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Physico-chemical characterisation of micron-sized silver powder and nano silver suspension

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Study accomplished under the authority of European Precious Metals Federation a.i.s.b.l. (EPMF)
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SUMMARY

Covance was commissioned by the European Precious Metals Federation (EPMF) to conduct in vivo toxicokinetics study in which they will compare different silver forms, including a micron-sized Ag powder (AgMP; particle size ~ 0,33 µm) and a nano silver suspension (AgNP; particle size ~ 15 nm).

Before starting the study, EPMF was in a need of data on the dissolution behavior and stability of these silver forms in the test vehicles (1% w/v methylcellulose (MC) in water and 5% w/v glucose in water) as well as a number of chemical characteristics.

The following questions are of concern:

- *Is there nano Ag and/or ionic Ag in the AgMP as supplied / when formulated in the vehicles?*
- *Is there ionic Ag in the AgNP as supplied / when formulated in the vehicles?*

In chapter 2 of this report, the results of the physico-chemical characterization (SEM, DLS, XRD, Ag, nano Ag and ionic Ag) of the nano suspension and micro silver powder test items are reported. These results confirm the data related to the expected size obtained from the suppliers. For the AgMP formulated in the vehicles, very small amounts (< 0,01%) of nano and ionic Ag as compared to total Ag are observed. For the Ag nano suspension, the ionic Ag amounted ~ 5 % of the total Ag concentration.

- *What is the sedimentation / agglomeration behavior of the test articles in the test vehicles?*

At the start, the optimal dilution of the AgNP/AgMP to be measured with dynamic light scattering was assessed (chapter 3). Thereafter, the following solutions were prepared for the sedimentation and agglomeration study (chapter 4):

	In 1% MC (oral), 400 ml	In 5% glucose (i.v.), 200 ml
AgMP formulations to be tested	1.8 mg/mL	4 mg/mL
	50 mg/mL	
AgNP formulations to be tested*	0.18 mg/mL	0.5 mg/mL
	18 mg/mL	
Test duration	11 days	24 hours
Test temperature	2 - 8 °C	2 - 8 °C
Timepoints for measurement	0h, 2-6h, 24h, d5, d8, d11	0h, 2-6h, 24h

* conc. expressed as mg Ag/mL (AgNP suspension contains ~10% Ag)

Dynamic light scattering was used to assess changes in particle size at the different time points of AgNP and AgMP in the vehicles after resuspension (ultrasonic bath and stirring). The DLS measurements in the 1 % MC solutions needed to be diluted in water to obtain qualitative results, especially for the AgNP problems of DLS detection were encountered. Overall no differences in average size (DLS) were observed between the treatment of 5 or 15 minutes ultrasonication of the formulations (followed by at least 20 min stirring). This was also confirmed by SEM/STEM images taken at the different timepoints. However, the results of the total Ag determination indicated a low recovery (< 50 %) for the AgMP formulations in 1 % MC, whilst this was not observed for the AgNP in 1 % MC (recovery > 80%). Supplementary tests were performed, showing that the lower recovery was not a function over time, but rather was caused by insufficient stirring while preparing the formulations themselves (see chapter 5). For the formulations prepared in 5 % glucose, the results of the average size (DLS), total Ag and SEM/STEM images indicated no change over the timepoints measured. Only the ionic Ag content in the Ag NP formulation decreased significantly (from 2,5 to 0,2 %)

TABLE OF CONTENTS

Distribution List I

Summary II

Table of Contents III

List of Figures V

List of Tables VII

CHAPTER 1 Introduction 1

CHAPTER 2 physico-chemical characterisation of test items 3

2.1. Introduction 3

2.2. Description WORK PACKAGE I 3

2.2.1. OBJECTIVE 3

2.2.2. ACTIONS – TASK DESCRIPTION 4

2.3. Results PHYSICO-CHEMICAL CHARACTERISATION Ag micro powder 5

2.3.1. Scanning electron microscope analysis 5

2.3.2. Crystallinity - X-ray Powder Diffraction analysis 7

2.3.3. Dynamic Light scattering analysis 8

2.3.4. nano and ionic Ag determination 8

2.4. Results PHYSICO-CHEMICAL CHARACTERISATION Ag nano suspension 9

2.4.1. Scanning electron microscope analysis 9

2.4.2. total and ionic Ag determination 11

2.4.3. Dynamic Light scattering analysis 11

CHAPTER 3 : PHYSICO-CHEMICAL CHARACTERISATION - pre-testing 13

3.1. Introduction 13

3.2. Optimal conditions Dynamic Light scattering analysis 13

3.2.1. 5 % glucose test vehicles 14

3.2.2. 1 % methyl cellulose test vehicles 15

CHAPTER 4 PHYSICO-CHEMICAL CHARACTERISATION – agglomeration/sedimentation study 17

4.1. Introduction 17

4.2. Timeline agglomeration/sedimentation study 17

4.3. Results 1 % w/v Methylcellulose 19

4.3.1. DLS Results 19

4.3.2. zeta potential and pH 23

4.3.3. Total and ionic Ag concentration 24

4.3.4. SEM/STEM 26

4.4. Results 5 % w/v glucose 28

4.4.1. DLS Results 28

4.4.2. zeta potential and pH 30

4.4.3. Total and ionic Ag concentration _____ 30
4.4.4. SEM/STEM _____ 32

CHAPTER 5 sedimentation study _____ 34

5.1. Introduction *34*

5.2. Results *35*

CHAPTER 6 Conclusion _____ 36

Annex A : DLS _____ 37

Annex B: SEM / STEM _____ 42

Annex C : zeta potential _____ 66

Annex D : sedimentation testing _____ 70

LIST OF FIGURES

Figure 1: SEM image Ag micro powder _____ 5
Figure 2: SEM image Ag micro powder. _____ 6
Figure 3 : XRD diffractogram of Ag micro powder (Empyrean, Co-tube) _____ 7
Figure 4 : XRD diffractogram of Ag micro powder (Empyrean, Co-tube), lattice parameter of 4,086 Angström corresponds to pure silver. _____ 7
Figure 5: dynamic light scattering analysis of Ag micro powder. _____ 8
Figure 6: SEM image Ag nano suspension (Carbon SEM stub) _____ 9
Figure 7: STEM image Ag nano suspension (Cu grid) _____ 10
Figure 8: dynamic light scattering analysis of Ag nano powder suspension. _____ 11
Figure 9: dynamic light scattering analysis of Ag nano powder suspension. _____ 14
Figure 10: dynamic light scattering analysis of Ag micro powder. _____ 15
Figure 11: dynamic light scattering analysis of Ag nano powder suspension _____ 15
Figure 12: dynamic light scattering analysis of Ag micro powder. _____ 16
Figure 13: Z average (nm) results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose. _____ 20
Figure 14: Z average (nm) results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose (measurements at 0 and 4 hours were performed in 2000*dilution, other time points in 1000*dilution). _____ 21
Figure 15: Z average (nm) results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose. ____ 22
Figure 16: Z average (nm) results of 18 mg/ml Ag nano suspension in 1% methylcellulose. _____ 23
Figure 17: recovery of total Ag from micro powder and nano suspension in 1% methyl cellulose solutions. _____ 25
Figure 18: STEM image Ag nanosuspension (Cu grid) _____ 26
Figure 19: STEM image 0,18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 26
Figure 20: STEM image 18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 26
Figure 21: STEM image 0,18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 11, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 27
Figure 22: STEM image 18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 11 (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 27
Figure 23: SEM image Ag micro powder _____ 27
Figure 24: SEM image 1,8 mg Ag/ml micro powder in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 27
Figure 25: SEM image 50 mg Ag/ml micro powder in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 28
Figure 26: SEM image 1,8 mg Ag/ml micro powder in 1 % MC (Cu grid) day 11, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 28
Figure 27: SEM image 50 mg Ag/ml micro powder in 1 % MC (Cu grid) day 11 (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 28
Figure 28: Z average (nm) results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. _____ 29
Figure 29: Z average (nm) results of 0,5 mg/ml Ag nano suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. _____ 30
Figure 30: recovery of total Ag from micro powder and nano suspension in 5% glucose solutions. 31
Figure 31: STEM image Ag nanosuspension (Cu grid) _____ 32
Figure 32: STEM image 0,5 mg Ag/ml nanosuspension in 5 % glucose day 0 (Cu grid), (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 32

Figure 33: STEM image 0,5 mg Ag/ml nanosuspension in 5 % glucose (Cu grid) day 1, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 32
Figure 34: SEM image Ag micro powder _____ 32
Figure 35: SEM image 4 mg Ag/ml micro powder in 5 % glucose day 0 (Cu grid), (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 33
Figure 36: SEM image 4 mg Ag/ml micro powder in 5 % glucose (Cu grid) day 1, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication. _____ 33
Figure 37: recovery of total Ag from micro powder and nano suspension in 1% methyl cellulose solutions. _____ 34

LIST OF TABLES

Table 1 : statistical evaluation of SEM image Ag micro powder (Figure 1). 5
Table 2 : statistical evaluation of SEM image Ag micro powder (Figure 2). 6
Table 3 : dynamic light scattering results of Ag micro powder. 8
Table4 : results of nano and ionic Ag determination (* samples in 1 % methyl cellulose were very harsh to filtrate manually over 0,1 µm filter, results are to be considered as indicative). 9
Table 5 : statistical evaluation of SEM image Ag nano suspension (Figure 6). 10
Table 6 : statistical evaluation of STEM image Ag nano suspension (Figure 7). 10
Table 7 : results of nano and ionic Ag determination. 11
Table 8 : dynamic light scattering results of Ag nano powder suspension. 12
Table 9 : dynamic light scattering results of Ag nano powder suspension. 14
Table 10 : dynamic light scattering results of Ag micro powder. 15
Table 11 : dynamic light scattering results of Ag nano powder suspension. 16
Table 12 : dynamic light scattering results of Ag micro powder. 16
Table 13 : Z average (nm) results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 19
Table 14 : Z average (nm) results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication first measurements (measurements at 0 and 4 hours were performed in 2000*dilution, other time points in 1000*dilution). 20
Table 15 : Z average (nm) results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 21
Table 16 : Z average (nm) results of 18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 22
Table 17 : Zeta potential results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 23
Table 18 : Zeta potential results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 23
Table 19 : Zeta potential results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 24
Table 20 : Zeta potential results of 18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 24
Table 21 : pH measurements in 1% methyl cellulose solutions. 24
Table 22 : influence of the heating step in the determination of total Ag. 25
Table23 : results of ionic Ag determination (3 kDa filtration + ICP-OES). 25
Table 24 : Z average (nm) results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 28
Table 25 : Z average (nm) results of 0,5 mg/ml Ag nano suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 29
Table 26 : Zeta potential results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 30
Table 27 : Zeta potential results of 0,5 mg/ml Ag nano powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication. 30
Table 28 : pH measurements in 5% glucose solutions. 30
Table29 : results of ionic Ag determination (3 kDa filtration + ICP-OES). 31
Table 30 : results of total Ag determination in 1% MC (viscosity 400 cP). 35
Table 31 : results of total Ag determination in 0,1% MC (viscosity 400 cP). 35

CHAPTER 1 INTRODUCTION

Covance was commissioned by the European Precious Metals Federation (EPMF) to conduct in vivo toxicokinetics study in which they will compare different silver forms, including a micron-sized Ag powder (AgMP; particle size ~ 0,33 µm) and a nano silver suspension (AgNP; particle size ~ 15 nm).

Before starting the study, EPMF was in a need of data on the dissolution behavior and stability of these silver forms in the test vehicles (1% w/v methylcellulose (MC) in water and 5% w/v glucose in water) as well as a number of chemical characteristics.

The following questions were of concern

- What is the sedimentation / agglomeration behavior of the test articles in the test vehicles?
- Is there nano Ag and/or ionic Ag in the AgMP as supplied / when formulated in the vehicles?
- Is there ionic Ag in the AgNP as supplied / when formulated in the vehicles?

In this proposal the physico-chemical characterization of nano and micro silver in 2 test vehicles (1% w/v methylcellulose (MC) in water and 5% w/v glucose in water) are reported.

CHAPTER 2 PHYSICO-CHEMICAL CHARACTERISATION OF TEST ITEMS

2.1. INTRODUCTION

EPMF is in need for physico-chemical characterisation of a micron-sized Ag powder (AgMP; particle size ~ 0,33 µm) and nano silver suspension (AgNP; particle size ~ 15 nm) in 2 test vehicles (1% w/v methylcellulose (MC) in water and 5% w/v glucose in water).

2.2. DESCRIPTION WORK PACKAGE I

2.2.1. OBJECTIVE

Evaluate the test items supplied as such (the following information was supplied from Arche):

- **micron-sized Ag powder (AgMP; particle size ~ 0,33 µm);**
  - High purity (> 99%), crystalline, sub-micron silver powder of highly uniform spheroidal shape
  - PSD: D10 = 0.24 µm, D50 = 0.33 µm, D90 = 0.52 µm
  - Relative surface area = 2.24 m^2/g (BET)
  - Density = 3.5 kg/dm^3

CONFIDENTIAL INFORMATION BOX

- **nano silver suspension (AgNP; particle size ~ 15 nm).**
  - agpure® W10 (https://ras-ag.com/agpure-w10/); official OECD (NM-300K silver) and BAM CRM

CONFIDENTIAL INFORMATION BOX

  - Contains **9.96 % Ag**, 74.3 % water
  - pH (dispersion): 7.0 - 9.0
  - Mean particle size = 15 nm, D99 = 20 nm (not defined for every batch but correlation PSD with UV-Vis spectrum validated)
  - Ag content and particle nr shown to be stable up to 12 months
  - Relative surface area = 2.24 m^2/g (BET)
  - Density = 1.1 kg/dm^3
  - Negative zeta potential

2.2.2. ACTIONS – TASK DESCRIPTION

The following characteristics needed to be determined

- AgMP powder
  - SEM/STEM analysis
  - Crystallinity XRD analysis
  - DLS

- Ag MP formulations in the test vehicles, immediately after preparation of the formulations:
  - nano Ag fraction : filtration (Anodisc, 0,1µm) < 100 nm + ICP-OES
  - ionic fraction : filtration (preconditioned VIVASPIN, 3.000 MWCO) < 3 kDa + ICP-OES

- AgNP suspension
  - SEM/STEM analysis
  - Total Ag concentration : acid digestion (HCl/HNO_3) + ICP-OES
  - Ionic Ag fraction : 3 kDa filtration + ICP-OES

2.3. RESULTS PHYSICO-CHEMICAL CHARACTERISATION AG MICRO POWDER

2.3.1. SCANNING ELECTRON MICROSCOPE ANALYSIS

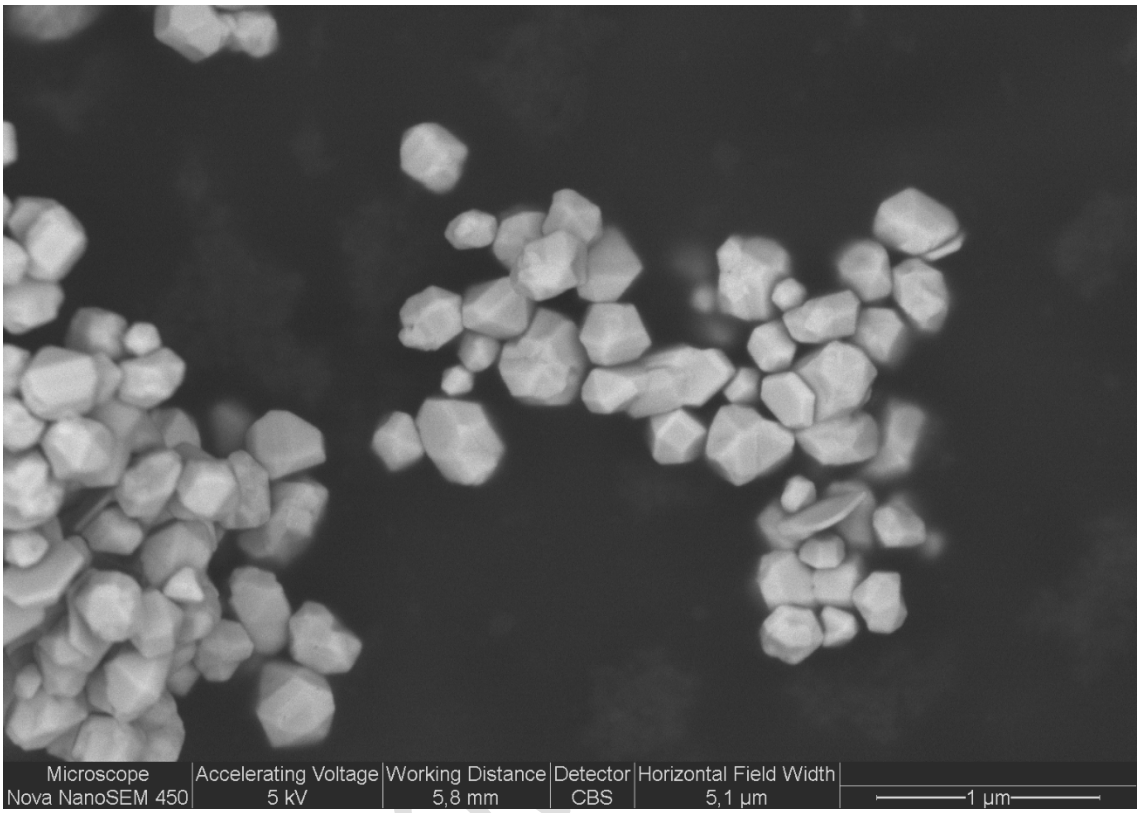


Figure 1: SEM image Ag micro powder

Based on the SEM image (Figure 1), the following statistical evaluation was performed on the size of the Ag micro powder.

SEM image	
Number particles (#)	24
Minimum size (nm)	119
10th percentile (nm)	139
Median (nm)	**265**
90th percentile (nm)	370
Maximum	423

Table 1 : statistical evaluation of SEM image Ag micro powder (Figure 1).

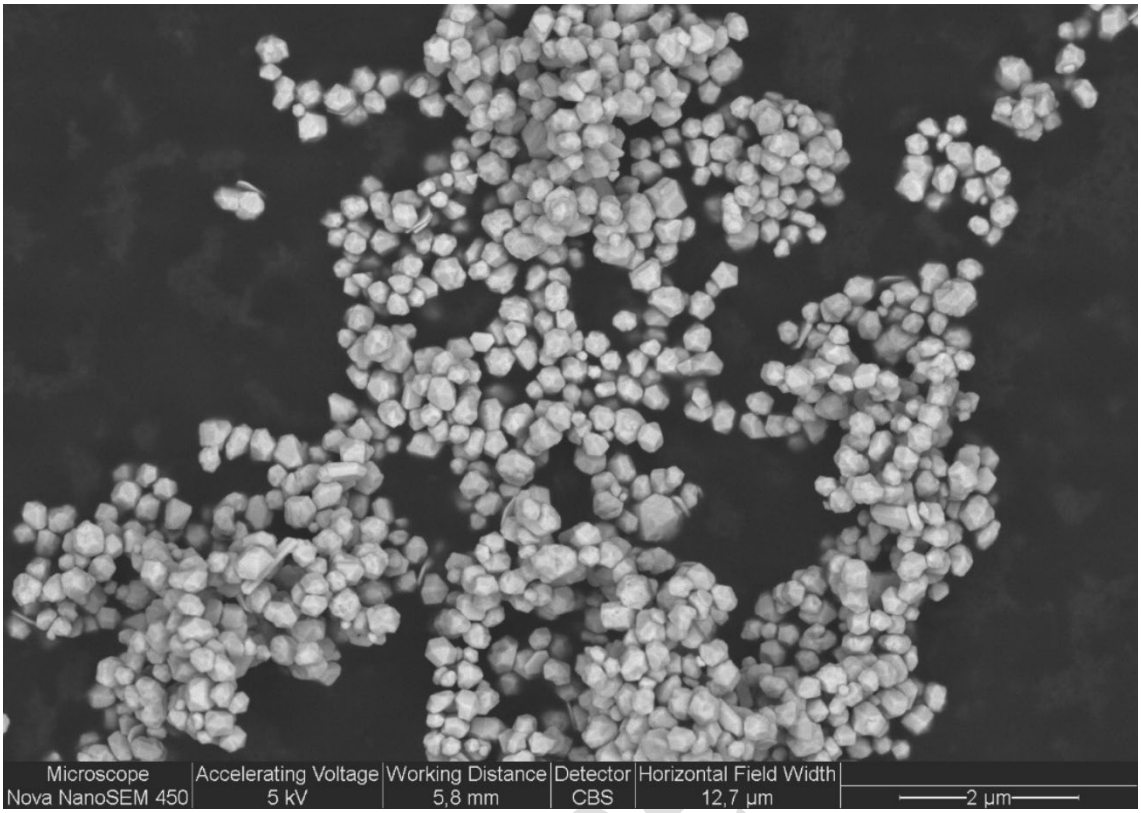


Figure 2: SEM image Ag micro powder.

Based on the SEM image (Figure 2), the following statistical evaluation was performed on the size of the Ag micro powder.

SEM image	
Number particles (#)	30
Minimum size (nm)	149
10^{th} percentile (nm)	198
Median (nm)	**314**
90^{th} percentile (nm)	410
Maximum	480

Table 2 : statistical evaluation of SEM image Ag micro powder (Figure 2).

2.3.2. CRYSTALLINITY - X-RAY POWDER DIFFRACTION ANALYSIS

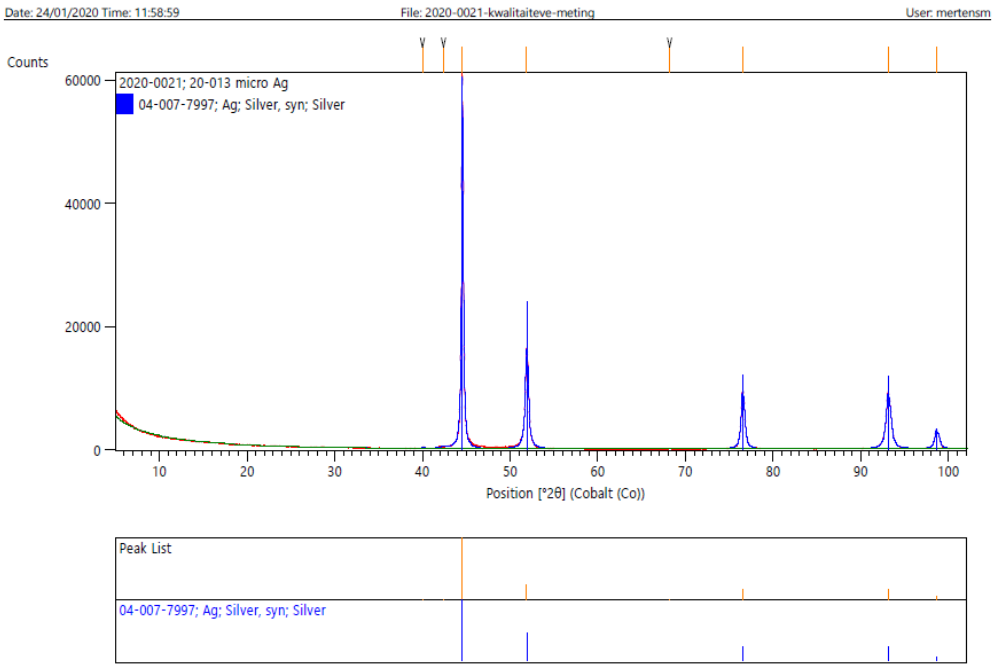


Figure 3 : XRD diffractogram of Ag micro powder (Empyrean, Co-tube)

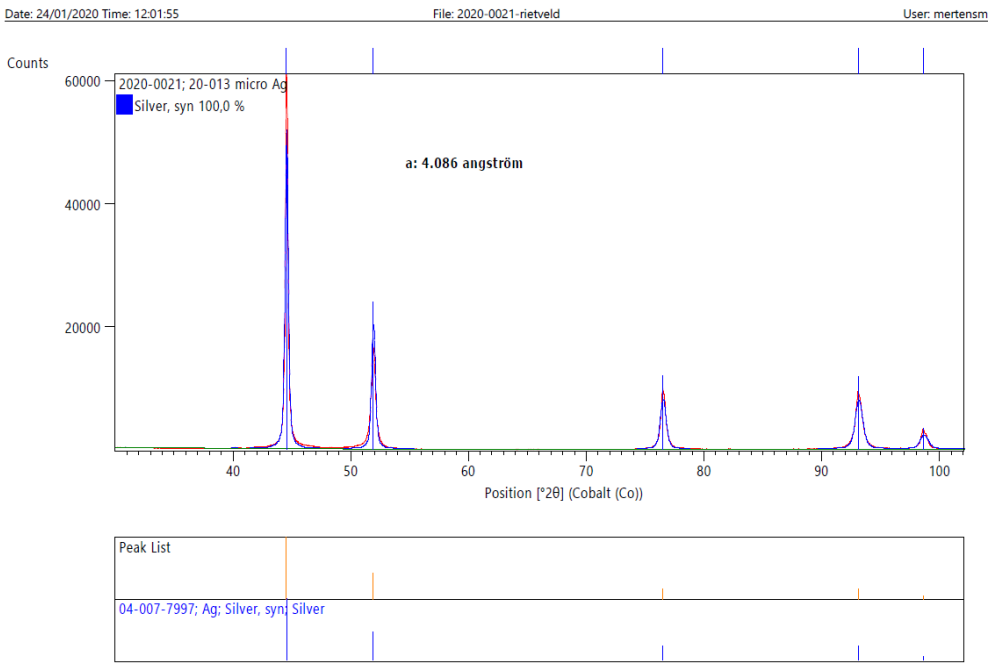


Figure 4 : XRD diffractogram of Ag micro powder (Empyrean, Co-tube), lattice parameter of 4,086 Angström corresponds to pure silver.

