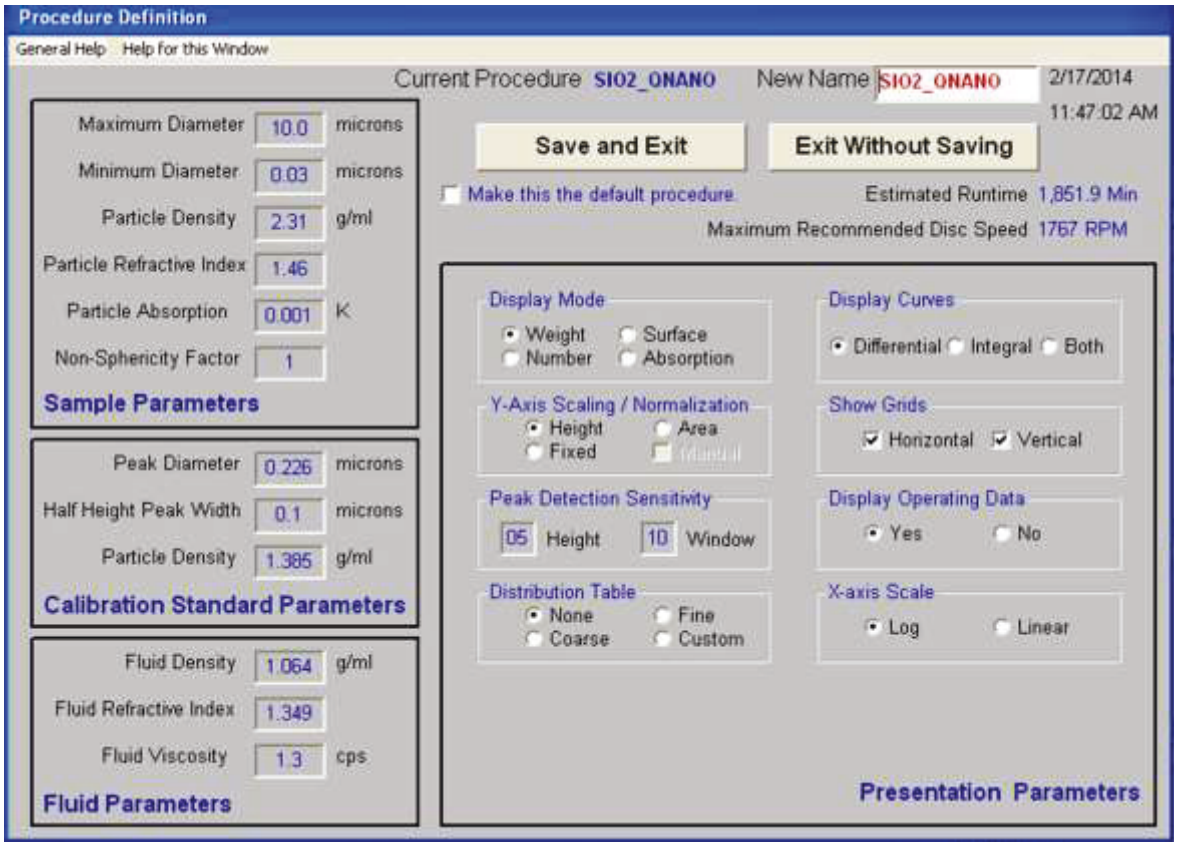

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Figure 1. Procedure definition window. It contains the parameters used for Silica measurements. Note that the parameters for the 'Calibration Standard Parameters' subsection depend on the specific calibration standard being used at each laboratory.

7.4 Measurements

7.4.1 Selecting the procedure

Click on the button 'Choose Procedure'. Select the procedure corresponding to the material and then click on the button 'Change to Selected Procedure'.

7.4.2 Measurements

A defined volume of the sample should be sampled with a syringe (with needle). The sample needs to be representative of the complete sample or volume. Typically a volume of 0.1 to 0.2 mL should be sampled for analysis. The volume of the sample is to be defined according to the concentration of the MNM in the sample (a larger volume of sample is required if the concentration of MNM is low). A maximum volume of 1 mL can be tested.

1. Click on the button 'Operate Analyser' from the main menu.

2. Type on the 'Sample' line: "name of your sample". Click ENTER.

2.a The software will ask for the calibration standard: Take 0.1 mL of the calibration standard with a syringe (with needle). Place the syringe in the orifice for sample injection. At the same time press the 'space bar' on the keyboard and inject the calibration standard.

Note: All measured peaks should have their intensity higher than 10 % in the blue line (see Figure 2).

2.b When the measurement is completed, introduce your sample with a syringe (with needle). Place the syringe in the orifice for sample injection. At the same time press the 'space bar' on the keyboard and inject the calibration standard.