2.3.3. DYNAMIC LIGHT SCATTERING ANALYSIS

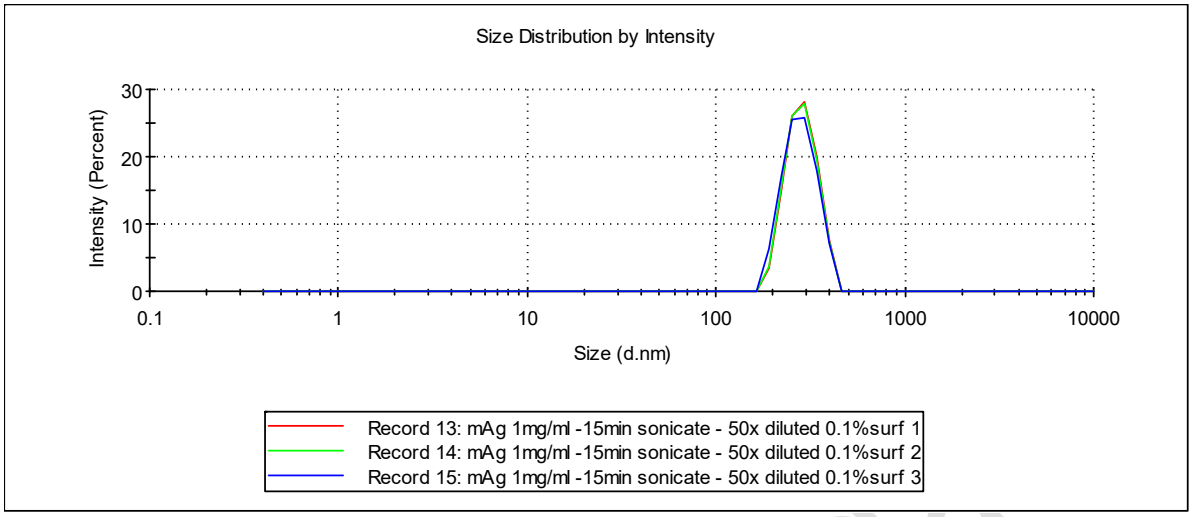


Figure 5: dynamic light scattering analysis of Ag micro powder.

Based on the DLS analysis, the following statistical evaluation was performed on the size (Z-average) of the Ag micro powder.

Record	Z-Ave (nm)	PdI	Intensity Mean (nm)
13	339,3	0,273	287,2
14	339,5	0,303	285,8
15	333,1	0,29	281

Table 3 : dynamic light scattering results of Ag micro powder.

2.3.4. NANO AND IONIC AG DETERMINATION

The following analysis were performed on the Ag MP formulations in the 3 test vehicles immediately after preparation of the formulations :

- nano Ag fraction : filtration (Anodisc, 0,1µm) < 100 nm + ICP-OES
- ionic fraction : filtration (preconditioned VIVASPIN, 3.000 MWCO) < 3 kDa + ICP-OES

The determination of nano Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ insert sample into a BD Luer-Lok syringe of 10 ml;
- ✓ attach the 25 mm Easy Pressure Syringe Filter Holder with the Anodisc Circle with Support Ring, 25 mm, 0.1 µm pore size;
- ✓ dilute 500 µl filtrate in *aqua regia* (12 % HCl + 4 % HNO_3) up to 5 ml and measure with ICP-AES.

The determination of ionic Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ a subsample of 1 ml was taken with an automatic pipet and transferred to a VIVAspin 6 tube.

- ✓ Centrifuge 15 minutes at 4000 rpm and discard the filtrate
- ✓ A subsample of 5 ml was taken with an automatic pipet and transferred to the pre-treated VIVAspin 6 tube.
- ✓ Centrifuge 120 minutes at 4000 rpm and collect the filtrate
- ✓ dilute 500 µl filtrate in *aqua regia* (12 % HCl + 4 % HNO_3) up to 5 ml and measure with ICP-AES.

sample	Day	< 100 nm fraction µg Ag /l	%	Ionic fraction µg Ag/l	%
1.8 mg/ml in 1 % MC	0	(90149)*	(5,0%)*	67	0,0037%
1.8 mg/ml in 1 % MC	11			34	0,0019%
50 mg/mL in 1 % MC	0	(6319)*	(0,013%)*	74	0,0001%
50 mg/mL in 1 % MC	11			178	0,0004%
4 mg/mL in 5 % glucose	0	40	0,001%	41	0,0010%
4 mg/mL in 5 % glucose	1			97	0,0024%

Table4 : results of nano and ionic Ag determination (samples in 1 % methyl cellulose were very harsh to filtrate manually over 0,1 µm filter, results are to be considered as indicative).*

2.4. RESULTS PHYSICO-CHEMICAL CHARACTERISATION AG NANO SUSPENSION

2.4.1. SCANNING ELECTRON MICROSCOPE ANALYSIS

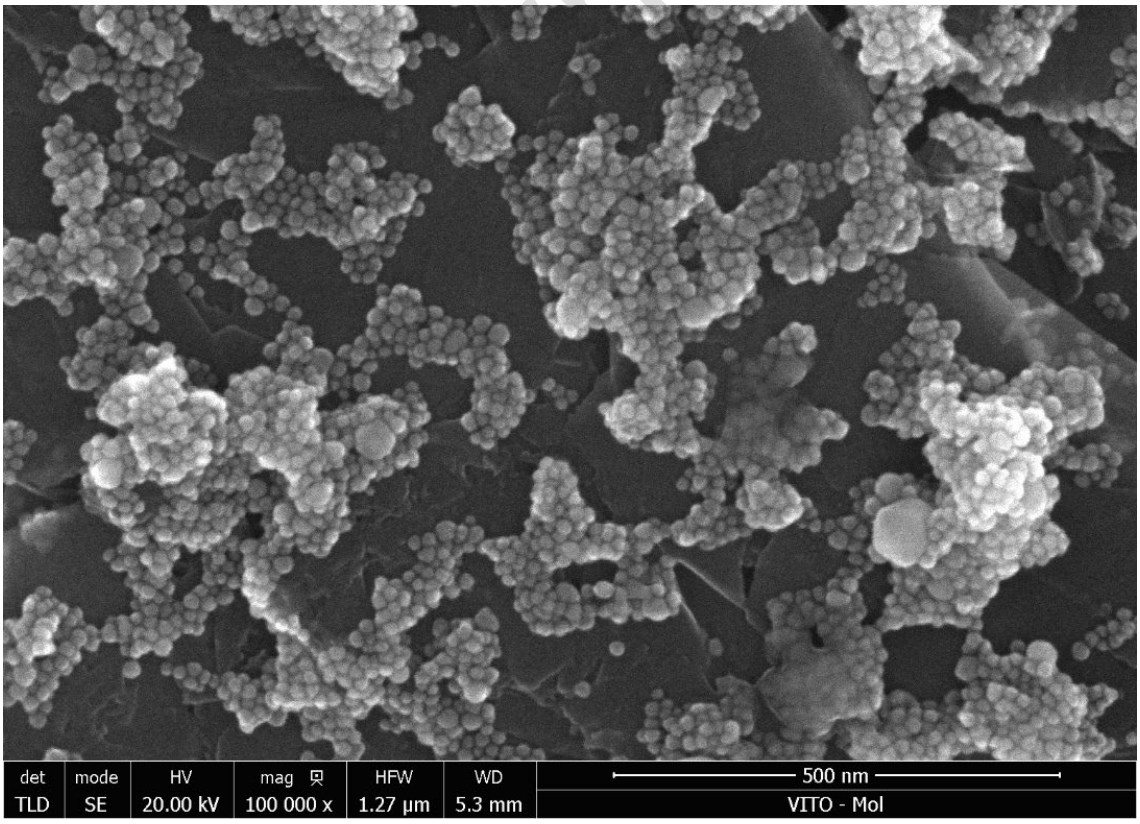


Figure 6: SEM image Ag nano suspension (Carbon SEM stub)

Based on the SEM image (Figure 6), the following statistical evaluation was performed on the size of the Ag nano suspension.

SEM image	
Number particles (#)	30
Minimum size (nm)	13
10^{th} percentile (nm)	13
Median (nm)	**17**
90^{th} percentile (nm)	37
Maximum	76

Table 5 : statistical evaluation of SEM image Ag nano suspension (Figure 6).

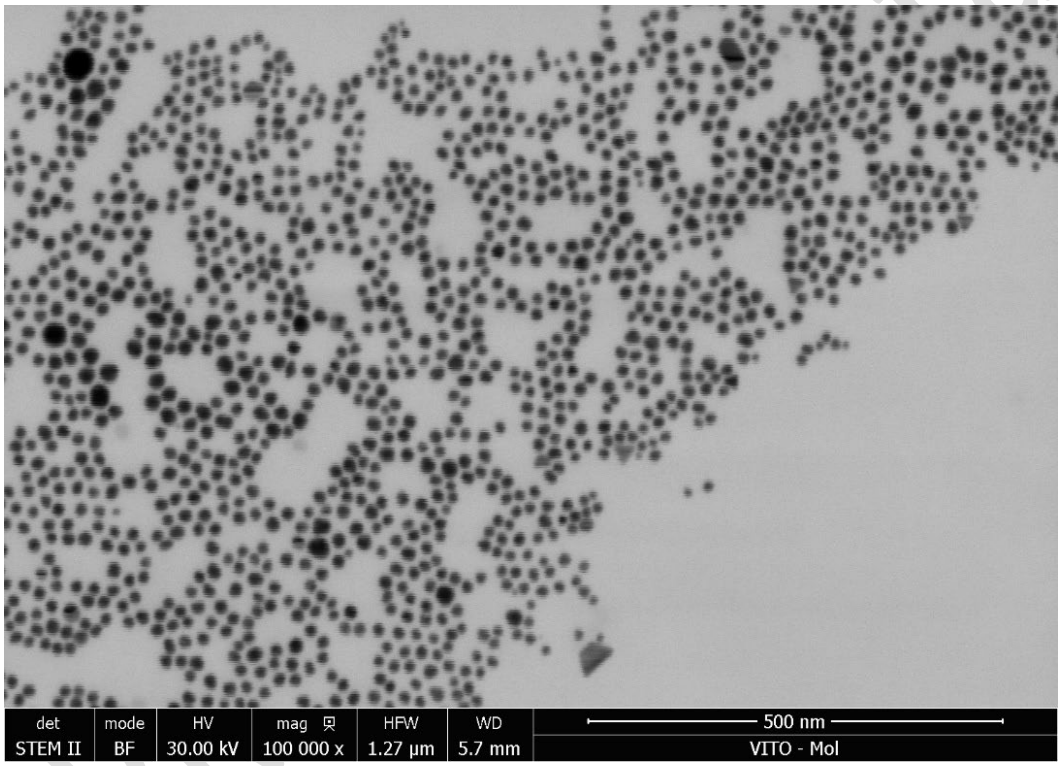


Figure 7: STEM image Ag nano suspension (Cu grid)

Based on the SEM image (Figure 7), the following statistical evaluation was performed on the size of the Ag nano suspension.

STEM image	
Number particles (#)	35
Minimum size (nm)	7
10^{th} percentile (nm)	10
Median (nm)	**13**
90^{th} percentile (nm)	21
Maximum	38

Table 6 : statistical evaluation of STEM image Ag nano suspension (Figure 7).

2.4.2. TOTAL AND IONIC AG DETERMINATION

The following analysis were performed on the Ag nano suspension solution:

- Total Ag concentration : diluted in HNO_3 + ICP-OES
- Ionic Ag fraction : 3 kDa filtration + ICP-OES

The determination of total Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ a subsample of 500 µl was taken with an automatic pipet (eppendorf research plus 100-1000 µl) and transferred to a 50 ml PP Digitube.
- ✓ 5 ml of nitric Acid 67-69% Optima Grade Fisher Chemicals is added and made up to 50 ml with ultrapure water
- ✓ diluted further in *aqua regia* (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

The determination of ionic Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ a subsample of 1 ml was taken with an automatic pipet and transferred to a VIVAspin 6 tube.
- ✓ Centrifuge 15 minutes at 4000 rpm and discard the filtrate
- ✓ A subsample of 5 ml was taken with an automatic pipet and transferred to the pre-treated VIVAspin 6 tube.
- ✓ Centrifuge 120 minutes at 4000 rpm and collect the filtrate
- ✓ Add to 500 µl filtrate 500 µl of nitric Acid 67-69% and dilute with Milli-Q water up to 5 ml
- ✓ dilute further in *aqua regia* (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

sample	Total Ag g/l	Ionic Ag g/l
AgNP suspension contains ~ 10% Ag	99,6	5,3

Table 7 : results of nano and ionic Ag determination.

2.4.3. DYNAMIC LIGHT SCATTERING ANALYSIS

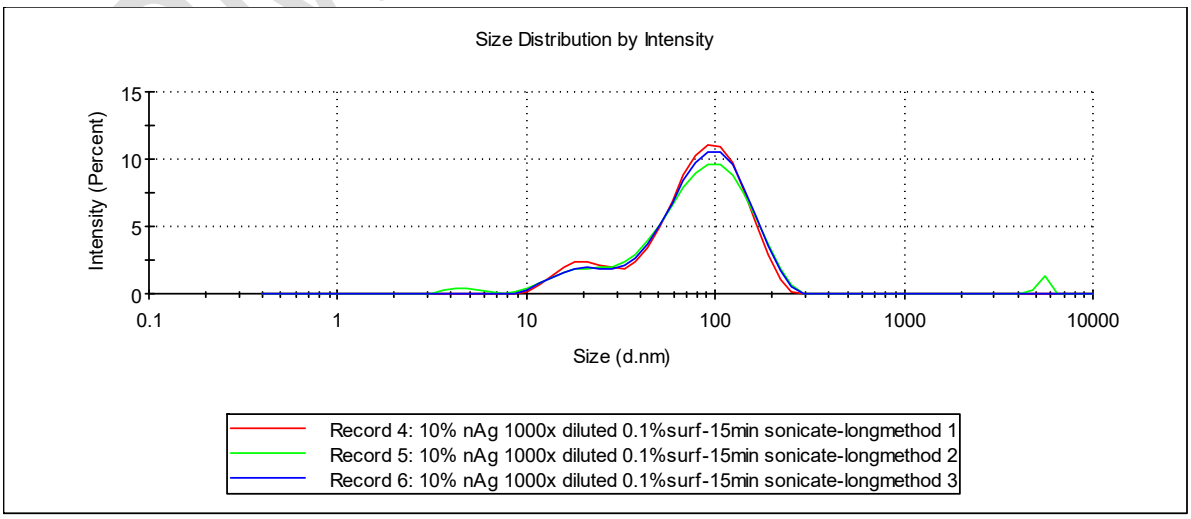


Figure 8: dynamic light scattering analysis of Ag nano powder suspension.

Record	Z-Ave (nm)	PdI	Intensity Mean (nm)
4	83,57	0,245	87,33
5	80,14	0,287	172,8
6	79,59	0,247	90,57

Table 8 : dynamic light scattering results of Ag nano powder suspension.

CHAPTER 3 : PHYSICO-CHEMICAL CHARACTERISATION - PRE-TESTING

3.1. INTRODUCTION

At the start, the optimal dilution of the AgNP/AgMP to be measured with DLS was assessed. The following test solutions were prepared by mixing the test vehicle stock solutions (i.e., 1 % MC (viscosity 400 cP), respectively 5 % glucose) with the appropriate amount of sample (i.e., Ag MP powder, respectively Ag NP suspension) in the precleaned vessels.

	In 1% MC (oral)	In 5% glucose (i.v.)
AgMP formulations to be tested	1.8 mg/mL	4 mg/mL
	50 mg/mL	
AgNP formulations to be tested*	0.18 mg/mL	0.5 mg/mL
	18 mg/mL	

* conc. expressed as mg Ag/mL (AgNP suspension contains ~10% Ag)

3.2. OPTIMAL CONDITIONS DYNAMIC LIGHT SCATTERING ANALYSIS

The Zetasizer Nano ZSP, Dynamic Light Scattering was used to measure particle size. DLS measures the diffusion of particles moving under Brownian motion and converts this to size and a size distribution using the Stokes-Einstein relationship. The ZSP also incorporates a zeta potential analyzer and pH determination.

Size is obtained from the correlation function by using various algorithms. In this case the following approach was chosen : fit a single exponential to the correlation function to obtain the mean size (z-average diameter) and an estimate of the width of the distribution (polydispersity index) (this is called the Cumulants analysis).

Method Size:

- ✓ temp 25°C;
- ✓ 120 sec equilibration time;
- ✓ Measurement duration: automatic;
- ✓ Number of measurements: 3.

The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move it. Those ions beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable.

Method Zeta potential:

- ✓ temp 25°C
- ✓ 60 sec equilibration time;
- ✓ measurement duration: min runs: 20 /max: 50;
- ✓ number of measurements: 3, delay between: 60 sec

In the paragraphs hereafter only the optimal DLS conditions are shown for the different solutions .

3.2.1. 5 % GLUCOSE TEST VEHICLES

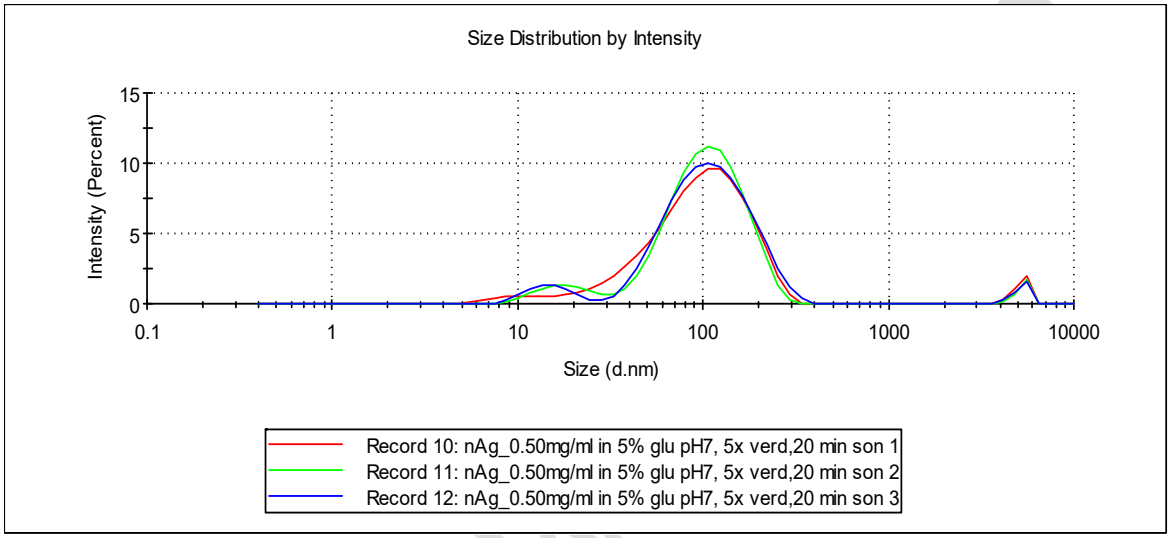


Figure 9: dynamic light scattering analysis of Ag nano powder suspension.

Record	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
10	**83,73**	0,44	272,7
11	**83,16**	0,53	231,5
12	**89,3**	0,366	241,1

Table 9 : dynamic light scattering results of Ag nano powder suspension.

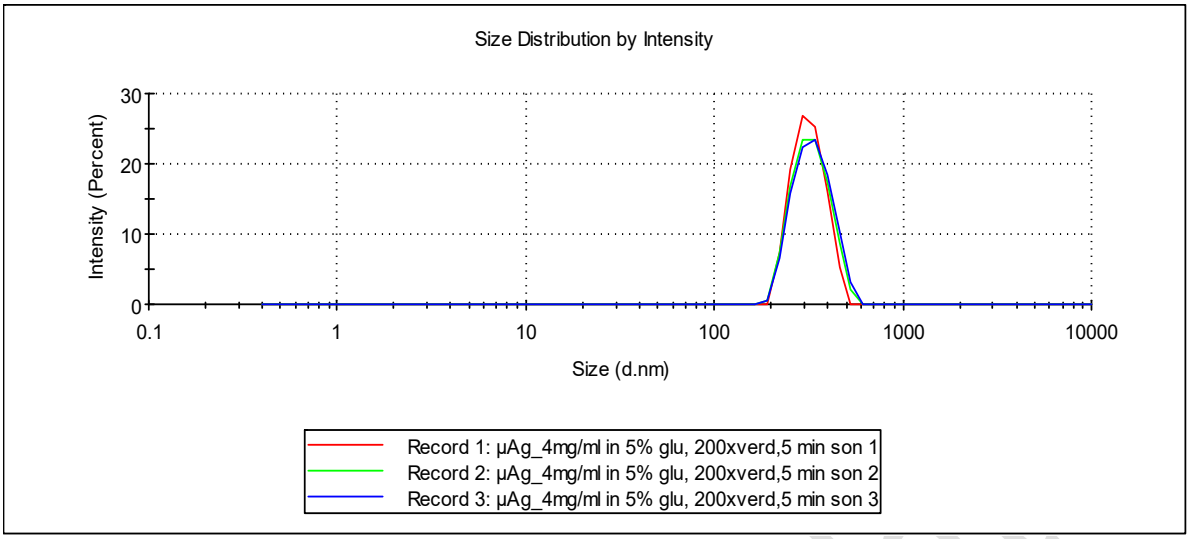


Figure 10: dynamic light scattering analysis of Ag micro powder.

Record	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
1	**372,1**	0,309	318,6
2	**373,5**	0,269	329,9
3	**373**	0,265	336,7

Table 10 : dynamic light scattering results of Ag micro powder.

3.2.2. 1 % METHYL CELLULOSE TEST VEHICLES

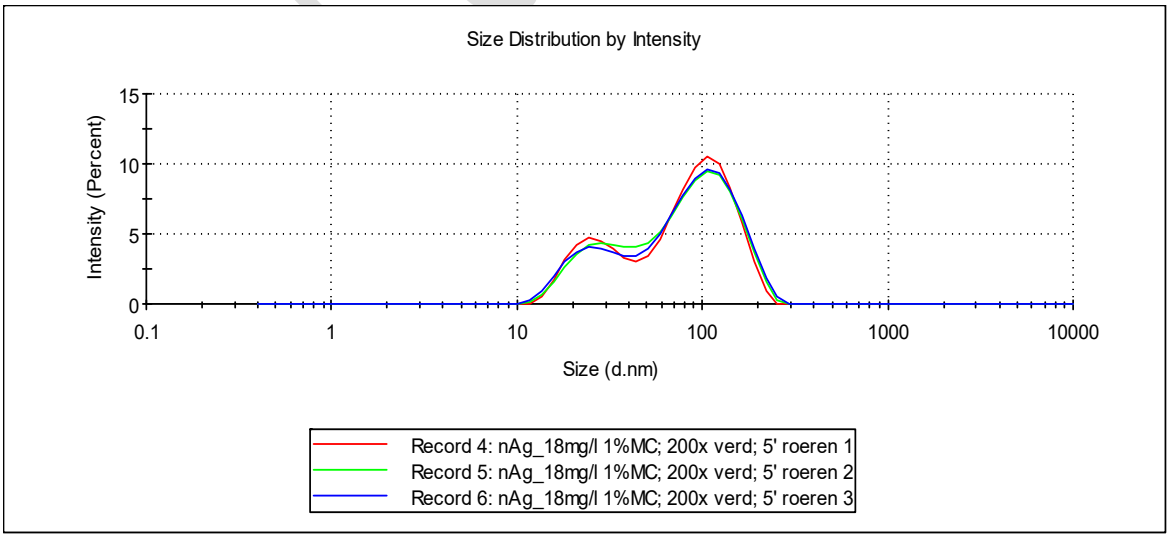


Figure 11: dynamic light scattering analysis of Ag nano powder suspension

Record	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
4	**98,09**	0,236	84,75
5	**95,99**	0,233	85,87
6	**91,04**	0,221	87,62

Table 11 : dynamic light scattering results of Ag nano powder suspension.

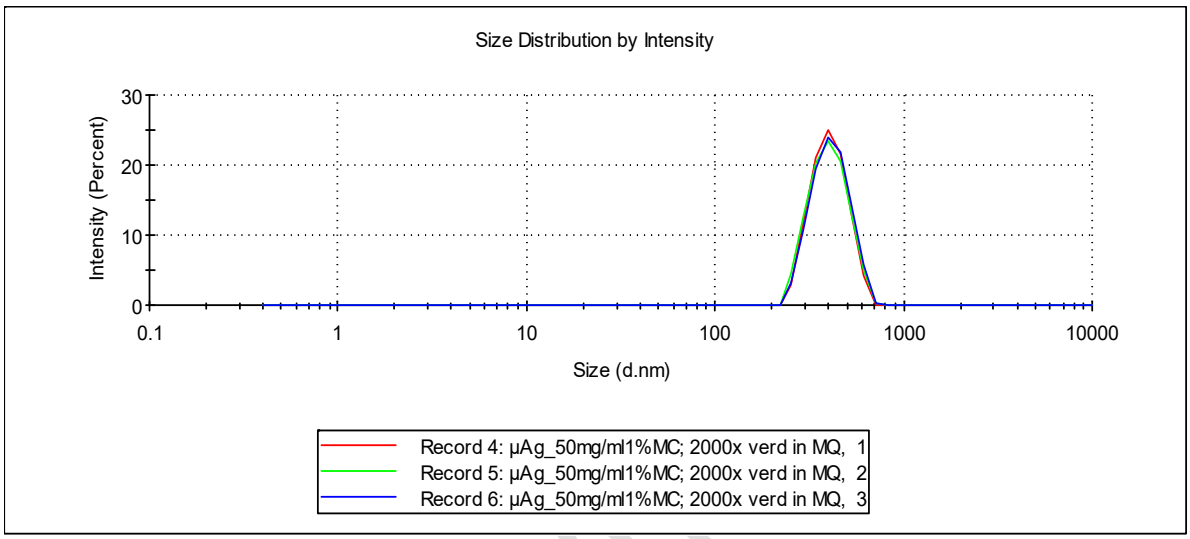


Figure 12: dynamic light scattering analysis of Ag micro powder.

Record	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
4	**465,7**	0,259	409,1
5	**457,9**	0,272	408,4
6	**454,3**	0,247	417,5

Table 12 : dynamic light scattering results of Ag micro powder.

No appropriate DLS results could be found in the 1 % MC solutions, therefore a dilution with Milli Q water was needed. The outcome of the pre-testing (optimal DLS working range) was :

- Ag MP 50 mg/ml in 1 % MC : dilute 2000 times in Milli-Q water
- Ag NP 18 mg/ml in 1 % MC : dilute 200 times in Milli-Q water
- Ag MP 1,8 mg/ml in 1 % MC : dilute 80 times in Milli-Q water
- Ag NP 0,18 mg/ml in 1 % MC : dilute 2 times in Milli-Q water

- Ag MP 4 mg/ml in 5 % glucose : dilute 200 times in 5 % glucose
- Ag NP 0,5 mg/ml 5 % glucose : dilute 5 times in 5 % glucose

CHAPTER 4 PHYSICO-CHEMICAL CHARACTERISATION – AGGLOMERATION/SEDIMENTATION STUDY

4.1. INTRODUCTION

The solutions summarised in the table hereunder were prepared in precleaned (with diluted HNO_3 solution and MQ water) polypropylene vessels.

	In 1% MC (oral), 400 ml	In 5% glucose (i.v.), 200 ml
AgMP formulations to be tested	1.8 mg/mL	4 mg/mL
	50 mg/mL	
AgNP formulations to be tested*	0.18 mg/mL	0.5 mg/mL
	18 mg/mL	
Test duration	11 days	24 hours
Test temperature	2 - 8 °C	2 - 8 °C
Timepoints for measurement	0h, 2-6h, 24h, d5, d8, d11	0h, 2-6h, 24h

* conc. expressed as mg Ag/mL (AgNP suspension contains ~10% Ag)

1% MC: Methyl cellulose Methocel A4C, VWR, cas nr: 9004-67-5 , 20g / 2l
5% Glucose: D(+)-Glucose monohydrate, Merck, cas nr: 14431-43-7, 50 g/l

From these freshly prepared solutions and after resuspending while stirring, aliquot samples of 25 ml for the 6 different timepoints in case of 1 % MC and 3 timepoints in case of 5 % glucose were taken by a 25 ml pipet in duplicate and stored in the fridge.

4.2. TIMELINE AGGLOMERATION/SEDIMENTATION STUDY

The following timepoints and analysis were performed in the agglomeration/sedimentation study.

Summary of measurement for Ag MP in each 1 % MC stock solution (4 in total)

	0h	4h	24h	d5	d8	d11
DLS	x	X	x	x	x	x
Total Ag	x					x
SEM/STEM	x					x
pH	x					x
Zeta potential	x					x

Summary of measurement for Ag NP in each 1 % MC stock solution (4 in total)

	0h	4h	24h	d5	d8	d11
DLS	x	x	x	x	x	x

Total Ag	x					x
SEM/STEM	x					x
pH	x					x
Zeta potential	x					x

Summary of measurement for Ag MP in each 5 % glucose stock solution (2 in total)

	0h	4h	24h
DLS	x	x	x
Total Ag	x		x
SEM/STEM	x		x
pH	x		x
Zeta potential	x		x

Summary of measurement for Ag NP in each 5 % glucose stock solution (2 in total)

	0h	4h	24h
DLS	x	x	x
Total Ag	x		x
SEM/STEM	x		x
pH	x	x	x
Zeta potential	x		X

- Timepoint 0h for 1 % MC:
  - 4 test solutions were prepared, an ultrasonic treatment for **5 min** was performed and subsequently magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.
  - 4 test solutions were prepared, an ultrasonic treatment for **15 min** was performed and subsequently magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.
  - Between the timepoints, the solutions were stored at fridge temperature.
  - For the other timepoints and prior to the DLS measurements, the daily aliquots of the stock solutions were taken out from the fridge, then the stock solutions were ultrasonic treated for 5 resp. 15 min and were magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.
  - The dilution factor for the DLS measurements was changed from timepoint 24h onwards to:
    - ✓ Ag MP 50 mg/ml in 1 % MC : dilute 1000 times in Milli-Q water, vortex
    - ✓ Ag NP 18 mg/ml in 1 % MC : dilute 200 times in Milli-Q water, vortex
    - ✓ Ag MP 1,8 mg/ml in 1 % MC : dilute 30 times in Milli-Q water, vortex
    - ✓ Ag NP 0,18 mg/ml in 1 % MC : dilute 4 times in Milli-Q water, stir 5 min

- Timepoint 0h for 5 % glucose:
  - 2 test solutions were prepared, an ultrasonic treatment for **5 min** was performed and subsequently magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.

- 2 test solutions were prepared, an ultrasonic treatment for **15 min** was performed and subsequently magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.
- Between the timepoints, the solutions were stored at fridge temperature.
- For the other timepoints and prior to the DLS measurements, the daily aliquots of the stock solutions were taken out from the fridge, then the stock solutions were ultrasonic treated for 5 resp. 15 min and were magnetically stirred for (at least) 20 min. From this solution a test portion was taken for DLS measurement which was further diluted (see § 3.2.2). Also, whilst stirring a test portion of 1 ml was taken for total Ag determination.

- the following analysis were supplementary performed (see also tables above):
  - pH in the 1 % MC and 5 % glucose solutions at start and endpoint.
  - Zeta potential and SEM/STEM at the start and end of the experiment on micron-sized Ag powder and nano silver suspension.

4.3. RESULTS 1 % W/V METHYLCELLULOSE

4.3.1. DLS RESULTS

The individual DLS results are summarized in Annex A and are summarized in the tables and figures hereunder.

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	616	48	626	46
4	593	18	619	14
24	612	28	584	18
72	648	28	663	28
168	629	30	706	13
264	660	26	650	21

Table 13 : Z average (nm) results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

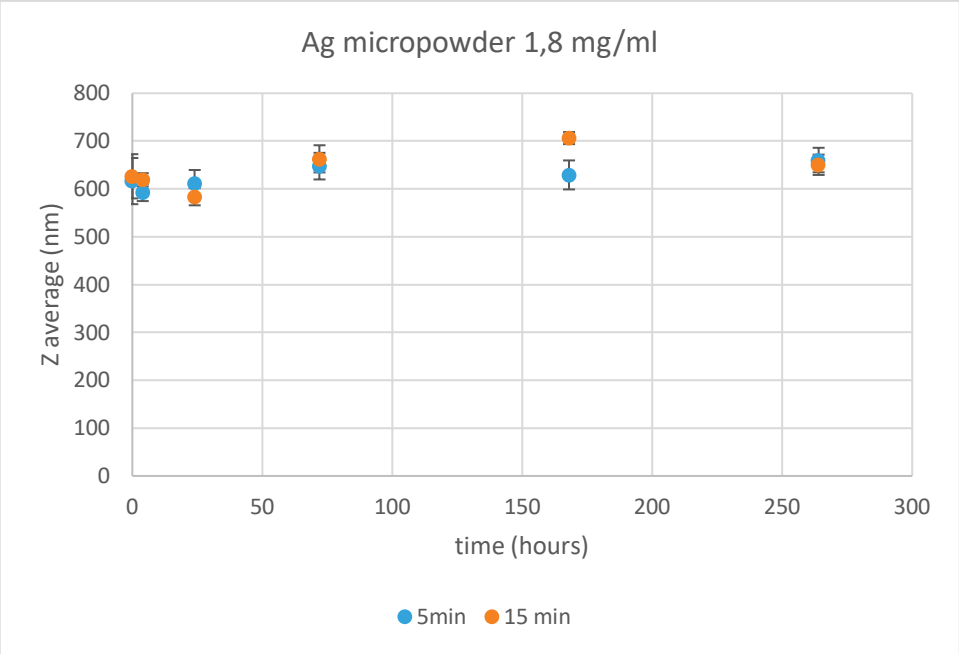


Figure 13: Z average (nm) results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose.

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	655	2	686	51
4	756	65	678	49
24	545	32	564	32
72	475	2	612	34
168	499	13	519	29
264	490	21	501	1

*Table 14 : Z average (nm) results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication first measurements (measurements at 0 and 4 hours were performed in 2000*dilution, other time points in 1000*dilution).*

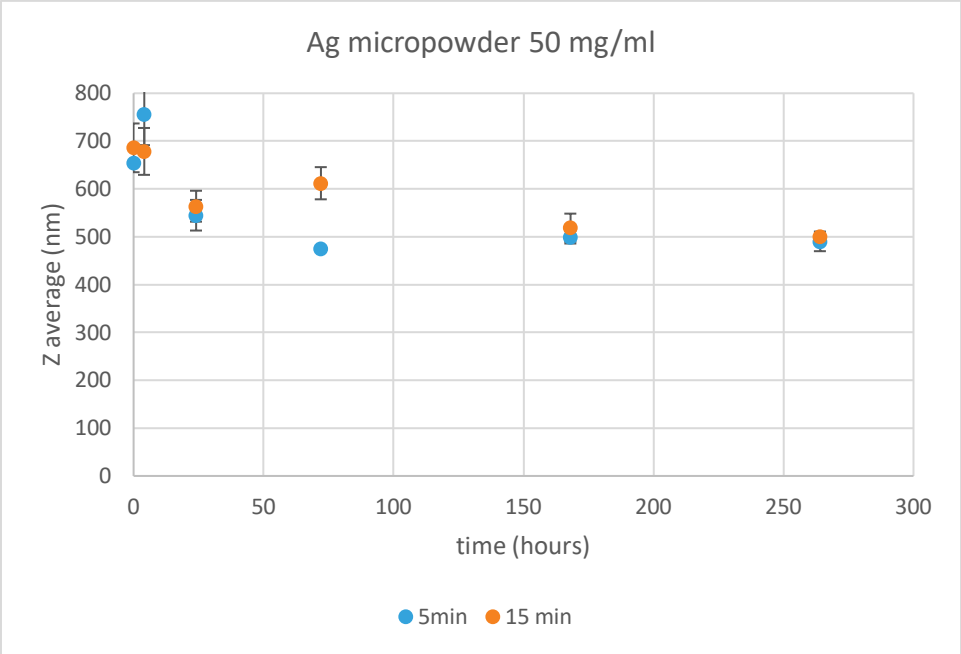


*Figure 14: Z average (nm) results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose (measurements at 0 and 4 hours were performed in 2000*dilution, other time points in 1000*dilution).*

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	283	113	277	32
4	245	8	264	55
24	167	54	128	11
72	133	26	130	26
168	284	10	286	9
264	186	108	158	47

Table 15 : Z average (nm) results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

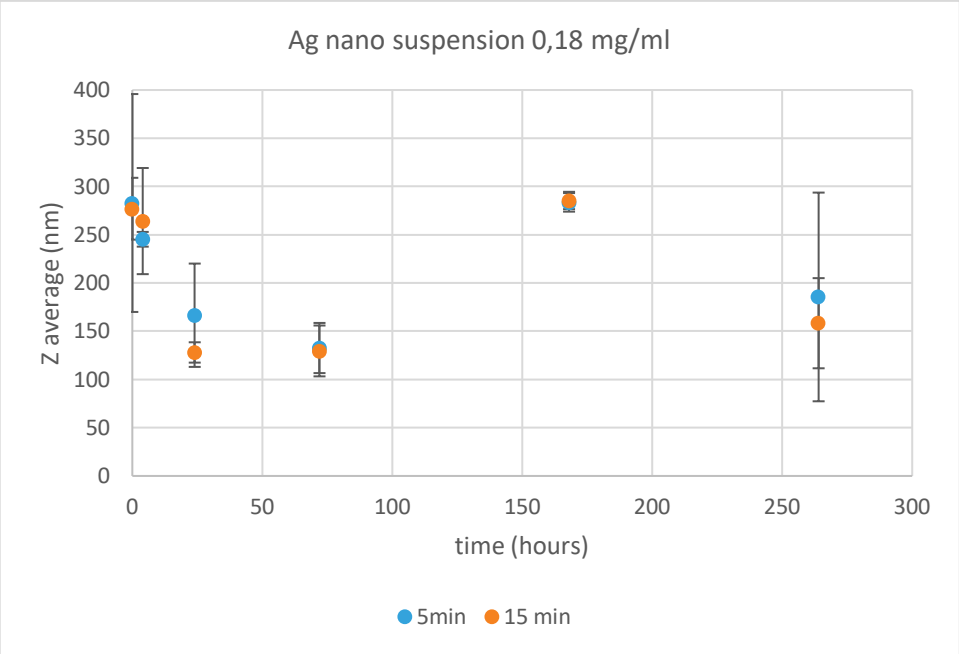


Figure 15: Z average (nm) results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose.

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	67	5	86	4
4	95	12	91	8
24	140	9	115	5
72	78	2	80	32
168	90	20	87	3
264	81	3	84	10

Table 16 : Z average (nm) results of 18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

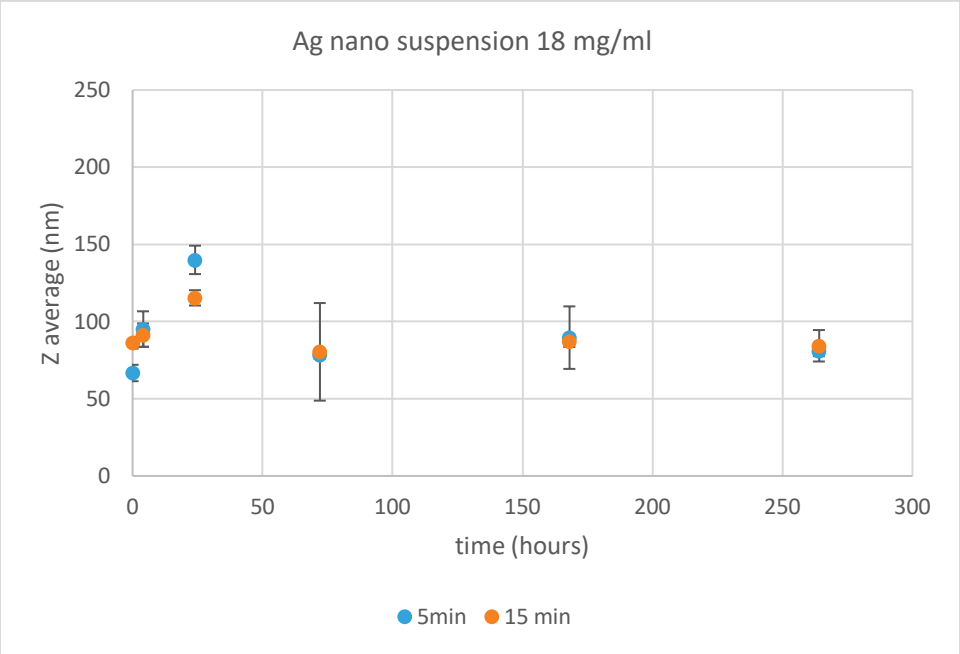


Figure 16: Z average (nm) results of 18 mg/ml Ag nano suspension in 1% methylcellulose.

4.3.2. ZETA POTENTIAL AND PH

The results of the zeta potential measurements are summarized in the tables hereunder.

Time (hours)	Dilution	ZP (mV) 5 min	2 *st.dev.	dilution	ZP (mV) 15 min	2 *st.dev.
0	80	-3,6	0,9	80	-3,4	0,1
24	30	-0,7	0,3	30	-0,8	0,6
264	30	-2,7	0,6	30	-2,7	0,3

Table 17 : Zeta potential results of 1,8 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

Time (hours)	dilution	ZP (mV) 5 min	2 *st.dev.	dilution	ZP (mV) 15 min	2 *st.dev.
0	200	-6,6	0,7	200	1,6	1,7
24	750	-1,2	0,9	1000	-0,7	0,4
264	200	-13,2	0,4	200	-12,0	0,1

Table 18 : Zeta potential results of 50 mg/ml Ag micro powder suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

Time (hours)	dilution	ZP (mV) 5 min	2 *st.dev.	dilution	ZP (mV) 15 min	2 *st.dev.
0	2	-0,3	0,5			
24	-	-0,6	0,0	-	-0,6	0,2
264	-	-0,6	0,1	-	-0,7	0,3

Table 19 : Zeta potential results of 0,18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

Time (hours)	dilution	ZP (mV) 5 min	2 *st.dev.	dilution	ZP (mV) 15 min	2 *st.dev.
0	200	-4,2	1,0	200	-2,8	0,6
24	50	-1,6	0,2	50	-1,5	0,3
264	200	-2,3	0,1	50	-1,7	0,1

Table 20 : Zeta potential results of 18 mg/ml Ag nano suspension in 1% methylcellulose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

The results of the pH measurements are summarized in the table hereunder.

sample	pH 0 hours	pH 272 hours
µAg in 1%MC: 1,8 mg/ml-5' son, 20' roeren	6,98	6,63
µAg in 1%MC: 1,8 mg/ml-15' son, 20' roeren	7,05	6,56
µAg in 1%MC: 50 mg/ml-5' son, 20' roeren	7,16	7,22
µAg in 1%MC: 50 mg/ml-15' son, 20' roeren	7,37	7,18
nAg in 1%MC: 0,18 mg/ml-5' son, 20' roeren	7,48	7,38
nAg in 1%MC: 0,18 mg/ml-15' son, 20' roeren	7,51	7,41
nAg in 1%MC: 18 mg/ml-5' son, 20' roeren	7,51	7,47
nAg in 1%MC: 18 mg/ml-15' son, 20' roeren	7,47	7,46
Control pH 7	7,03	7,02

Table 21 : pH measurements in 1% methyl cellulose solutions.

4.3.3. TOTAL AND IONIC AG CONCENTRATION

For the determination of total Ag in the solutions, a sample of 1 ml was taken whilst magnetically stirring after the treatment of 5 or 15 minutes ultrasonication and at least 20 min stirring. The determination of total Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ After the stirring, a subsample of 1 ml is taken with an automatic pipet (eppendorf research plus 100-1000 µl) and transferred to a 50 ml PP Digitube.
- ✓ 5 ml of nitric Acid 67-69% Optima Grade Fisher Chemicals is added.
- ✓ The tubes are covered with a clear screw cap and placed into a SCP Science Digiprep MS heating block 120 minutes at 105°C.
- ✓ After cooling down the samples are made up to 50 ml with ultrapure water, diluted further in Aqua regia (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

A comparison was made between heating in a heating block for 120 minutes at 105°C and no heating. The results (ratio of result no heating versus result after heating) are summarized in the table hereunder.

sample	Recovery no heating versus heating @105°C

µAg in 1%MC: 1,8 mg/ml	101 ± 1 % (n=3)
µAg in 1%MC: 50 mg/ml	95 ± 5 % (n=3)
nAg in 1%MC: 0,18 mg/ml	103 ± 2 % (n=2)
nAg in 1%MC: 18 mg/ml	89 ± 8 % (n=2)

Table 22 : influence of the heating step in the determination of total Ag.

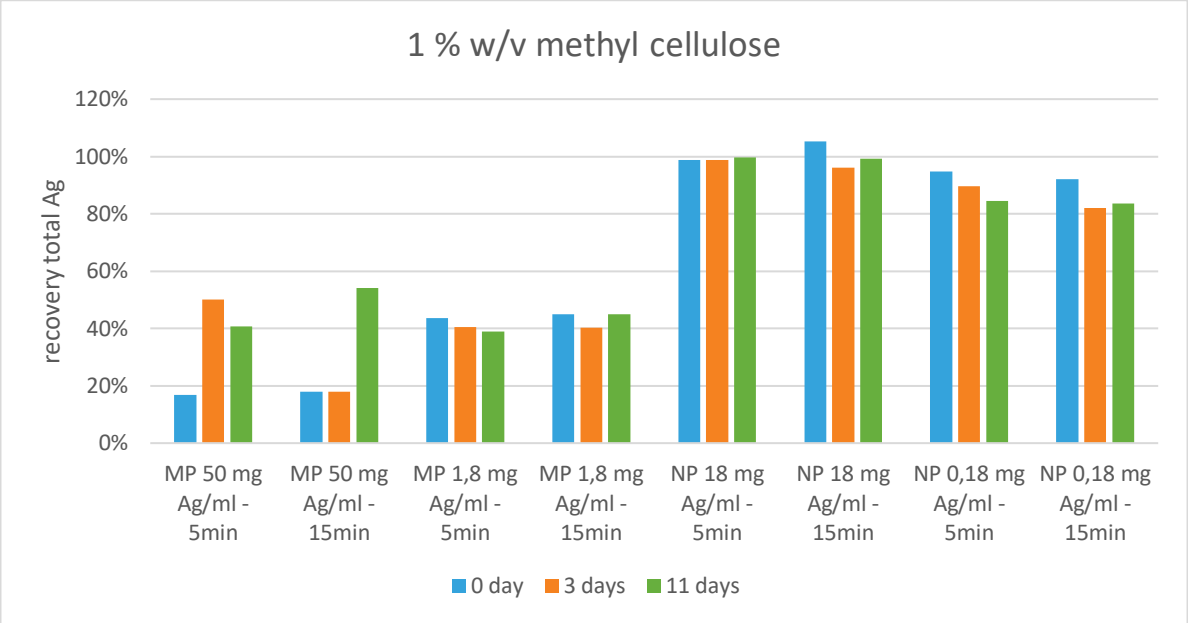


Figure 17: recovery of total Ag from micro powder and nano suspension in 1% methyl cellulose solutions.

The determination of ionic Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ a subsample of 1 ml was taken with an automatic pipet and transferred to a VIVAspin 6 tube.
- ✓ Centrifuge 15 minutes at 4000 rpm and discard the filtrate
- ✓ A subsample of 5 ml was taken with an automatic pipet and transferred to the pre-treated VIVAspin 6 tube.
- ✓ Centrifuge 120 minutes at 4000 rpm and collect the filtrate
- ✓ Add to 500 µl filtrate 500 µl of nitric Acid 67-69% and dilute with Milli-Q water up to 5 ml
- ✓ dilute further in *aqua regia* (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

sample	Day	Ionic fraction µg Ag/l	%
100 mg/ml (product)		5327300	5,3%
18 mg/ml in 1 % MC	11	863960	4,8%
0,18 mg/ml in 1 % MC	11	121	0,1%

Table23 : results of ionic Ag determination (3 kDa filtration + ICP-OES).

4.3.4. SEM/STEM

All individual images are included in Annex B.