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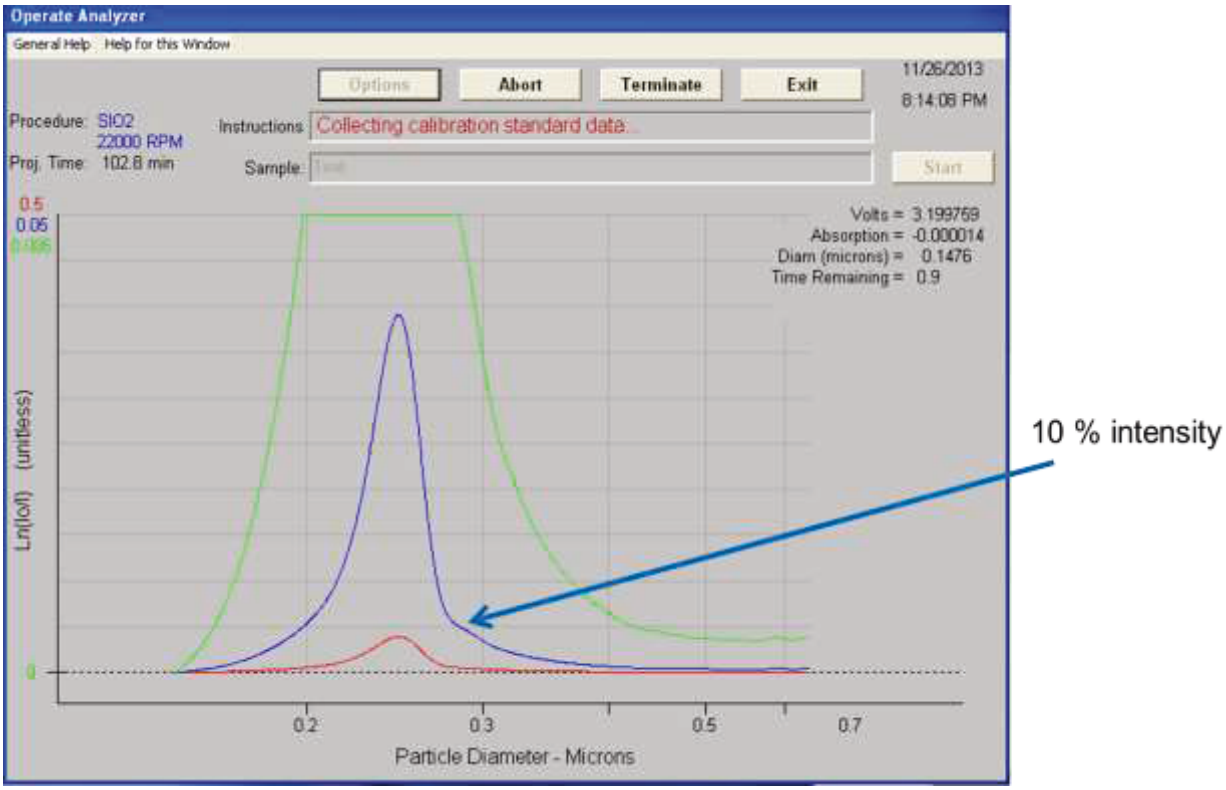

Figure 2. Curve of the calibration standard. A good calibration is obtained when the blue curve is above the 10 % intensity level (first line from bottom-up, see arrow).

7.5 Cleaning the disk after use

This procedure initiates after the disk is stopped (Go to the main menu and press STOP). After the disk stops rotating, open the lid. With the 20 mL syringe insert the attached plastic tube and collect part of the sucrose gradient. Throw it in a container (i.e. glass or plastic beaker). Open the cap of the disk, clean it carefully with the soft tissue (e.g., Tork premium soft tissue). Continue with the syringe collecting the rest of the sucrose gradient. Add a few millilitres of deionized water or ethanol, rotate a little bit the disk, and extract the water with the syringe. Use soft tissue to clean and dry the inside of the disk.

Make special attention to the extreme end of the disc (where the rubber seal is placed) because the measured particles sediment there and it may take a few cleaning cycles with the soft tissue and deionized water/ethanol until most of the particles are removed.

7.6 Determining the Particle Size Distribution

From the main menu click on the 'Retrieve Distribution' button and then click on the 'Choose Procedure' button. Select the procedure under which the measurements where done, and click on the 'OK' button. This will make

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the software go to the window of the procedure containing all the files measured under that procedure. Select the filename of the measured sample and click on the 'View Files' button.

Select the display weight mode. The PSD is determined from this mode (see references). A two-column table is automatically displayed with columns named Peaks and Half-Width.

7.7 Reporting the data

The Peaks and Half-Width of the each sample should be reported in an excel file with clearly identified sample name and parameters. The highest peak should be reported as the major peak, and any other peaks as minor peaks. If a peak appears only in some measurements, report that as an anomaly. In all cases determined peak and half-width should be reported.

This SOP uses in part references from:

1. IRMM Test protocol for the characterization of the particle size of colloidal silica candidate reference materials with Centrifugal Liquid Sedimentation (CLS)
2. JRC Interlaboratory comparison of methods for the measurement of particle size, effective particle density and zeta potential of silica nanoparticles in an aqueous solution
3. ISO 13318-1 Determination of particle size distribution by centrifugal sedimentation methods – Part 1: General principles and guidelines
4. ISO 13318-2 Determination of particle size distribution by centrifugal sedimentation methods – Part 2: Photocentrifuge method

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