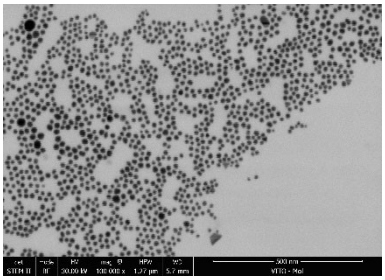


Figure 18: STEM image Ag nanosuspension (Cu grid)

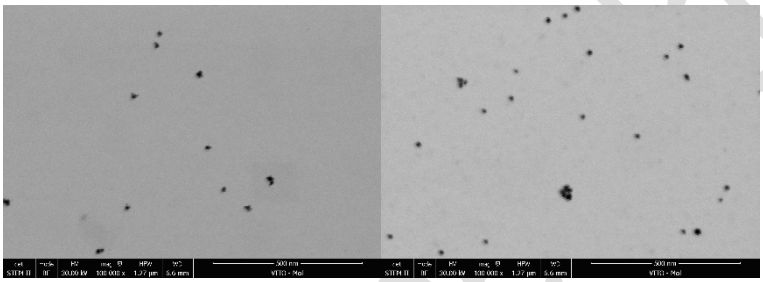


Figure 19: STEM image 0,18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

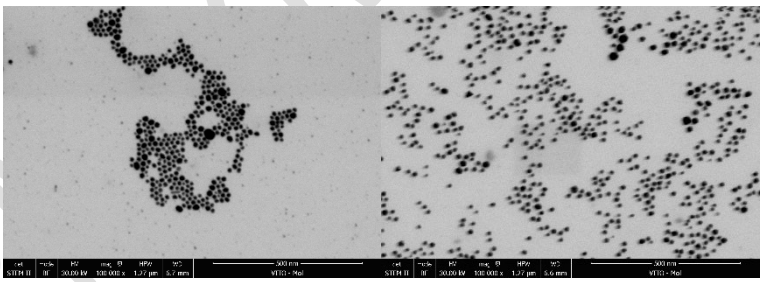


Figure 20: STEM image 18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

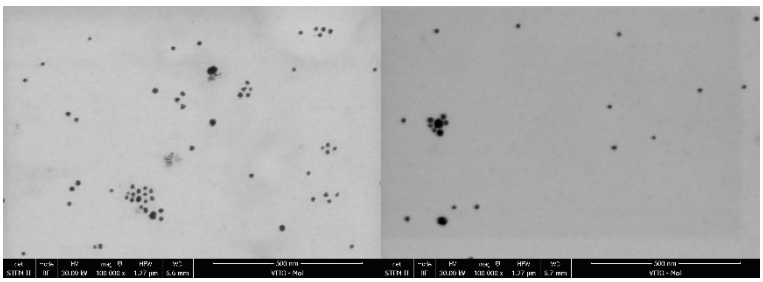


Figure 21: STEM image 0,18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 11, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

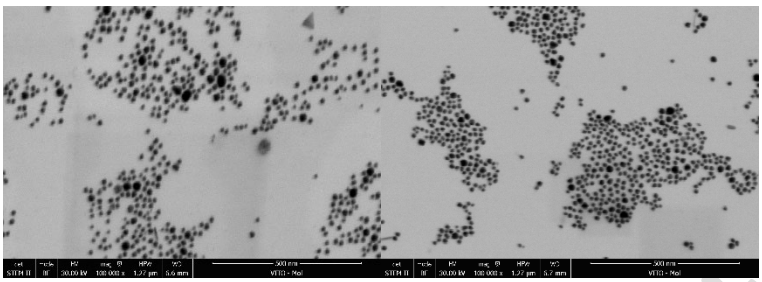


Figure 22: STEM image 18 mg Ag/ml nanosuspension in 1 % MC (Cu grid) day 11 (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

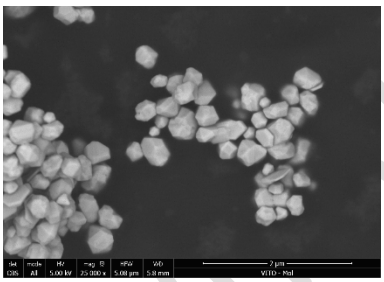


Figure 23: SEM image Ag micro powder

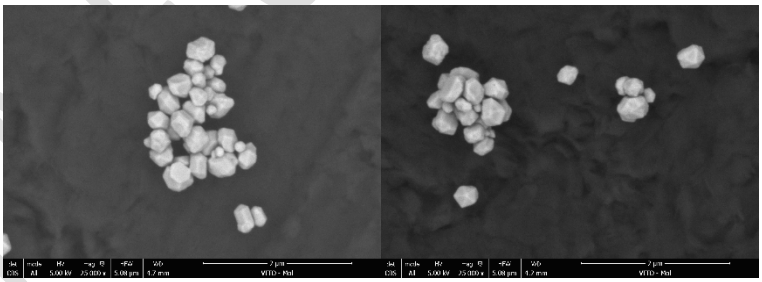


Figure 24: SEM image 1,8 mg Ag/ml micro powder in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

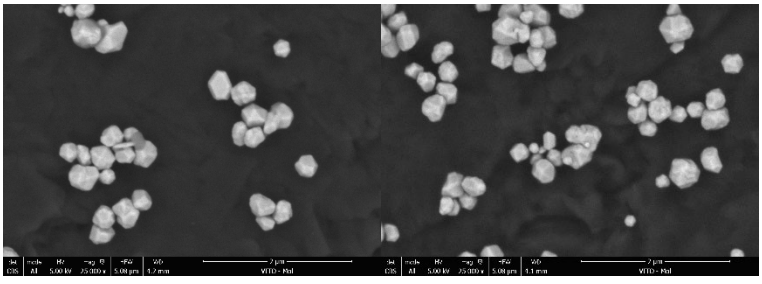


Figure 25: SEM image 50 mg Ag/ml micro powder in 1 % MC (Cu grid) day 0, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

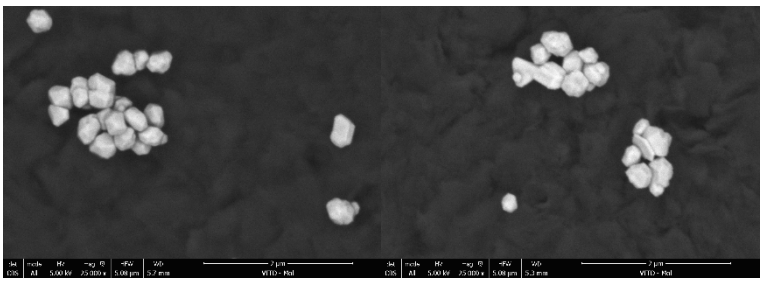


Figure 26: SEM image 1,8 mg Ag/ml micro powder in 1 % MC (Cu grid) day 11, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

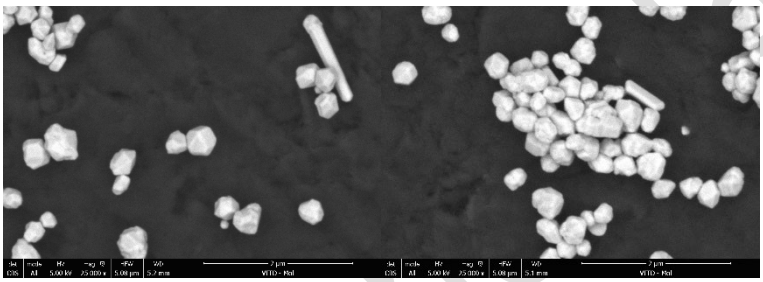


Figure 27: SEM image 50 mg Ag/ml micro powder in 1 % MC (Cu grid) day 11 (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

4.4. RESULTS 5 % W/V GLUCOSE

4.4.1. DLS RESULTS

The individual DLS results are summarized in Annex A and are summarized in the table and figures hereunder.

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	436	20	414	18
4	433	17	395	8
24	401	4	398	10

Table 24 : Z average (nm) results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

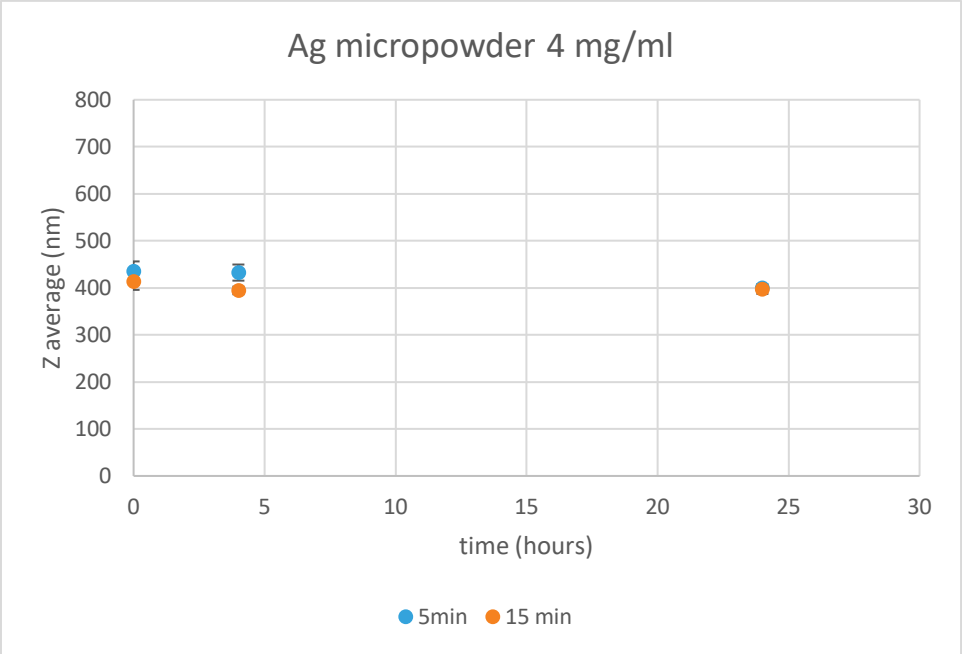


Figure 28: Z average (nm) results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

Time (hours)	Z average (nm) 5 min	2 *st.dev.	Z average (nm) 15 min	2 *st.dev.
0	99	8	106	6
4	100	1	102	28
24	106	4	103	9

Table 25 : Z average (nm) results of 0,5 mg/ml Ag nano suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

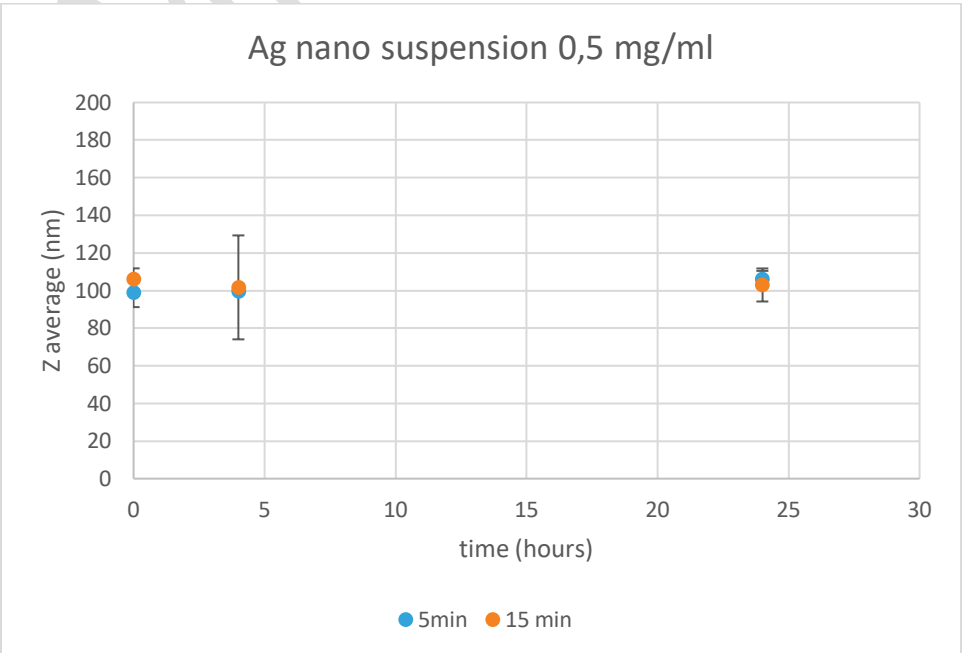


Figure 29: Z average (nm) results of 0,5 mg/ml Ag nano suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

4.4.2. ZETA POTENTIAL AND PH

The results of the zeta potential measurements are summarized in the tables hereunder.

Time (hours)	Dilution	ZP (mV) 5 min	2 *st.dev.	Dilution	ZP (mV) 15 min	2 *st.dev.
0	100	-34,6	1,3	100	-34,0	0,6
24	100	-35,6	0,6	100	-34,2	1,1

Table 26 : Zeta potential results of 4 mg/ml Ag micro powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

Time (hours)	dilution	ZP (mV) 5 min	2 *st.dev.	dilution	ZP (mV) 15 min	2 *st.dev.
0	-	-3,8	0,8	-	-4,1	0,3
24	-	-5,0	0,3	-	-4,7	0,4

Table 27 : Zeta potential results of 0,5 mg/ml Ag nano powder suspension in 5% glucose after resp. 5 minutes ultrasonication and 15 minutes ultrasonication.

The results of the pH measurements are summarized in the table hereunder.

Sample	pH 0 hours	pH 4 hours	pH 24 hours
µAg in 5% glu pH7: 4 mg/ml-5' son, 20' roeren	7,07	/	6,88
µAg in 5% glu pH7: 4 mg/ml-15' son, 20' roeren	6,98	/	6,77
nAg in 5% glu pH7: 0,5 mg/ml-5' son, 20' roeren	7,83	7,81	7,42
nAg in 5% glu pH7: 0,5 mg/ml-15' son, 20' roeren	7,74	7,78	7,48
controle glucose solution	6,93	/	7,02
Controle pH 7	7,01	7,03	7,03

Table 28 : pH measurements in 5% glucose solutions.

4.4.3. TOTAL AND IONIC AG CONCENTRATION

For the determination of total Ag in the solutions, a sample of 1 ml was taken whilst magnetically stirring after the treatment of 5 or 15 minutes ultrasonication and at least 20 min stirring. The determination of total Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ After the stirring, a subsample of 1 ml is taken with an automatic pipet (eppendorf research plus 100-1000 µl) and transferred to a 50 ml PP Digitube.
- ✓ 5 ml of nitric Acid 67-69% Optima Grade Fisher Chemicals is added.
- ✓ The tubes are covered with a clear screw cap and placed into a SCP Science Digiprep MS heating block 120 minutes at 105°C;

- ✓ After cooling down the samples are made up to 50 ml with ultrapure water, diluted further in Aqua regia (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

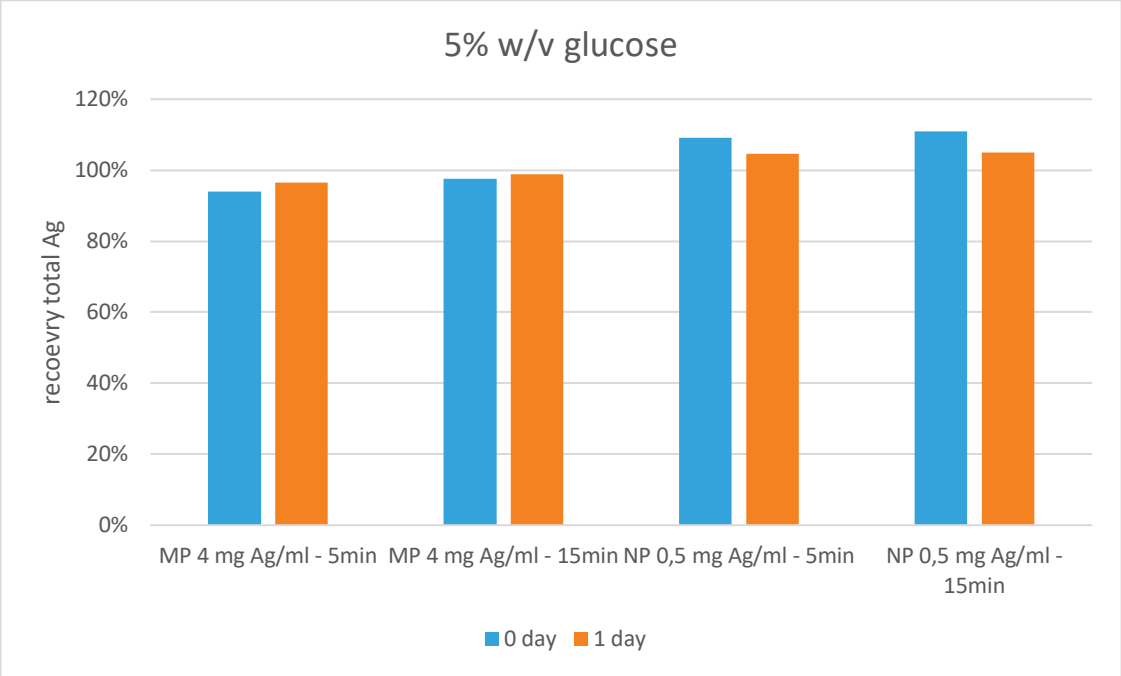


Figure 30: recovery of total Ag from micro powder and nano suspension in 5% glucose solutions.

The determination of ionic Ag was performed by ICP-AES (Agilent 5100 VDV, Ag 328.068 nm, correction with Ge as internal standard) according to the following procedure:

- ✓ a subsample of 1 ml was taken with an automatic pipet and transferred to a VIVAspin 6 tube.
- ✓ Centrifuge 15 minutes at 4000 rpm and discard the filtrate
- ✓ A subsample of 5 ml was taken with an automatic pipet and transferred to the pre-treated VIVAspin 6 tube.
- ✓ Centrifuge 120 minutes at 4000 rpm and collect the filtrate
- ✓ Add to 500 µl filtrate 500 µl of nitric Acid 67-69% and dilute with Milli-Q water up to 5 ml
- ✓ dilute further in *aqua regia* (12 % HCl + 4 % HNO_3) and measured with ICP-AES.

sample	Day	Ionic fraction µg Ag/l	%
NP 100 mg/ml (product)		5327300	5,3%
NP 0,5 mg/ml in 5 % Glu	0	12682	2,5%
NP 0,5 mg/ml in 5 % Glu	1	925	0,2%
MP 4 mg/mL in 5 % Glu	0	41	0,0010%
MP 4 mg/mL in 5 % Glu	1	97	0,0024%

Table29 : results of ionic Ag determination (3 kDa filtration + ICP-OES).

4.4.4. SEM/STEM

All individual images are included in Annex B.

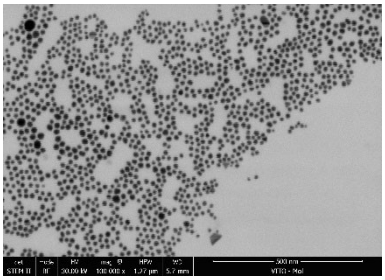


Figure 31: STEM image Ag nanosuspension (Cu grid)

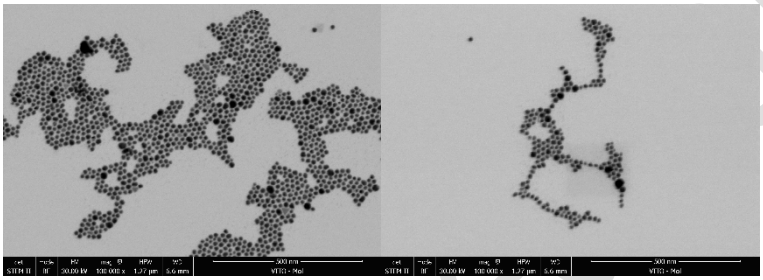


Figure 32: STEM image 0,5 mg Ag/ml nanosuspension in 5 % glucose day 0 (Cu grid), (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

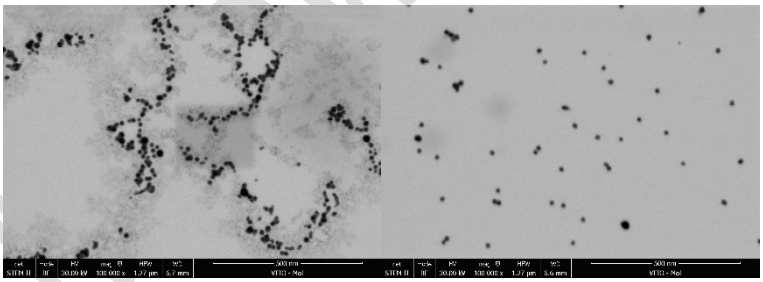


Figure 33: STEM image 0,5 mg Ag/ml nanosuspension in 5 % glucose (Cu grid) day 1, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

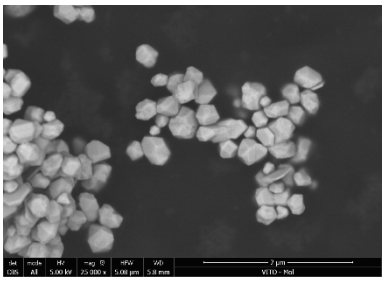


Figure 34: SEM image Ag micro powder

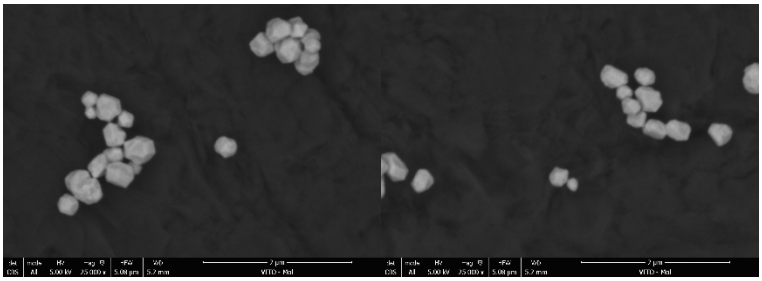


Figure 35: SEM image 4 mg Ag/ml micro powder in 5 % glucose day 0 (Cu grid), (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

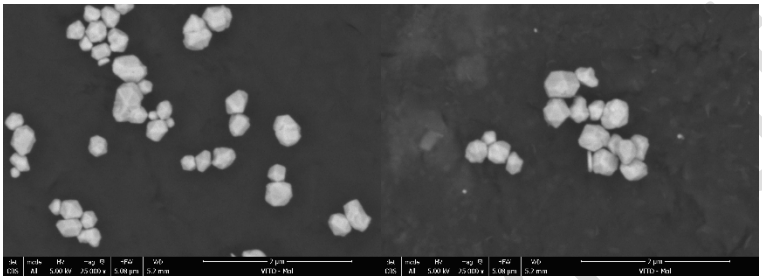


Figure 36: SEM image 4 mg Ag/ml micro powder in 5 % glucose (Cu grid) day 1, (left) 5 minutes ultrasonication, (right) 15 minutes ultrasonication.

CHAPTER 5 SEDIMENTATION STUDY

5.1. INTRODUCTION

The results of total Ag determination of the Ag micro powder in 1 % methyl cellulose indicated possible sedimentation during the preparation of the sample or the resuspension/homogenization process (see § 4.3.3).

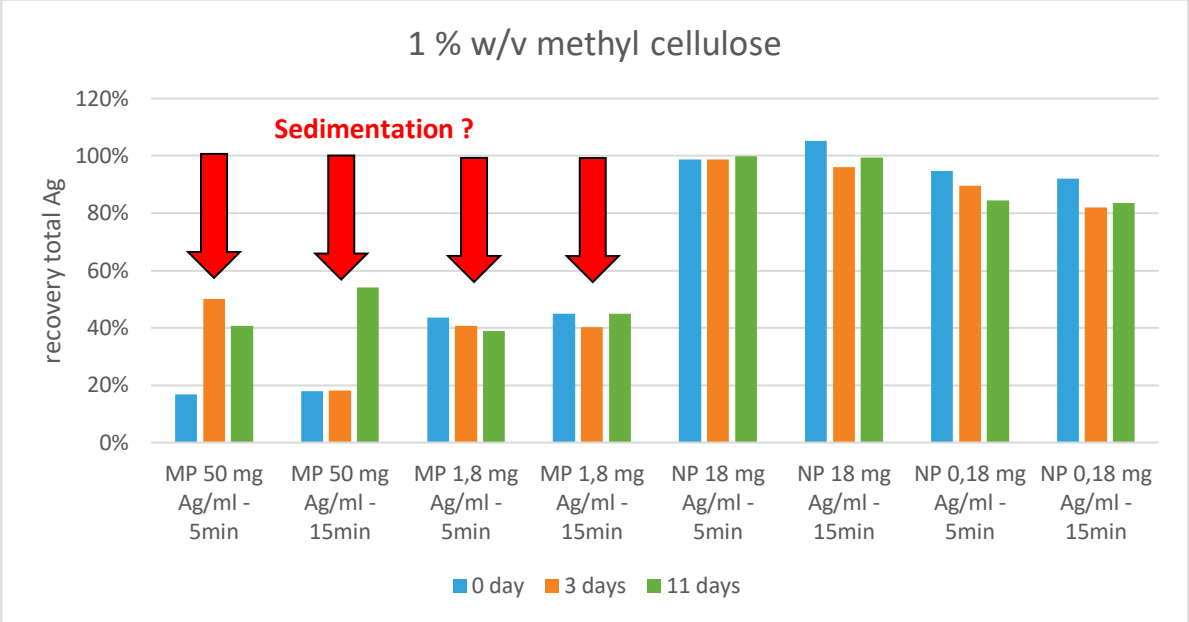


Figure 37: recovery of total Ag from micro powder and nano suspension in 1% methyl cellulose solutions.

To further study the possible sedimentation and improve sample pretreatment (stirring), the supplementary tests were proposed (in duplicate) on Ag micro powder in 1 % methyl cellulose.

- ✓ generate a suspension of µAg (50 mg/mL) in 0,1 % and 1 % MC (400 cP). Ensure the suspension is homogeneous.
- ✓ take a subsample of the homogenous suspensions for total Ag determination
- ✓ then, perform two additional treatments on each suspension:
  1. keep the suspension in a tube roller machine for 1h, resuspend again to homogeneity by magnetic stirring (5 min and 20 min) and take again a sample for total Ag determination at each timepoint
  2. put the suspension in the fridge for at least 12h (24h if possible?), resuspend again by magnetic stirring (20 min) to homogeneity and take a sample for total Ag determination.

For the magnetic stirring - as a rule of thumb - the rpm for stirring should give a vortex of approx. half the depth of the suspension to get a proper resuspension and homogenization.

5.2. RESULTS

The sample preparation showed that at least 4 hours of stirring was necessary to completely dissolve the 1 % MC solution.

Results of the testing are summarized hereunder (procedure and pictures can be found in annex D), all results are expressed in mg/g because the calculations were made using the weight of the 1 ml subsample.

	Volume	Time = 0	Tube roller 1 hour	Tube roller 1 hour	20 hours fridge	4 days fridge
			5 min. stirring	20 min. stirring	20 min. stirring	20 min. stirring
	ml	mg/g Ag	mg/g Ag	mg/g Ag	mg/g Ag	mg/g Ag
1% MC 50 mg/ml µAg bottle A sample 1	400	46,9	47,5	47,2	46,9	46,7
1% MC 50 mg/ml µAg bottle A sample 2	400	46,4	47,1	47,6	47,2	46,2
1% MC 50 mg/ml µAg bottle B sample 1	400	46,1	46,8	46,1	46,7	46,2
1% MC 50 mg/ml µAg bottle B sample 2	400	45,7	46,5	46,6	46,8	45,7

Table 30 : results of total Ag determination in 1% MC (viscosity 400 cP).

	Volume	Time = 0	Tube roller 1 hour	Tube roller 1 hour	20 hours fridge	4 days fridge
			5 min. stirring	20 min. stirring	20 min. stirring	20 min. stirring
	ml	mg/g Ag	mg/g Ag	mg/g Ag	mg/g Ag	mg/g Ag
0.1% MC 50 mg/ml µAg bottle A sample 1	400	45,9	46,0	46,5	46,8	45,6
0.1% MC 50 mg/ml µAg bottle A sample 2	400	45,5	46,4	46,6	45,9	45,8
0.1% MC 50 mg/ml µAg bottle B sample 1	400	45,0	46,5	47,2	46,8	45,9
0.1% MC 50 mg/ml µAg bottle B sample 2	400	45,9	46,1	46,3	46,0	45,6

Table 31 : results of total Ag determination in 0,1% MC (viscosity 400 cP).

The results of the tests show that if stirring is well performed, the suspension can repeatedly be re-homogenised. As the total silver content is constant after the 1h rolling+ resuspension, and after 20h in the fridge and resuspension for both suspensions, it was decided to continue the solubilization work in the 1% MC (400 cP) vehicle.

The following instruction was given for the preparation and resuspension : when a sample is being taken out of the fridge, stir for 20 min at 900 rpm to a homogeneous suspension and sample immediately (i.e., avoiding (partial) settling).

CHAPTER 6 CONCLUSION

In this report the answers for the following questions were substantiated by using different analytical techniques:

- *Is there nano Ag and/or ionic Ag in the AgMP as supplied / when formulated in the vehicles?*
- *Is there ionic Ag in the AgNP as supplied / when formulated in the vehicles?*

In chapter 2 of this report, the results of the physico-chemical characterization (SEM, DLS, XRD, Ag, nano Ag and ionic Ag) of the nano suspension and micro silver powder test items are reported. These results confirm the data related to the expected size obtained from the suppliers. For the AgMP formulated in the vehicles, very small amounts (< 0,01%) of nano and ionic Ag as compared to total Ag are observed. For the Ag nano suspension, the ionic Ag amounted ~ 5 % of the total Ag concentration.

- *What is the sedimentation / agglomeration behavior of the test articles in the test vehicles?*

At the start, the optimal dilution of the AgNP/AgMP to be measured with dynamic light scattering was assessed (chapter 3). Thereafter, the following solutions were prepared for the sedimentation and agglomeration study (chapter 4):

	In 1% MC (oral), 400 ml	In 5% glucose (i.v.), 200 ml
AgMP formulations to be tested	1.8 mg/mL	4 mg/mL
	50 mg/mL	
AgNP formulations to be tested*	0.18 mg/mL	0.5 mg/mL
	18 mg/mL	
Test duration	11 days	24 hours
Test temperature	2 - 8 °C	2 - 8 °C
Timepoints for measurement	0h, 2-6h, 24h, d5, d8, d11	0h, 2-6h, 24h

* conc. expressed as mg Ag/mL (AgNP suspension contains ~10% Ag)

Dynamic light scattering was used to assess changes in particle size at the different time points of AgNP and AgMP in the vehicles after resuspension (ultrasonic bath and stirring). The DLS measurements in the 1 % MC solutions needed to be diluted in water to obtain qualitative results, especially for the AgNP problems of DLS detection were encountered. Overall no differences in average size (DLS) were observed between the treatment of 5 or 15 minutes ultrasonication of the formulations (followed by at least 20 min stirring). This was also confirmed by SEM/STEM images taken at the different timepoints. However, the results of the total Ag determination indicated a low recovery (< 50 %) for the AgMP formulations in 1 % MC, whilst this was not observed for the AgNP in 1 % MC (recovery > 80%). Supplementary tests were performed, showing that the lower recovery was not a function over time, but rather was caused by insufficient stirring while preparing the formulations themselves (see chapter 5). For the formulations prepared in 5 % glucose, the results of the average size (DLS), total Ag and SEM/STEM images indicated no change over the timepoints measured. Only the ionic Ag content in the Ag NP formulation decreased significantly (from 2,5 to 0,2 %).

ANNEX A : DLS

Record	Sample Name	Measurement Date and Time	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
1	ǂAg_1.8mg/ml in1%MC_5' son_20'roeren_80x verd in MQ 1	maandag 3 februari 2020 11:38:42	644,3	0,529	382,6
2	ǂAg_1.8mg/ml in1%MC_5' son_20'roeren_80x verd in MQ 2	maandag 3 februari 2020 11:40:54	601,7	0,511	385,5
3	ǂAg_1.8mg/ml in1%MC_5' son_20'roeren_80x verd in MQ 3	maandag 3 februari 2020 11:43:07	603,3	0,505	352
4	ǂAg_1.8mg/ml in1%MC_15' son_20'roeren_80x verd in MQ 1	maandag 3 februari 2020 11:51:17	652,3	0,512	386,9
5	ǂAg_1.8mg/ml in1%MC_15' son_20'roeren_80x verd in MQ 2	maandag 3 februari 2020 11:53:40	619,2	0,487	376,7
6	ǂAg_1.8mg/ml in1%MC_15' son_20'roeren_80x verd in MQ 3	maandag 3 februari 2020 11:56:02	607,8	0,479	388,7
7	ǂAg_50mg/ml in1%MC_5' son_20'roeren_2000x verd in MQ 1	maandag 3 februari 2020 12:10:19	654,7	0,715	273,6
8	ǂAg_50mg/ml in1%MC_5' son_20'roeren_2000x verd in MQ 2	maandag 3 februari 2020 12:12:22	655,5	0,717	280,9
9	ǂAg_50mg/ml in1%MC_5' son_20'roeren_2000x verd in MQ 3	maandag 3 februari 2020 12:14:24	653,9	0,741	262,2
10	ǂAg_50mg/ml in1%MC_15' son_20'roeren_2000x verd in MQ 1	maandag 3 februari 2020 12:22:00	658,1	0,719	288,8
11	ǂAg_50mg/ml in1%MC_15' son_20'roeren_2000x verd in MQ 2	maandag 3 februari 2020 12:24:03	691,6	0,697	260,8
12	ǂAg_50mg/ml in1%MC_15' son_20'roeren_2000x verd in MQ 3	maandag 3 februari 2020 12:26:05	708,1	0,704	274,1
13	nAg_0.18mg/ml in1%MC_5' son_20'roeren_2x verd in MQ-5'son 1	maandag 3 februari 2020 12:51:19	348	0,727	838,9
14	nAg_0.18mg/ml in1%MC_5' son_20'roeren_2x verd in MQ-5'son 2	maandag 3 februari 2020 12:53:42	253,4	1	1050
15	nAg_0.18mg/ml in1%MC_5' son_20'roeren_2x verd in MQ-5'son 3	maandag 3 februari 2020 12:56:05	247,3	1	1125
22	nAg_0.18mg/ml in1%MC_15' son_20'roeren_2x verd in MQ 1	maandag 3 februari 2020 14:02:52	294	1	1373
23	nAg_0.18mg/ml in1%MC_15' son_20'roeren_2x verd in MQ 2	maandag 3 februari 2020 14:05:15	262,2	1	1242
24	nAg_0.18mg/ml in1%MC_15' son_20'roeren_2x verd in MQ 3	maandag 3 februari 2020 14:07:38	274,7	1	1438
16	nAg_18mg/ml in1%MC_5' son_20'roeren_200x verd in MQ 1	maandag 3 februari 2020 13:35:55	66,89	0,673	378,7
17	nAg_18mg/ml in1%MC_5' son_20'roeren_200x verd in MQ 2	maandag 3 februari 2020 13:38:08	69,22	0,475	344,7
18	nAg_18mg/ml in1%MC_5' son_20'roeren_200x verd in MQ 3	maandag 3 februari 2020 13:40:21	63,92	0,652	386,9
19	nAg_18mg/ml in1%MC_15' son_20'roeren_200x verd in MQ 1	maandag 3 februari 2020 13:46:56	88,44	0,305	87,41
20	nAg_18mg/ml in1%MC_15' son_20'roeren_200x verd in MQ 2	maandag 3 februari 2020 13:48:58	84,85	0,295	91,08
21	nAg_18mg/ml in1%MC_15' son_20'roeren_200x verd in MQ 3	maandag 3 februari 2020 13:51:01	85,63	0,293	89,75
Record	Sample Name	Measurement Date and Time	Z-Ave (d.nm)	PdI	Intensity Mean (d.nm)
49	ǂAg_1,8mg/ml in 1%MC-5'son-20'roer-80x verd in MQ-4h 1	maandag 3 februari 2020 16:12:23	598,1	0,408	425,4
50	ǂAg_1,8mg/ml in 1%MC-5'son-20'roer-80x verd in MQ-4h 2	maandag 3 februari 2020 16:14:35	597,3	0,485	390,9
51	ǂAg_1,8mg/ml in 1%MC-5'son-20'roer-80x verd in MQ-4h 3	maandag 3 februari 2020 16:16:48	582,2	0,419	408,6
52	ǂAg_1,8mg/ml in 1%MC-15'son-20'roer-80x verd in MQ-4h 1	maandag 3 februari 2020 16:31:08	626,7	0,414	439,3
53	ǂAg_1,8mg/ml in 1%MC-15'son-20'roer-80x verd in MQ-4h 2	maandag 3 februari 2020 16:33:30	617,3	0,427	413,7
54	ǂAg_1,8mg/ml in 1%MC-15'son-20'roer-80x verd in MQ-4h 3	maandag 3 februari 2020 16:35:53	613,2	0,45	404,1

55	ǂAg_50mg/ml in 1%MC-5'son-20'roer-2000x verd in MQ-4h 1	maandag 3 februari 2020 16:43:56	**740,6**	0,839	262
56	ǂAg_50mg/ml in 1%MC-5'son-20'roer-2000x verd in MQ-4h 2	maandag 3 februari 2020 16:46:09	**734,5**	0,736	260,3
57	ǂAg_50mg/ml in 1%MC-5'son-20'roer-2000x verd in MQ-4h 3	maandag 3 februari 2020 16:48:22	**793,2**	0,705	247,1
58	ǂAg_50mg/ml in 1%MC-15'son-20'roer-2000x verd in MQ-4h 1	maandag 3 februari 2020 16:53:43	**703,2**	0,764	288,8
59	ǂAg_50mg/ml in 1%MC-15'son-20'roer-2000x verd in MQ-4h 2	maandag 3 februari 2020 16:55:46	**678**	0,742	268,9
60	ǂAg_50mg/ml in 1%MC-15'son-20'roer-2000x verd in MQ-4h 3	maandag 3 februari 2020 16:57:49	**654,2**	0,651	268,7
61	nAg_0.18mg/ml in 1%MC-5'son-20'roer-2x verd in MQ-4h 1	maandag 3 februari 2020 17:06:03	**489,7**	0,707	334,5
62	nAg_0.18mg/ml in 1%MC-5'son-20'roer-2x verd in MQ-4h 2	maandag 3 februari 2020 17:08:36	**248**	1	950,4
63	nAg_0.18mg/ml in 1%MC-5'son-20'roer-2x verd in MQ-4h 3	maandag 3 februari 2020 17:11:09	**242,6**	1	1165
64	nAg_0.18mg/ml in 1%MC-15'son-20'roer-2x verd in MQ-4h 1	maandag 3 februari 2020 17:18:18	**263,5**	1	1203
65	nAg_0.18mg/ml in 1%MC-15'son-20'roer-2x verd in MQ-4h 2	maandag 3 februari 2020 17:20:21	**237**	1	1281
66	nAg_0.18mg/ml in 1%MC-15'son-20'roer-2x verd in MQ-4h 3	maandag 3 februari 2020 17:22:24	**292**	1	1089
67	nAg_18mg/ml in 1%MC-5'son-20'roer-200x verd in MQ-4h 1	maandag 3 februari 2020 17:30:55	**94,2**	0,27	87,88
68	nAg_18mg/ml in 1%MC-5'son-20'roer-200x verd in MQ-4h 2	maandag 3 februari 2020 17:33:28	**101,3**	0,238	82,14
69	nAg_18mg/ml in 1%MC-5'son-20'roer-200x verd in MQ-4h 3	maandag 3 februari 2020 17:36:01	**89,89**	0,26	86,25
70	nAg_18mg/ml in 1%MC-15'son-20'roer-200x verd in MQ-4h 1	maandag 3 februari 2020 17:42:01	**91,52**	0,345	218,1
71	nAg_18mg/ml in 1%MC-15'son-20'roer-200x verd in MQ-4h 2	maandag 3 februari 2020 17:44:35	**94,88**	0,275	103,2
72	nAg_18mg/ml in 1%MC-15'son-20'roer-200x verd in MQ-4h 3	maandag 3 februari 2020 17:47:08	**87,36**	0,373	200,5
Record	Sample Name	Measurement Date and Time	**Z-Ave (d.nm)**	PdI	Intensity Mean (d.nm)
4	ǂAg_1.8mg/ml 1%MC_5'son-20'roeren_30x verd_24h 1	dinsdag 4 februari 2020 11:41:25	**622,1**	0,347	476,6
5	ǂAg_1.8mg/ml 1%MC_5'son-20'roeren_30x verd_24h 2	dinsdag 4 februari 2020 11:43:48	**617,1**	0,302	528,8
6	ǂAg_1.8mg/ml 1%MC_5'son-20'roeren_30x verd_24h 3	dinsdag 4 februari 2020 11:46:10	**595,8**	0,271	528,4
13	ǂAg_1.8mg/ml in MC_15'son-20'roer-30x ve_5'roerrd 1	dinsdag 4 februari 2020 12:17:24	**590,4**	0,237	601,3
14	ǂAg_1.8mg/ml in MC_15'son-20'roer-30x ve_5'roerrd 2	dinsdag 4 februari 2020 12:19:47	**587,3**	0,197	651,9
15	ǂAg_1.8mg/ml in MC_15'son-20'roer-30x ve_5'roerrd 3	dinsdag 4 februari 2020 12:22:10	**573,5**	0,201	661,2
22	ǂAg_50mg/ml-24h-5'son-20'stir-1000xverd 1	dinsdag 4 februari 2020 12:58:02	**560,7**	0,516	336,3
23	ǂAg_50mg/ml-24h-5'son-20'stir-1000xverd 2	dinsdag 4 februari 2020 13:00:35	**545,8**	0,465	340,5
24	ǂAg_50mg/ml-24h-5'son-20'stir-1000xverd 3	dinsdag 4 februari 2020 13:03:08	**528,7**	0,398	361,2
31	ǂAg-50mg/ml-MC-24h-15'son-+20'roer-1000x verd 1	dinsdag 4 februari 2020 13:38:08	**576,9**	0,509	353,8
32	ǂAg-50mg/ml-MC-24h-15'son-+20'roer-1000x verd 2	dinsdag 4 februari 2020 13:40:52	**569**	0,478	349,3
33	ǂAg-50mg/ml-MC-24h-15'son-+20'roer-1000x verd 3	dinsdag 4 februari 2020 13:43:35	**545,8**	0,418	380,3
43	nAg_0.18mg/ml inMC-24h-5'son-20'roer-5x verd-5'roer 1	dinsdag 4 februari 2020 14:23:31	**164,3**	0,64	260,8
44	nAg_0.18mg/ml inMC-24h-5'son-20'roer-5x verd-5'roer 2	dinsdag 4 februari 2020 14:25:54	**141**	0,911	493,9
45	nAg_0.18mg/ml inMC-24h-5'son-20'roer-5x verd-5'roer 3	dinsdag 4 februari 2020 14:28:17	**194,4**	0,55	333,3
48	nAg-0.18mg/ml-15'son-20'roer-4x verd-5'roer 1	dinsdag 4 februari 2020 14:46:01	**129,3**	1	555,4
49	nAg-0.18mg/ml-15'son-20'roer-4x verd-5'roer 2	dinsdag 4 februari 2020 14:48:14	**132,5**	1	586

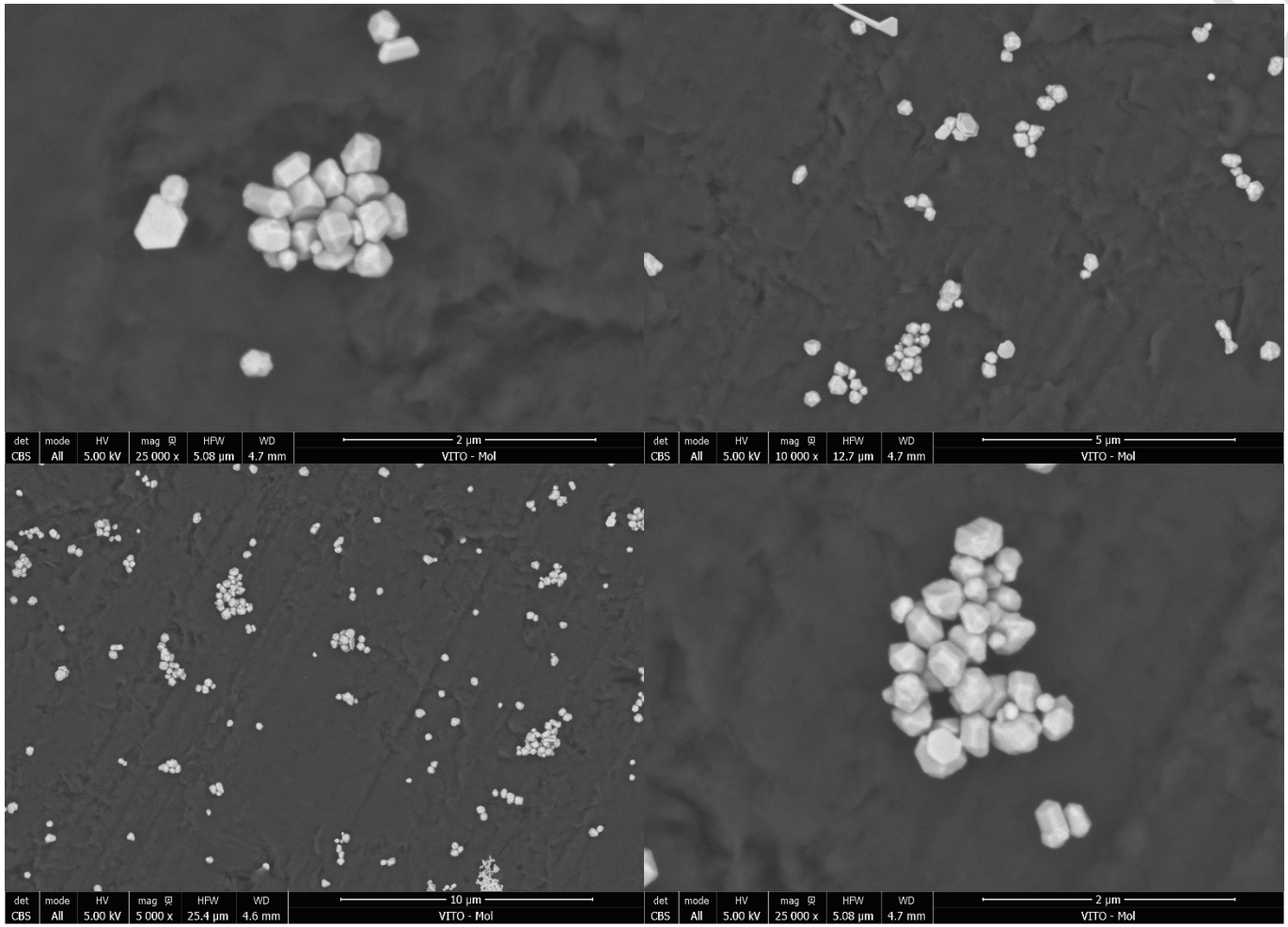
50	nAg-0.18mg/ml-15'son-20'roer-4x verd-5'roer 3	dinsdag 4 februari 2020 14:50:27	**122,2**	1	499,5
57	nAg_18mg/ml-5'son-20'roer-200xverd 1	dinsdag 4 februari 2020 15:21:23	**144,1**	0,317	139,6
58	nAg_18mg/ml-5'son-20'roer-200xverd 2	dinsdag 4 februari 2020 15:23:36	**135**	0,305	148,2
59	nAg_18mg/ml-5'son-20'roer-200xverd 3	dinsdag 4 februari 2020 15:25:49	**140,8**	0,268	145,4
69	nAg-18mg/ml-15'son-20'roer-200x verd 1	dinsdag 4 februari 2020 16:08:50	**115,8**	0,834	568,5
70	nAg-18mg/ml-15'son-20'roer-200x verd 2	dinsdag 4 februari 2020 16:11:23	**112,7**	0,9	587,2
71	nAg-18mg/ml-15'son-20'roer-200x verd 3	dinsdag 4 februari 2020 16:13:56	**117,6**	0,842	715
Record	Sample Name	Measurement Date and Time	**Z-Ave (d.nm)**	PdI	Intensity Mean (d.nm)
1	ǂAg_1.8mg/ml_5'son-20'roeren-30x verd 1	donderdag 6 februari 2020 12:05:07	**662,7**	0,363	526,8
2	ǂAg_1.8mg/ml_5'son-20'roeren-30x verd 2	donderdag 6 februari 2020 12:07:29	**644,9**	0,363	492,5
3	ǂAg_1.8mg/ml_5'son-20'roeren-30x verd 3	donderdag 6 februari 2020 12:09:52	**635,3**	0,341	521,8
4	ǂAg_1.8mg/ml_15'son-20'roeren-30x verd 1	donderdag 6 februari 2020 12:16:36	**675,6**	0,318	552,9
5	ǂAg_1.8mg/ml_15'son-20'roeren-30x verd 2	donderdag 6 februari 2020 12:18:58	**665,6**	0,333	549,1
6	ǂAg_1.8mg/ml_15'son-20'roeren-30x verd 3	donderdag 6 februari 2020 12:21:21	**647,6**	0,247	610,6
7	ǂAg_50mg/ml_5'son-20'roeren-1000x verd 1	donderdag 6 februari 2020 12:37:18	**474,9**	0,289	402,8
8	ǂAg_50mg/ml_5'son-20'roeren-1000x verd 2	donderdag 6 februari 2020 12:39:41	**476,5**	0,267	421,4
9	ǂAg_50mg/ml_5'son-20'roeren-1000x verd 3	donderdag 6 februari 2020 12:42:03	**474,2**	0,292	421,6
10	ǂAg_50mg/ml_15'son-20'roeren-1000x verd 1	donderdag 6 februari 2020 12:50:14	**627,8**	0,515	352,6
11	ǂAg_50mg/ml_15'son-20'roeren-1000x verd 2	donderdag 6 februari 2020 12:52:47	**613,7**	0,454	366,2
12	ǂAg_50mg/ml_15'son-20'roeren-1000x verd 3	donderdag 6 februari 2020 12:55:20	**594,3**	0,406	406,8
13	nAg_0.18mg/ml_5'son-20'roeren-4x verd-'roere 1	donderdag 6 februari 2020 13:30:12	**139,7**	0,924	558,5
14	nAg_0.18mg/ml_5'son-20'roeren-4x verd-'roere 2	donderdag 6 februari 2020 13:32:25	**140,5**	1	628,2
15	nAg_0.18mg/ml_5'son-20'roeren-4x verd-'roere 3	donderdag 6 februari 2020 13:34:37	**117,6**	1	530,1
16	nAg_0.18mg/ml_15'son-20'roeren-4x verd-5' roer 1	donderdag 6 februari 2020 13:43:18	**143,2**	0,969	590,3
17	nAg_0.18mg/ml_15'son-20'roeren-4x verd-5' roer 2	donderdag 6 februari 2020 13:45:41	**128,6**	1	719,5
18	nAg_0.18mg/ml_15'son-20'roeren-4x verd-5' roer 3	donderdag 6 februari 2020 13:48:04	**116,9**	0,946	510,2
19	nAg_18mg/ml_5'son-20'roeren-200x verd 1	donderdag 6 februari 2020 14:02:00	**77,4**	0,633	416,1
20	nAg_18mg/ml_5'son-20'roeren-200x verd 2	donderdag 6 februari 2020 14:04:03	**79,04**	0,652	419,9
21	nAg_18mg/ml_5'son-20'roeren-200x verd 3	donderdag 6 februari 2020 14:06:05	**125,9**	0,458	673,6
22	nAg_18mg/ml_15'son-20'roeren-200x verd 1	donderdag 6 februari 2020 14:12:04	**87,64**	0,209	77,5
23	nAg_18mg/ml_15'son-20'roeren-200x verd 2	donderdag 6 februari 2020 14:14:37	**91,27**	0,186	82,2
24	nAg_18mg/ml_15'son-20'roeren-200x verd 3	donderdag 6 februari 2020 14:17:10	**62,26**	0,502	194
Record	Sample Name	Measurement Date and Time	**Z-Ave (d.nm)**	PdI	Intensity Mean (d.nm)

7	ǂAg_1.8mg/ml; 5'son-20'roeren, 30x verd-hermeting 1	maandag 10 februari 2020 12:07:49	**628,8**	0,349	524,4
8	ǂAg_1.8mg/ml; 5'son-20'roeren, 30x verd-hermeting 2	maandag 10 februari 2020 12:10:12	**644,7**	0,351	524
9	ǂAg_1.8mg/ml; 5'son-20'roeren, 30x verd-hermeting 3	maandag 10 februari 2020 12:12:35	**614,4**	0,293	502,4
4	ǂAg_1.8mg/ml; 15'son-20'roeren, 30x verd 1	maandag 10 februari 2020 11:56:06	**711,2**	0,414	520,3
5	ǂAg_1.8mg/ml; 15'son-20'roeren, 30x verd 2	maandag 10 februari 2020 11:58:39	**708,5**	0,395	530,9
6	ǂAg_1.8mg/ml; 15'son-20'roeren, 30x verd 3	maandag 10 februari 2020 12:01:12	**699,2**	0,405	521,6
10	ǂAg_50mg/ml; 5'son-20'roeren, 1000x verd 1	maandag 10 februari 2020 12:18:54	**506,8**	0,379	396
11	ǂAg_50mg/ml; 5'son-20'roeren, 1000x verd 2	maandag 10 februari 2020 12:21:27	**495,8**	0,369	383,4
12	ǂAg_50mg/ml; 5'son-20'roeren, 1000x verd 3	maandag 10 februari 2020 12:24:00	**494,9**	0,358	378,6
13	ǂAg_50mg/ml; 15'son-20'roeren, 1000x verd 1	maandag 10 februari 2020 12:30:23	**520,4**	0,387	403,8
14	ǂAg_50mg/ml; 15'son-20'roeren, 1000x verd 2	maandag 10 februari 2020 12:32:55	**532,9**	0,418	374,2
15	ǂAg_50mg/ml; 15'son-20'roeren, 1000x verd 3	maandag 10 februari 2020 12:35:28	**503,6**	0,369	380,1
31	nAg_0.18mg/ml; 5'son-20'roeren, 4x verd-herrmeting 1	maandag 10 februari 2020 14:08:06	**287,5**	0,469	259
32	nAg_0.18mg/ml; 5'son-20'roeren, 4x verd-herrmeting 2	maandag 10 februari 2020 14:10:19	**278,2**	0,392	295,3
33	nAg_0.18mg/ml; 5'son-20'roeren, 4x verd-herrmeting 3	maandag 10 februari 2020 14:12:32	**285**	0,397	292,3
34	nAg_0.18mg/ml; 15'son-20'roeren, 4x verd-herrmeting 1	maandag 10 februari 2020 14:18:05	**281,8**	0,395	270,7
35	nAg_0.18mg/ml; 15'son-20'roeren, 4x verd-herrmeting 2	maandag 10 februari 2020 14:20:18	**284,2**	0,392	279,2
36	nAg_0.18mg/ml; 15'son-20'roeren, 4x verd-herrmeting 3	maandag 10 februari 2020 14:22:31	**290,5**	0,402	273,4
22	nAg_18mg/ml; 5'son-20'roeren, 200x verd 1	maandag 10 februari 2020 13:32:32	**82,04**	0,328	156,9
23	nAg_18mg/ml; 5'son-20'roeren, 200x verd 2	maandag 10 februari 2020 13:34:55	**101,1**	0,238	73,86
24	nAg_18mg/ml; 5'son-20'roeren, 200x verd 3	maandag 10 februari 2020 13:37:18	**85,65**	0,21	86,11
28	nAg_18mg/ml; 15'son-20'roeren, 200x verd-herrmeting 1	maandag 10 februari 2020 13:56:41	**85,02**	0,299	94,37
29	nAg_18mg/ml; 15'son-20'roeren, 200x verd-herrmeting 2	maandag 10 februari 2020 13:59:14	**87,64**	0,302	162,7
30	nAg_18mg/ml; 15'son-20'roeren, 200x verd-herrmeting 3	maandag 10 februari 2020 14:01:47	**88,25**	0,252	90,26
Record	Sample Name	Measurement Date and Time	**Z-Ave (d.nm)**	PdI	Intensity Mean (d.nm)
1	ǂAg_1.8mg/ml; 5'son-20'roeren-30x verd 1	donderdag 13 februari 2020 10:30:06	**675,1**	0,39	494,6
2	ǂAg_1.8mg/ml; 5'son-20'roeren-30x verd 2	donderdag 13 februari 2020 10:32:28	**651,7**	0,325	538,2
3	ǂAg_1.8mg/ml; 5'son-20'roeren-30x verd 3	donderdag 13 februari 2020 10:34:51	**654,3**	0,265	641,5
4	ǂAg_1.8mg/ml;15'son-20'roeren-30x verd 1	donderdag 13 februari 2020 10:48:59	**662,1**	0,309	557,2
5	ǂAg_1.8mg/ml;15'son-20'roeren-30x verd 2	donderdag 13 februari 2020 10:51:21	**641,3**	0,263	611,7
6	ǂAg_1.8mg/ml;15'son-20'roeren-30x verd 3	donderdag 13 februari 2020 10:53:44	**648**	0,183	670,4
15	ǂAg-50mg/ml;5'son-20'roer-1000xverd-herm 1	donderdag 13 februari 2020 11:34:48	**502**	0,285	431,8
16	ǂAg-50mg/ml;5'son-20'roer-1000xverd-herm 2	donderdag 13 februari 2020 11:37:32	**482,5**	0,269	457,5
17	ǂAg-50mg/ml;5'son-20'roer-1000xverd-herm 3	donderdag 13 februari 2020 11:40:15	**486,7**	0,203	499,8

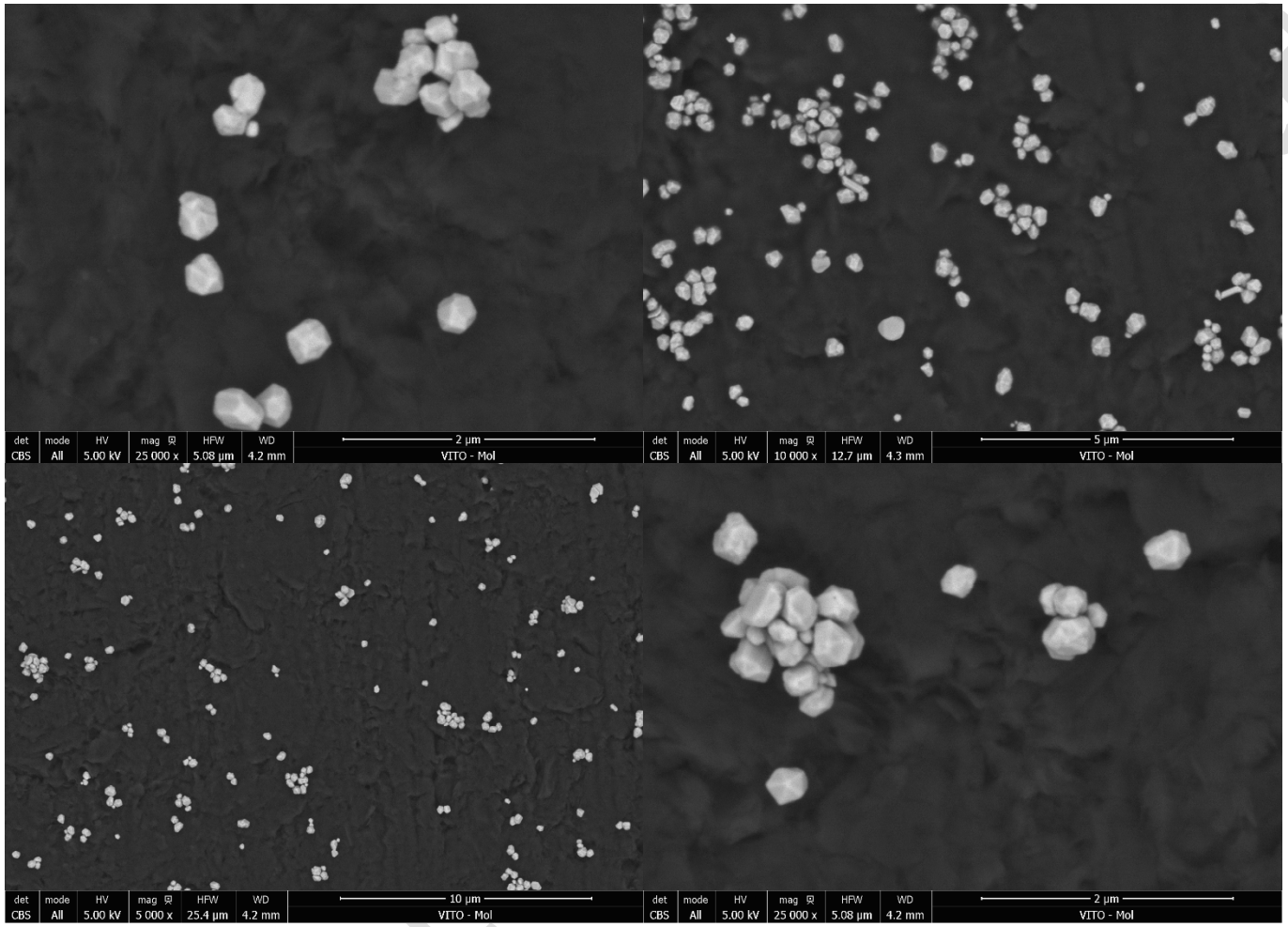
18	ﾅAg-50mg/ml;15'son-20'roer-1000xverd 1	donderdag 13 februari 2020 11:45:56	**500,6**	0,225	456,7
19	ﾅAg-50mg/ml;15'son-20'roer-1000xverd 2	donderdag 13 februari 2020 11:48:19	**500,8**	0,221	513,5
20	ﾅAg-50mg/ml;15'son-20'roer-1000xverd 3	donderdag 13 februari 2020 11:50:42	**500,2**	0,209	605,3
27	nAg_0.18mg/ml: 5'son-20'roer-4xverd-5'roer 1	donderdag 13 februari 2020 13:00:59	**171,3**	0,682	463,8
28	nAg_0.18mg/ml: 5'son-20'roer-4xverd-5'roer 2	donderdag 13 februari 2020 13:03:12	**140**	0,94	623,7
29	nAg_0.18mg/ml: 5'son-20'roer-4xverd-5'roer 3	donderdag 13 februari 2020 13:05:25	**245,3**	0,416	292,1
33	nAg 0.18mg/ml;15'son-20'roer-4xverd-5'roer-d10 1	donderdag 13 februari 2020 13:27:34	**181,3**	0,712	526,8
34	nAg 0.18mg/ml;15'son-20'roer-4xverd-5'roer-d10 2	donderdag 13 februari 2020 13:29:47	**159**	0,873	583,6
35	nAg 0.18mg/ml;15'son-20'roer-4xverd-5'roer-d10 3	donderdag 13 februari 2020 13:31:59	**134,6**	1	707,7
39	nAg-18mg/ml-5'son-20'roer-200xverd-10d 1	donderdag 13 februari 2020 14:05:33	**82,51**	0,29	154,8
40	nAg-18mg/ml-5'son-20'roer-200xverd-10d 2	donderdag 13 februari 2020 14:07:46	**79,91**	0,286	183,2
41	nAg-18mg/ml-5'son-20'roer-200xverd-10d 3	donderdag 13 februari 2020 14:09:58	**79,75**	0,287	88,98
44	nAg-18mg/ml-15'son-20'roer-200xverd-10d-herm 1	donderdag 13 februari 2020 14:24:04	**81,66**	0,242	76,11
45	nAg-18mg/ml-15'son-20'roer-200xverd-10d-herm 2	donderdag 13 februari 2020 14:26:06	**81,13**	0,241	74,63
46	nAg-18mg/ml-15'son-20'roer-200xverd-10d-herm 3	donderdag 13 februari 2020 14:28:09	**90,2**	0,182	73,13

ANNEX B: SEM / STEM

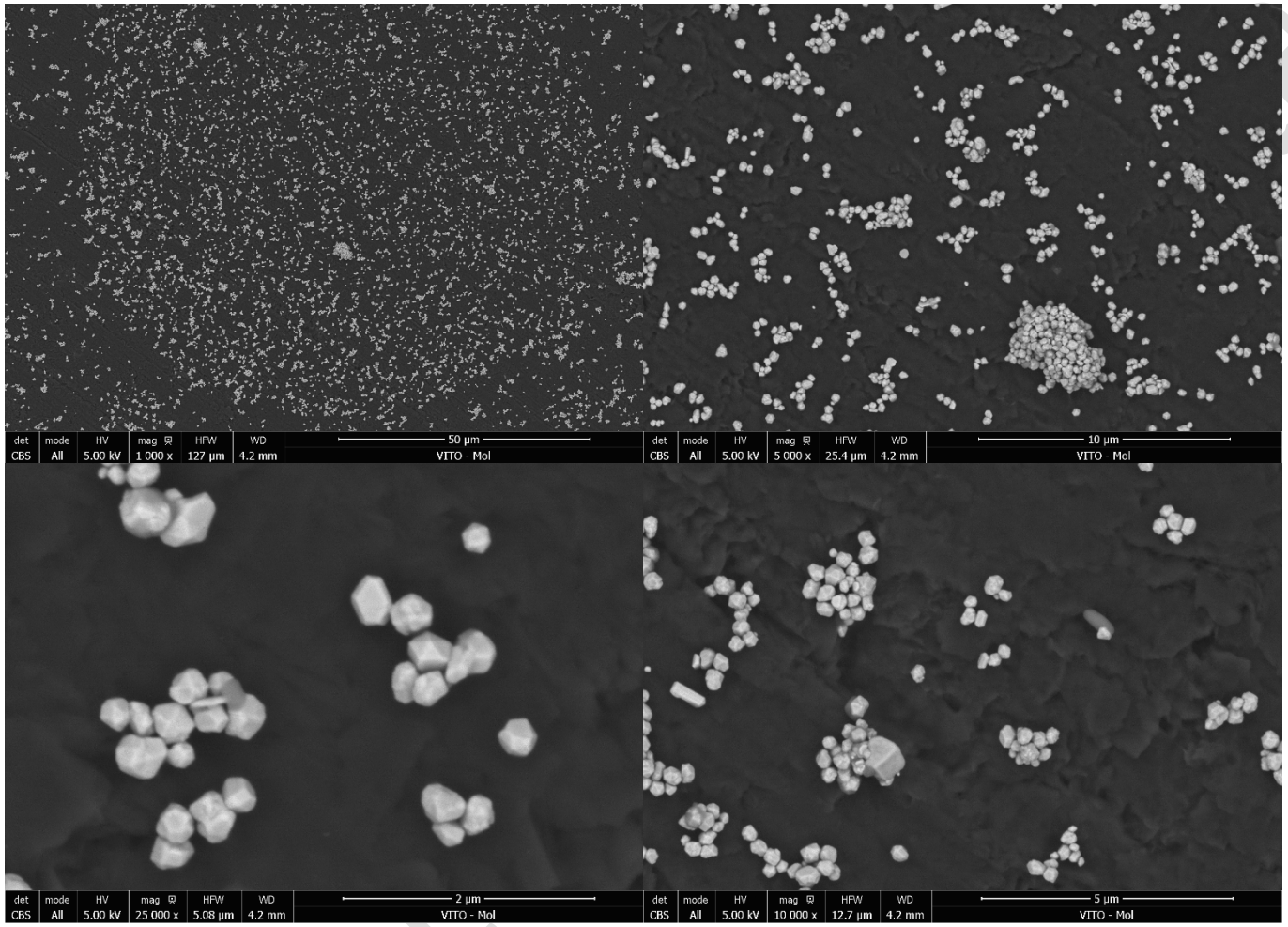
1 % w/v methyl cellulose 1,8 mg/l Ag micropowder (5 minutes ultrasonication), time point 0 h.



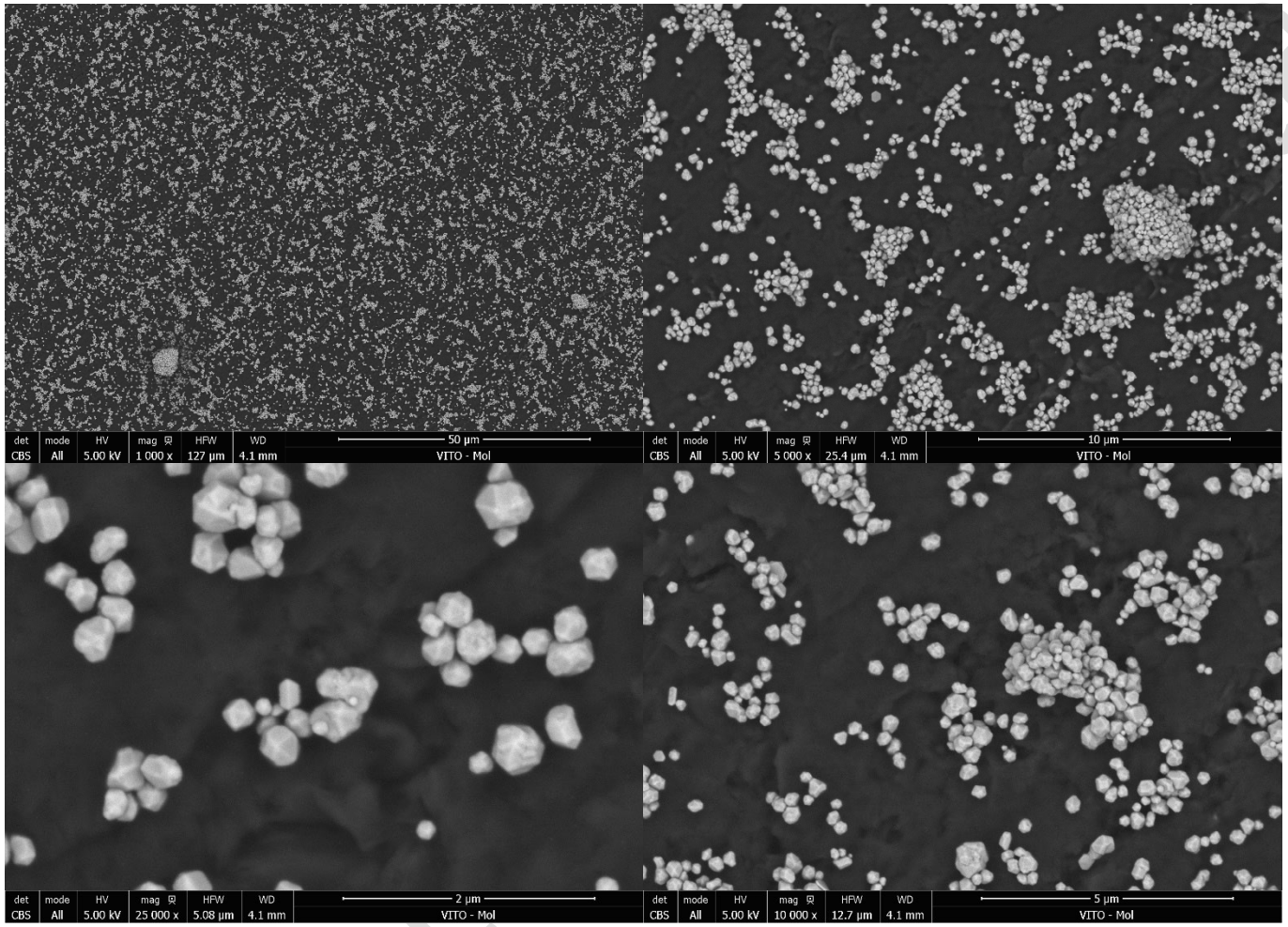
1 % w/v methyl cellulose 1,8 mg/l Ag micropowder (15 minutes ultrasonication), time point 0 h.



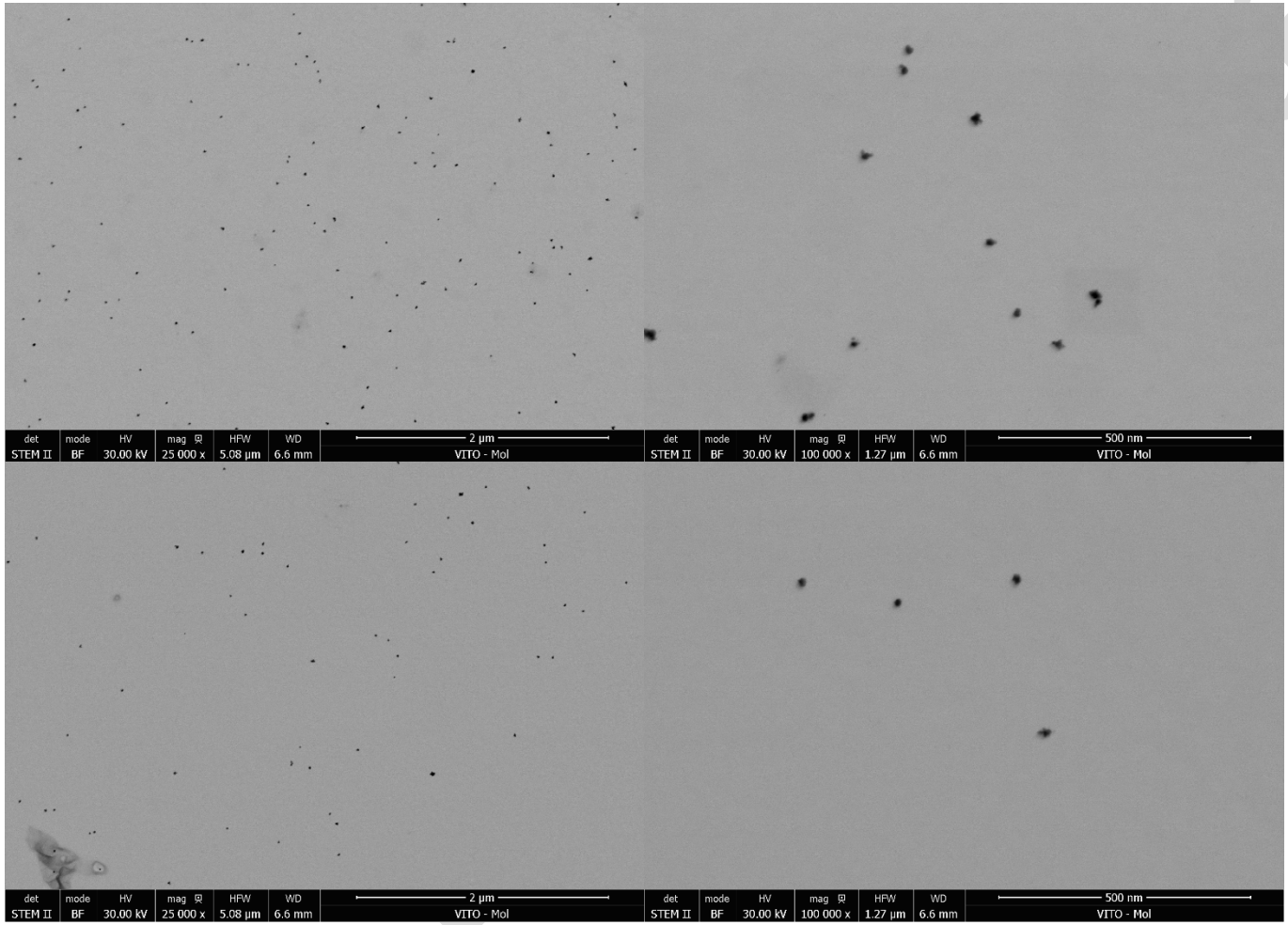
1 % w/v methyl cellulose 50 mg/l Ag micropowder (5 minutes ultrasonication), time point 0 h.



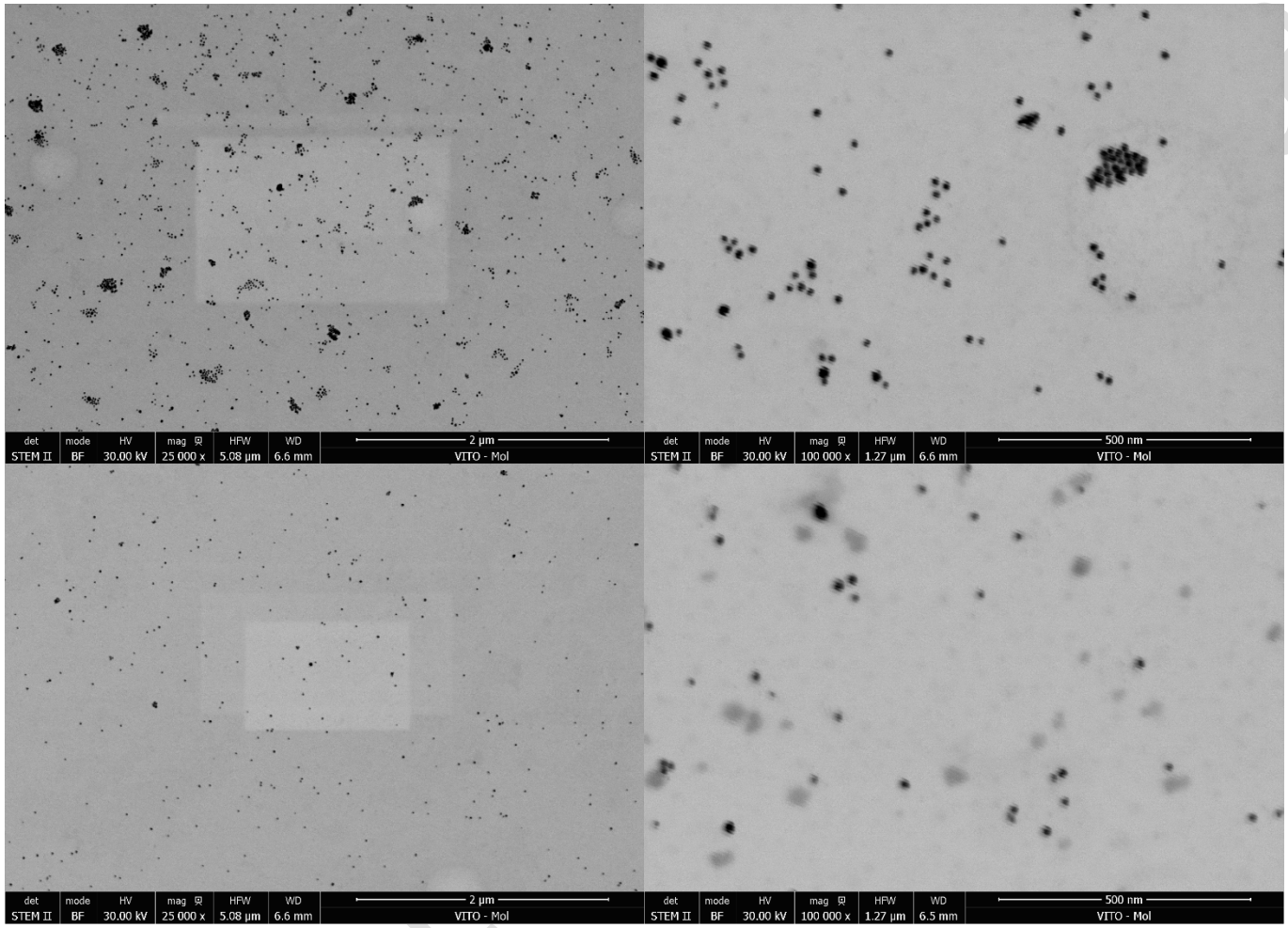
1 % w/v methyl cellulose 50 mg/l Ag micropowder (15 minutes ultrasonication), time point 0 h.



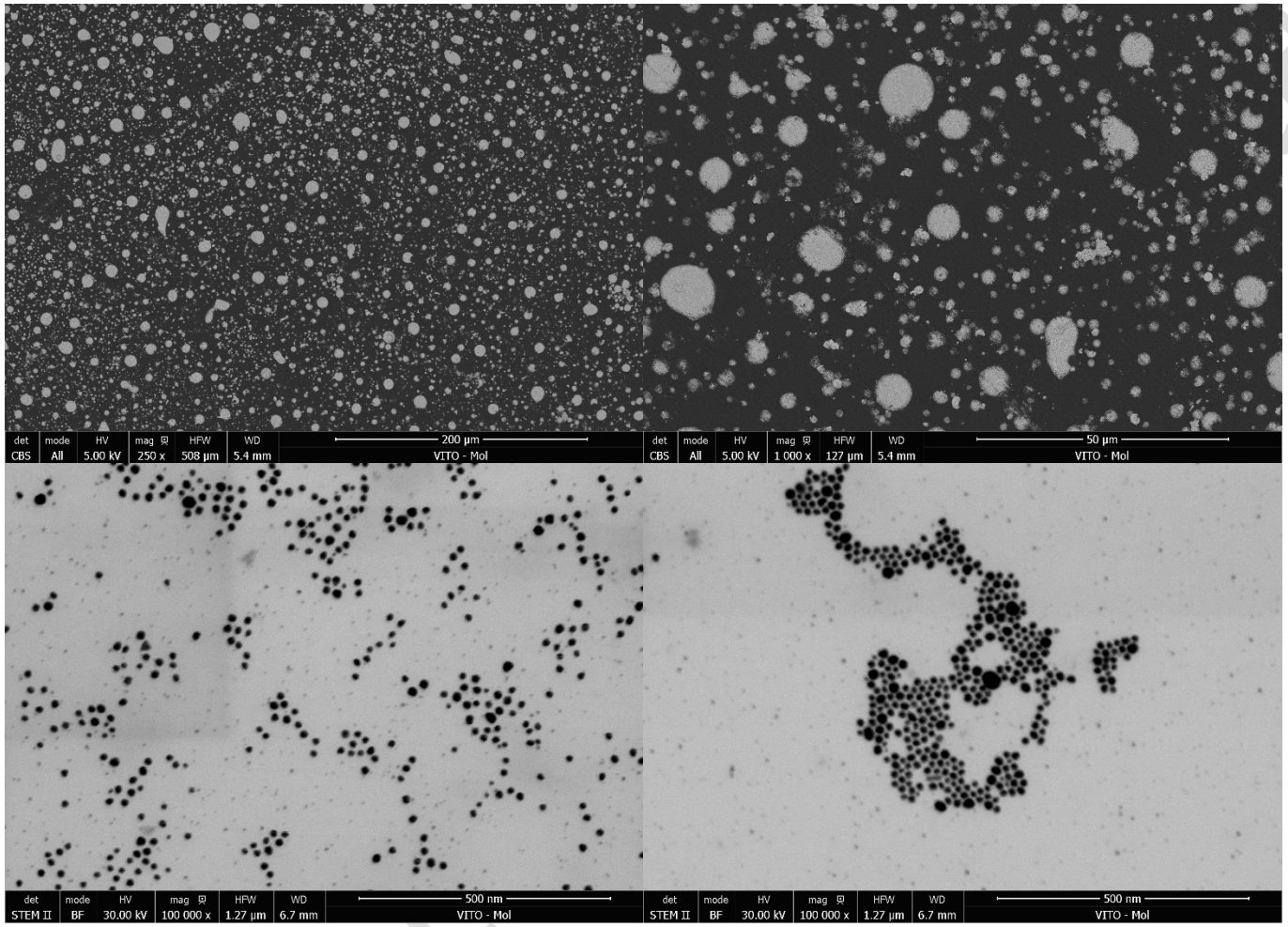
1 % w/v methyl cellulose 0,18 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 0 h.



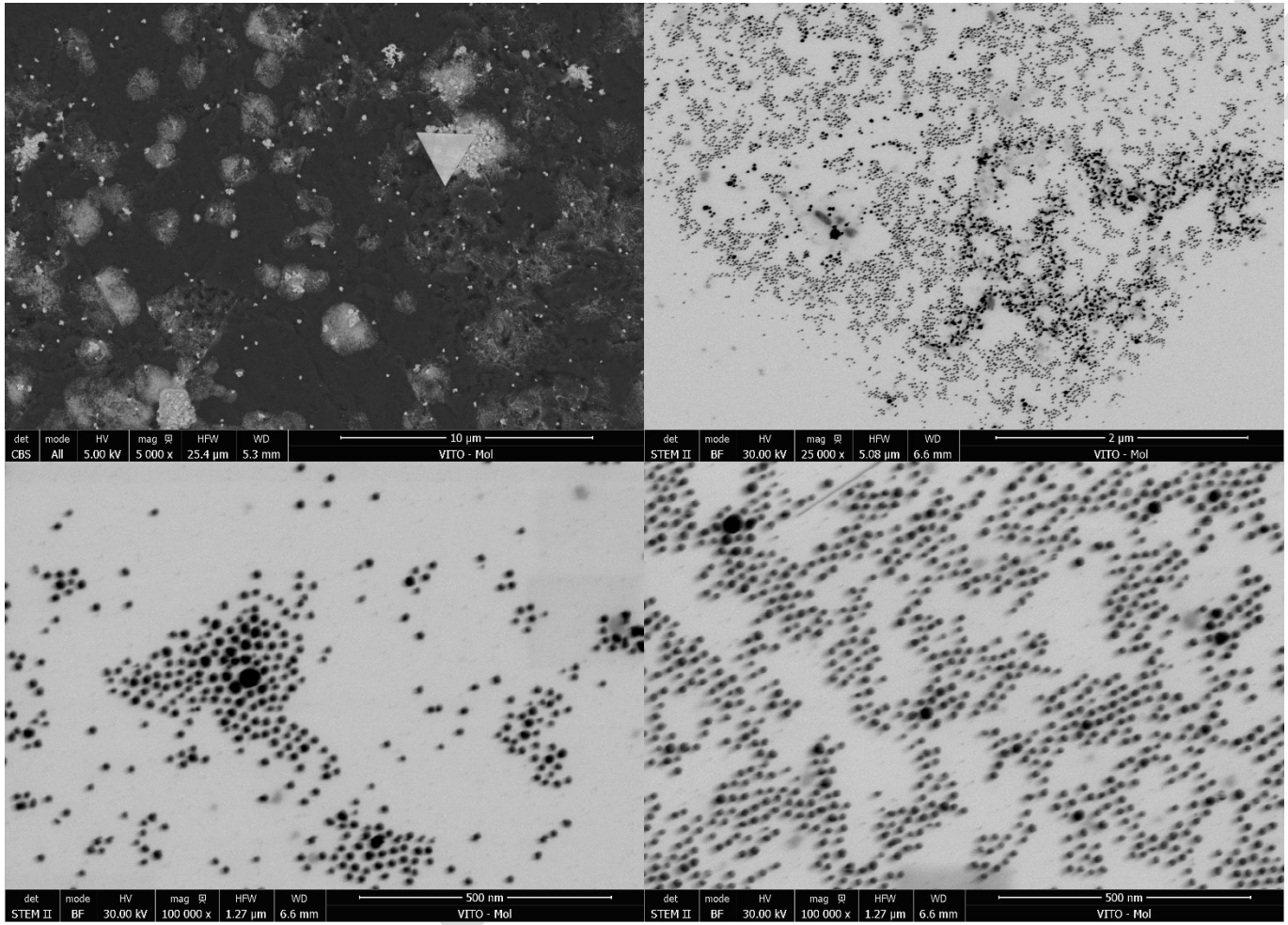
1 % w/v methyl cellulose 0,18 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 0 h.



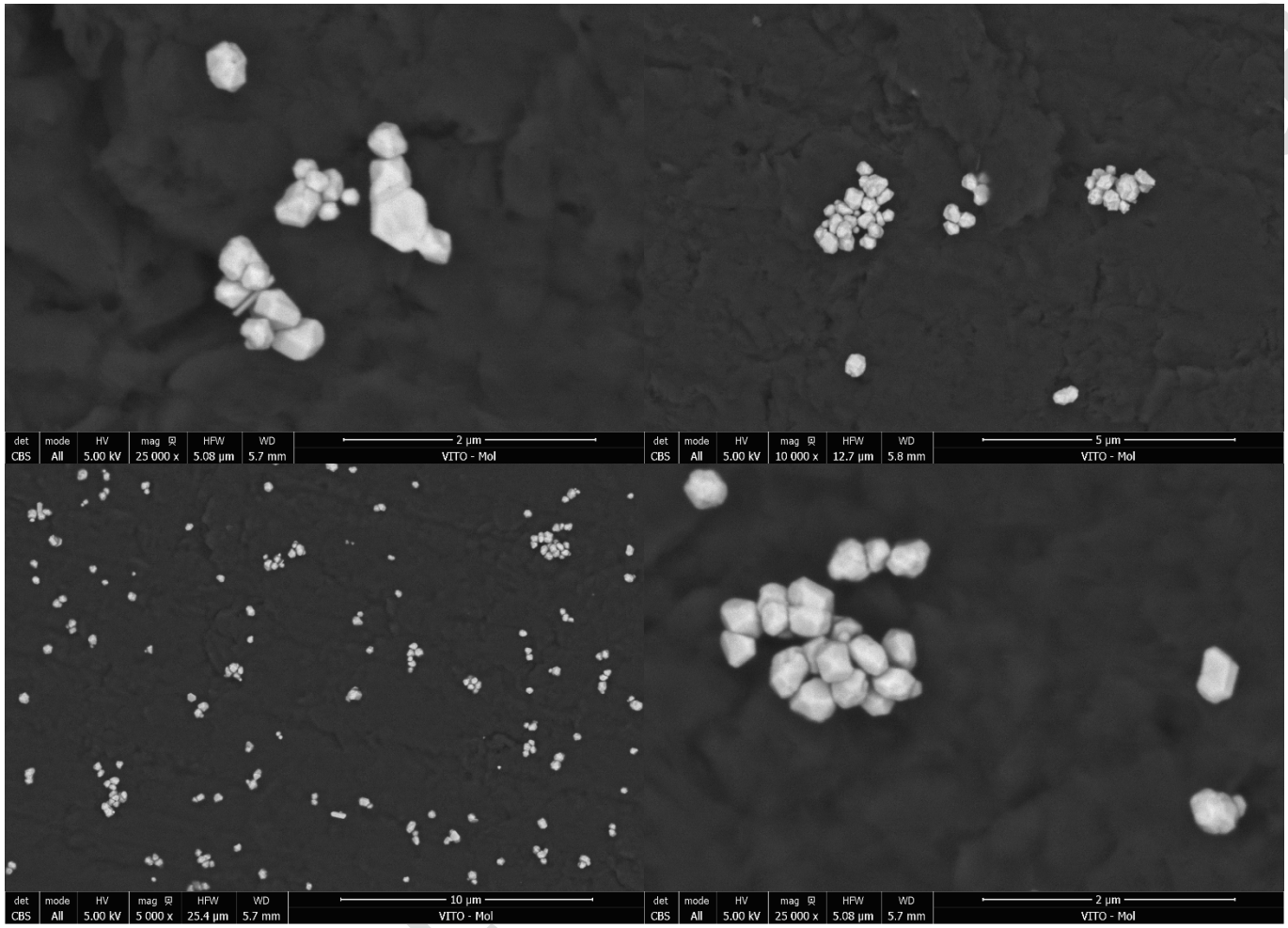
1 % w/v methyl cellulose 18 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 0 h.



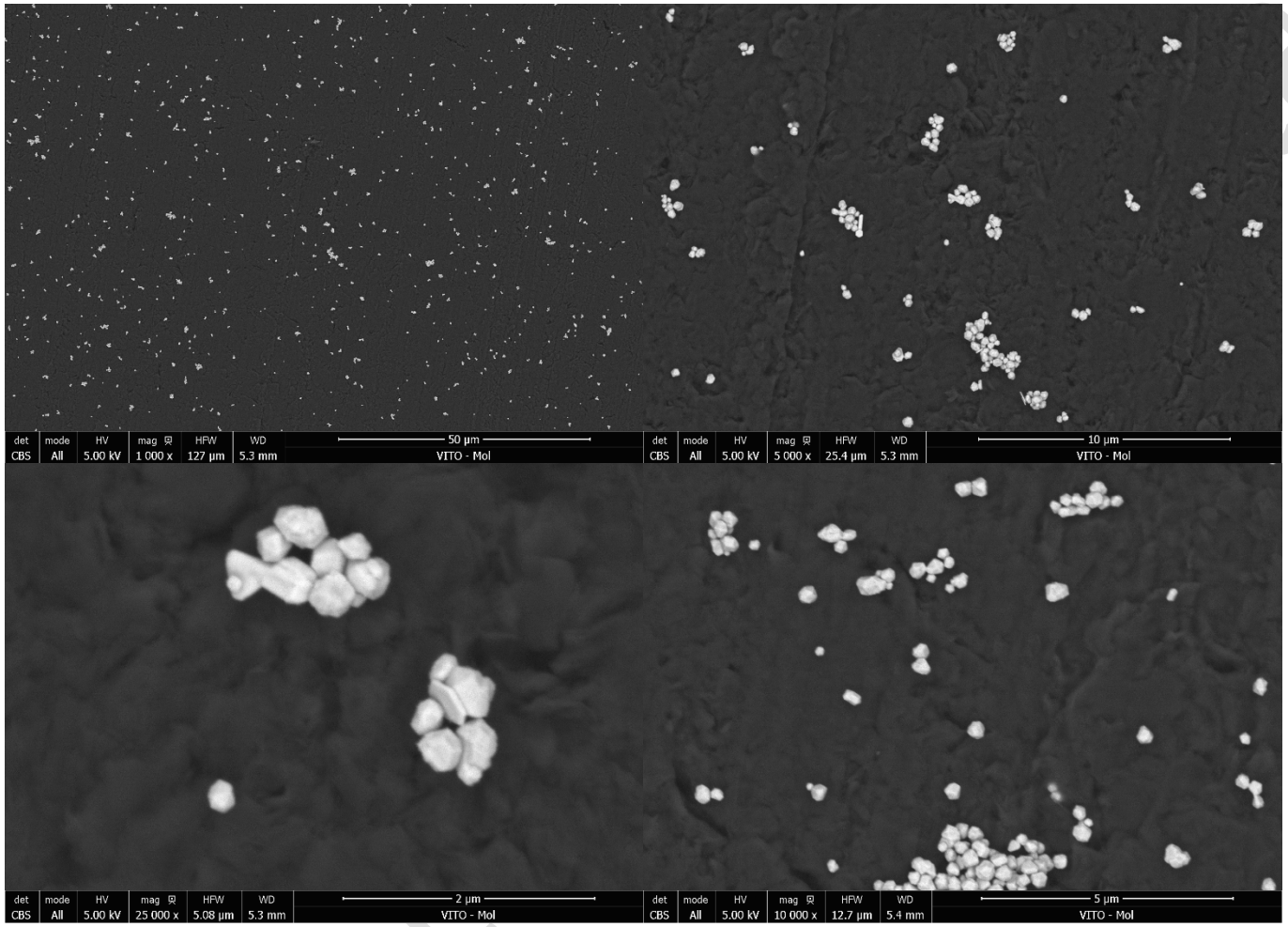
1 % w/v methyl cellulose 18 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 0 h.



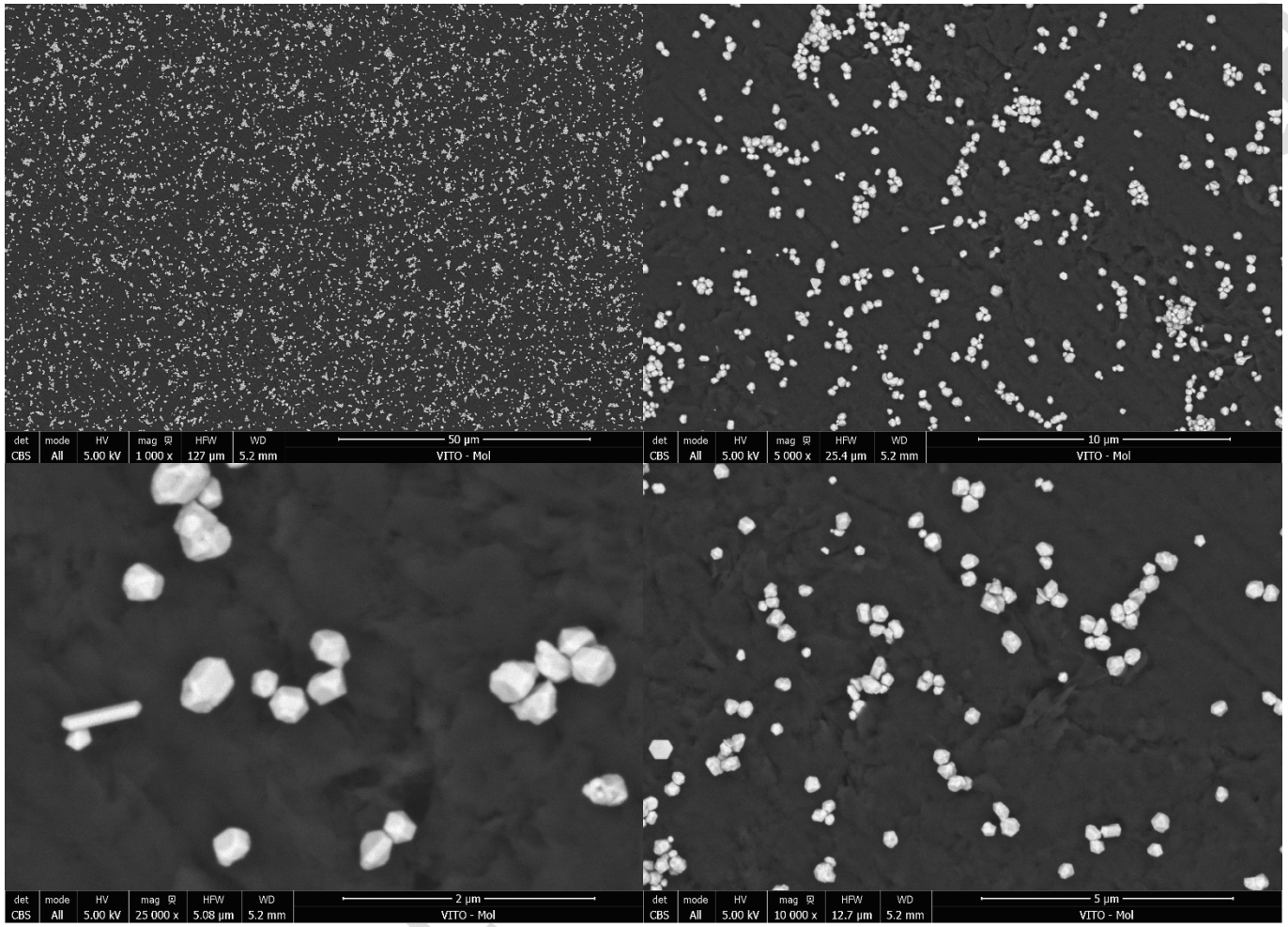
1 % w/v methyl cellulose 1,8 mg/l Ag micropowder (5 minutes ultrasonication), time point 264 h.



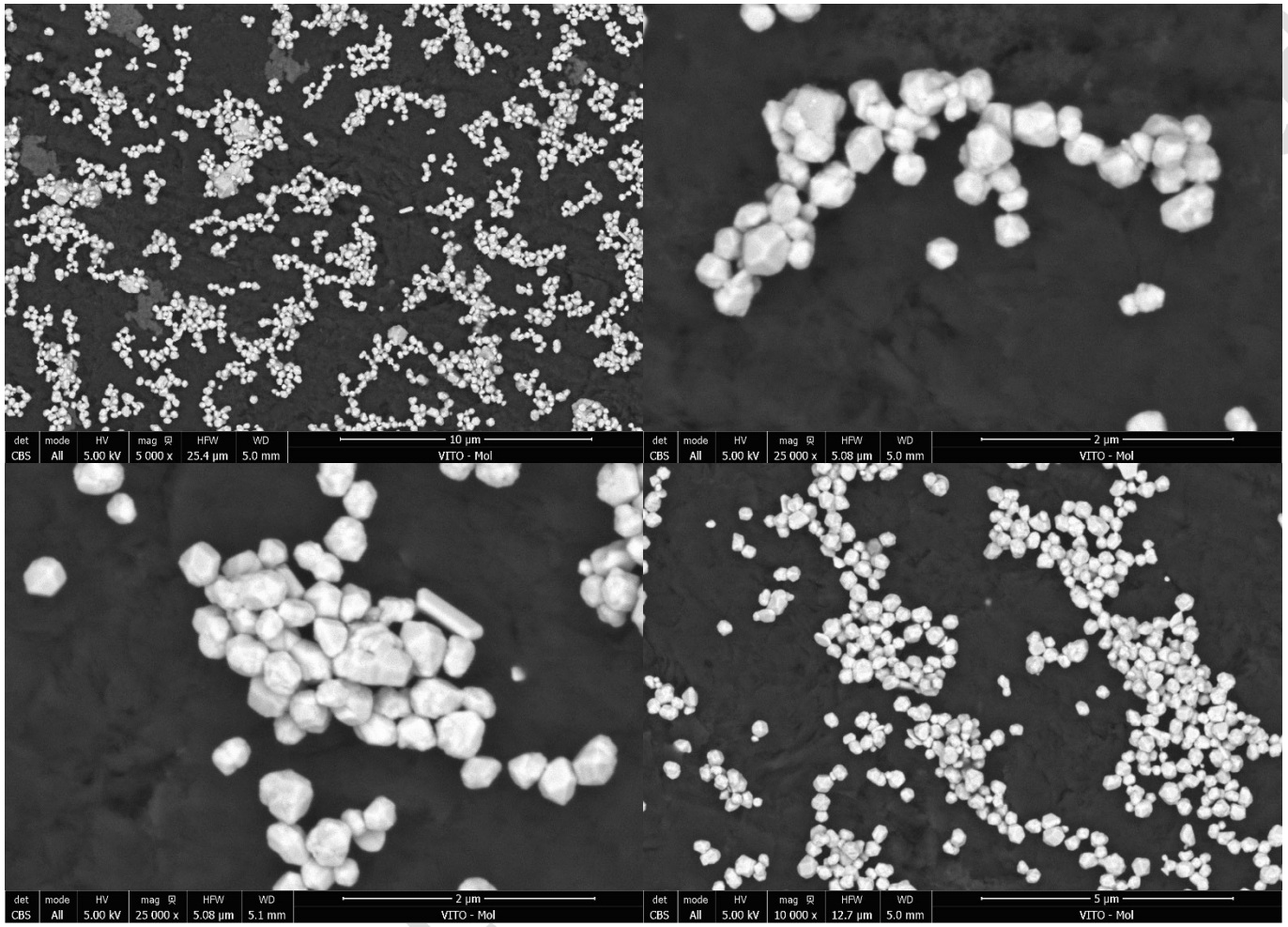
1 % w/v methyl cellulose 1,8 mg/l Ag micropowder (15 minutes ultrasonication), time point 264 h.



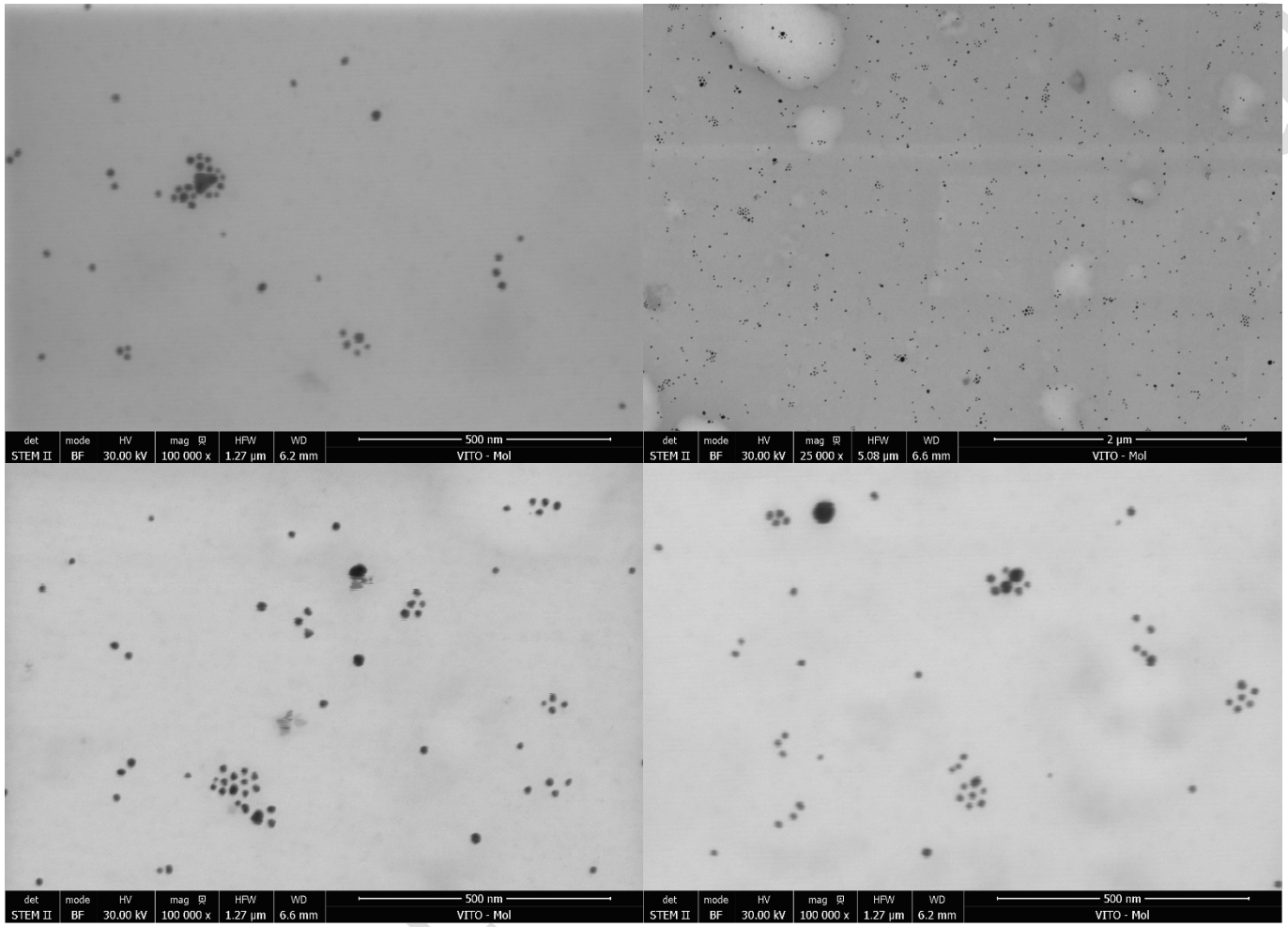
1 % w/v methyl cellulose 50 mg/l Ag micropowder (5 minutes ultrasonication), time point 264 h.



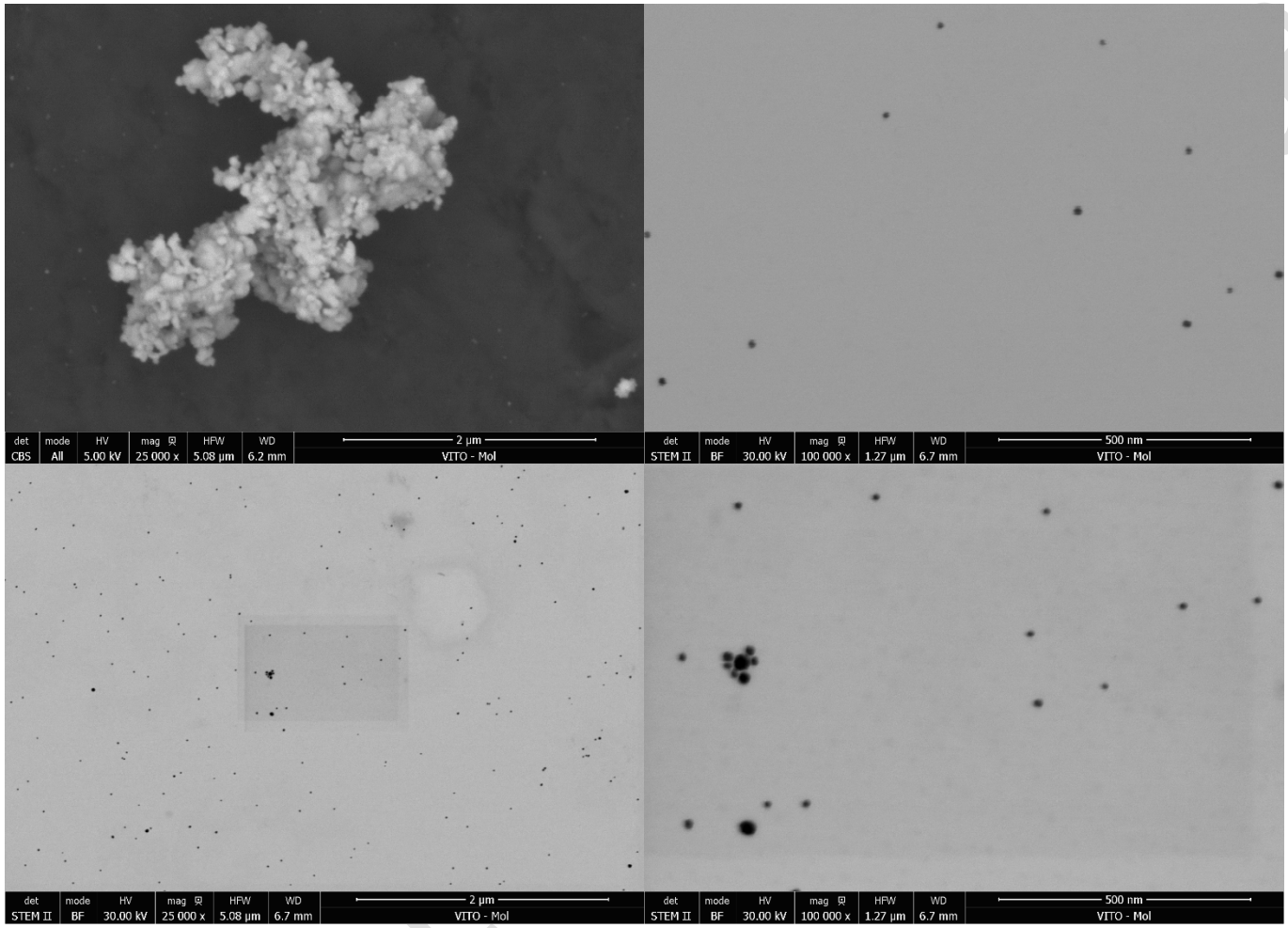
1 % w/v methyl cellulose 50 mg/l Ag micropowder (15 minutes ultrasonication), time point 264 h.



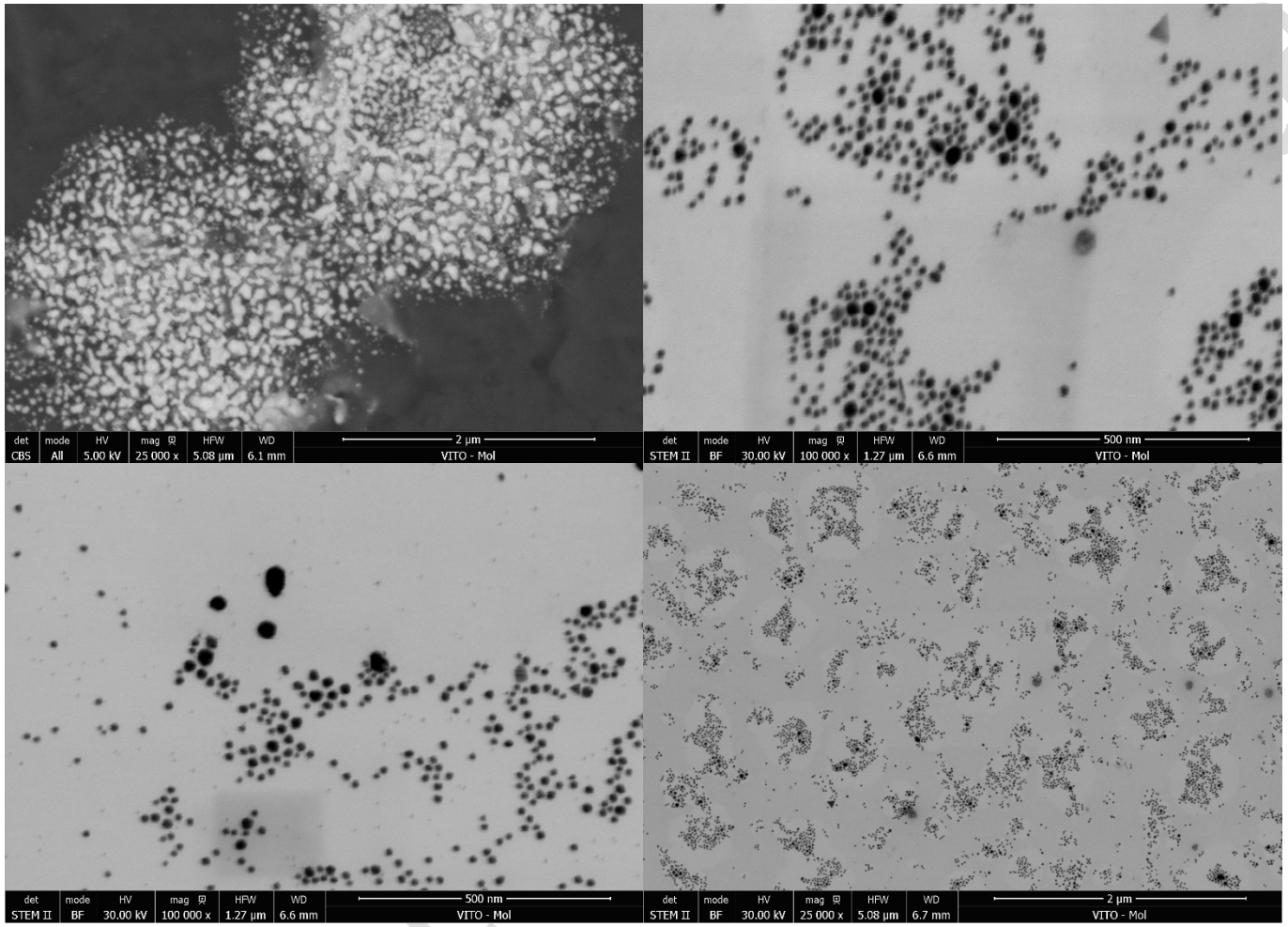
1 % w/v methyl cellulose 0,18 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 264 h.



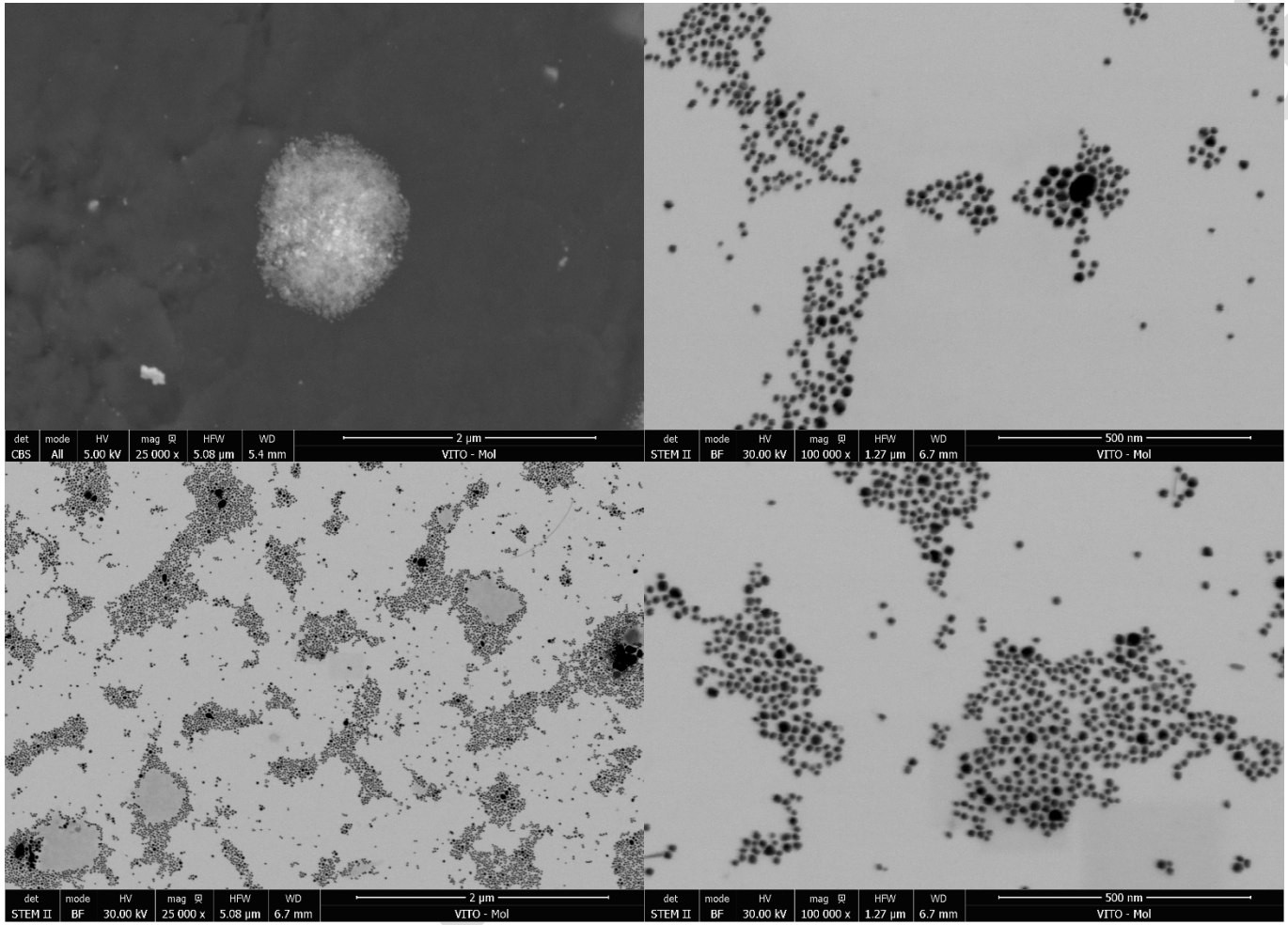
1 % w/v methyl cellulose 0,18 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 264 h.



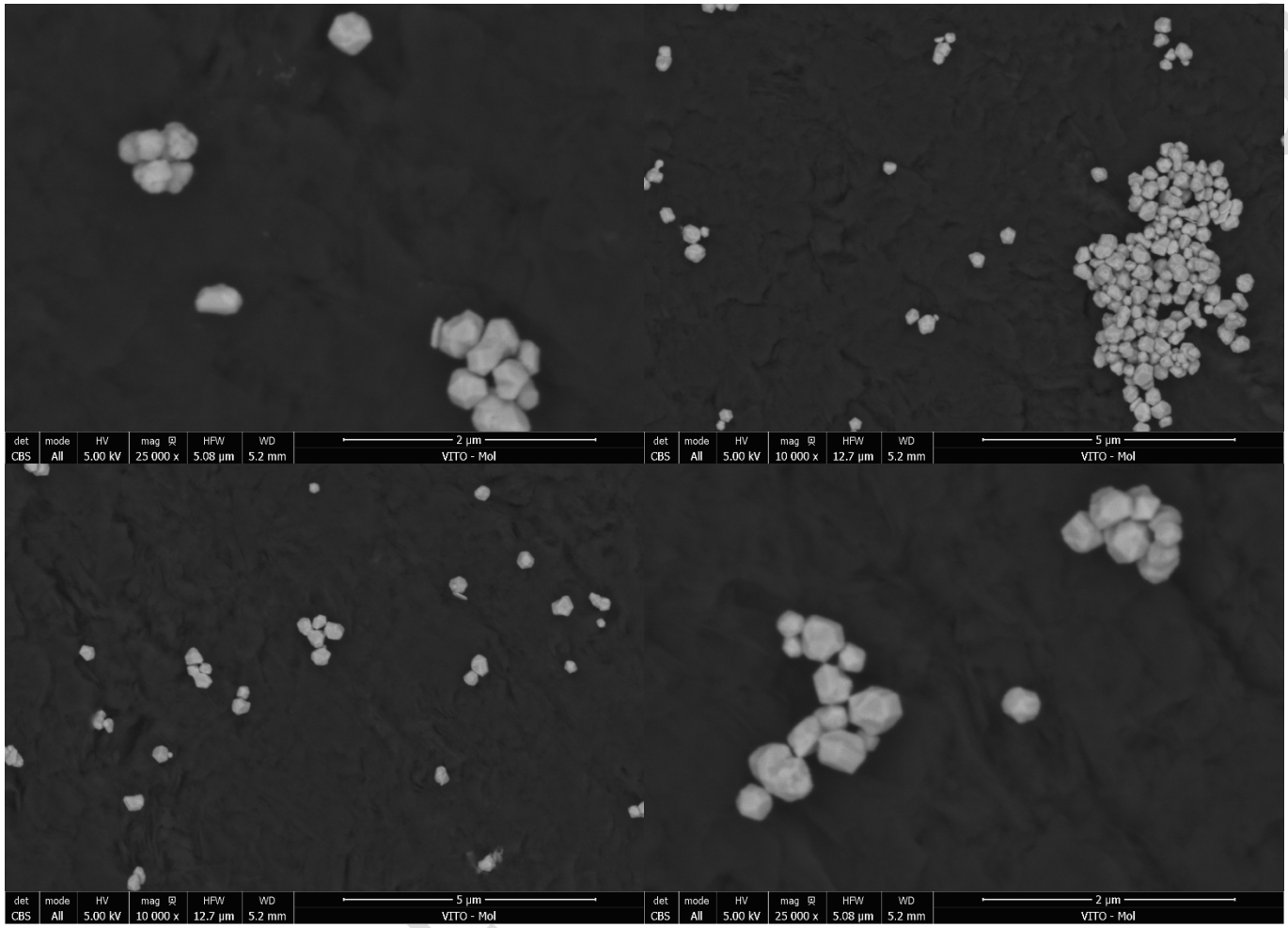
1 % w/v methyl cellulose 18 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 264 h.



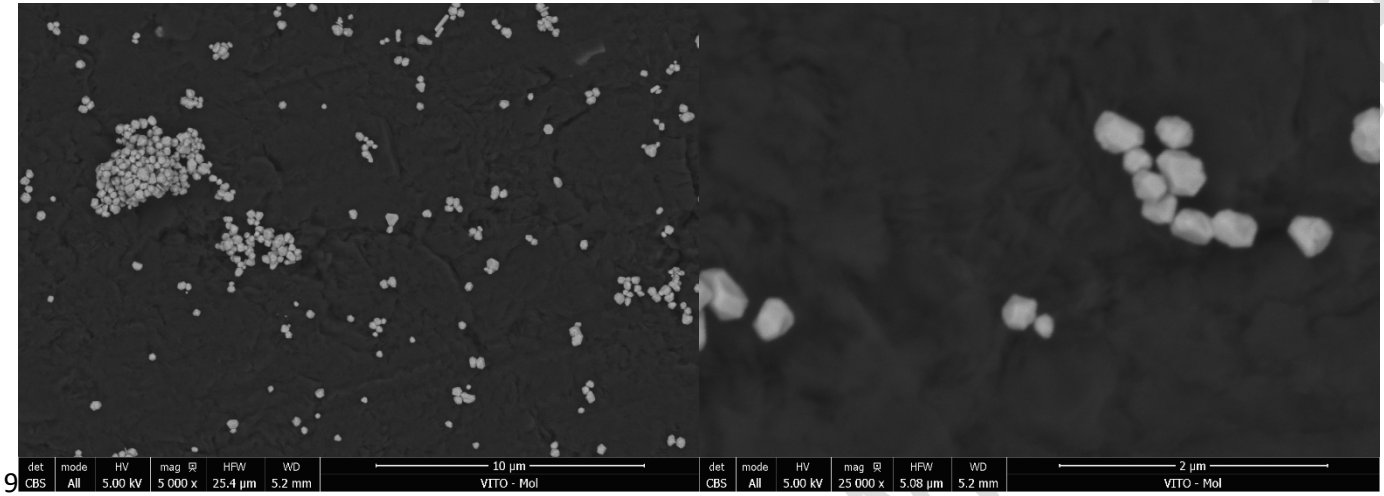
1 % w/v methyl cellulose 18 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 264 h.

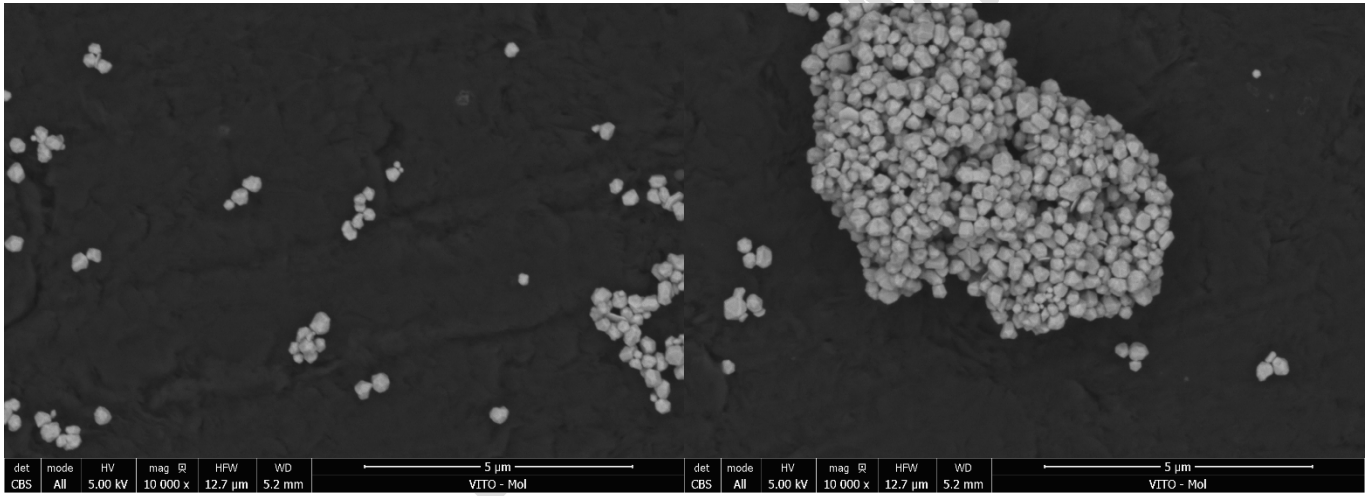


5 % w/v glucose 4 mg/l Ag micropowder suspension (5 minutes ultrasonication), time point 0 h.

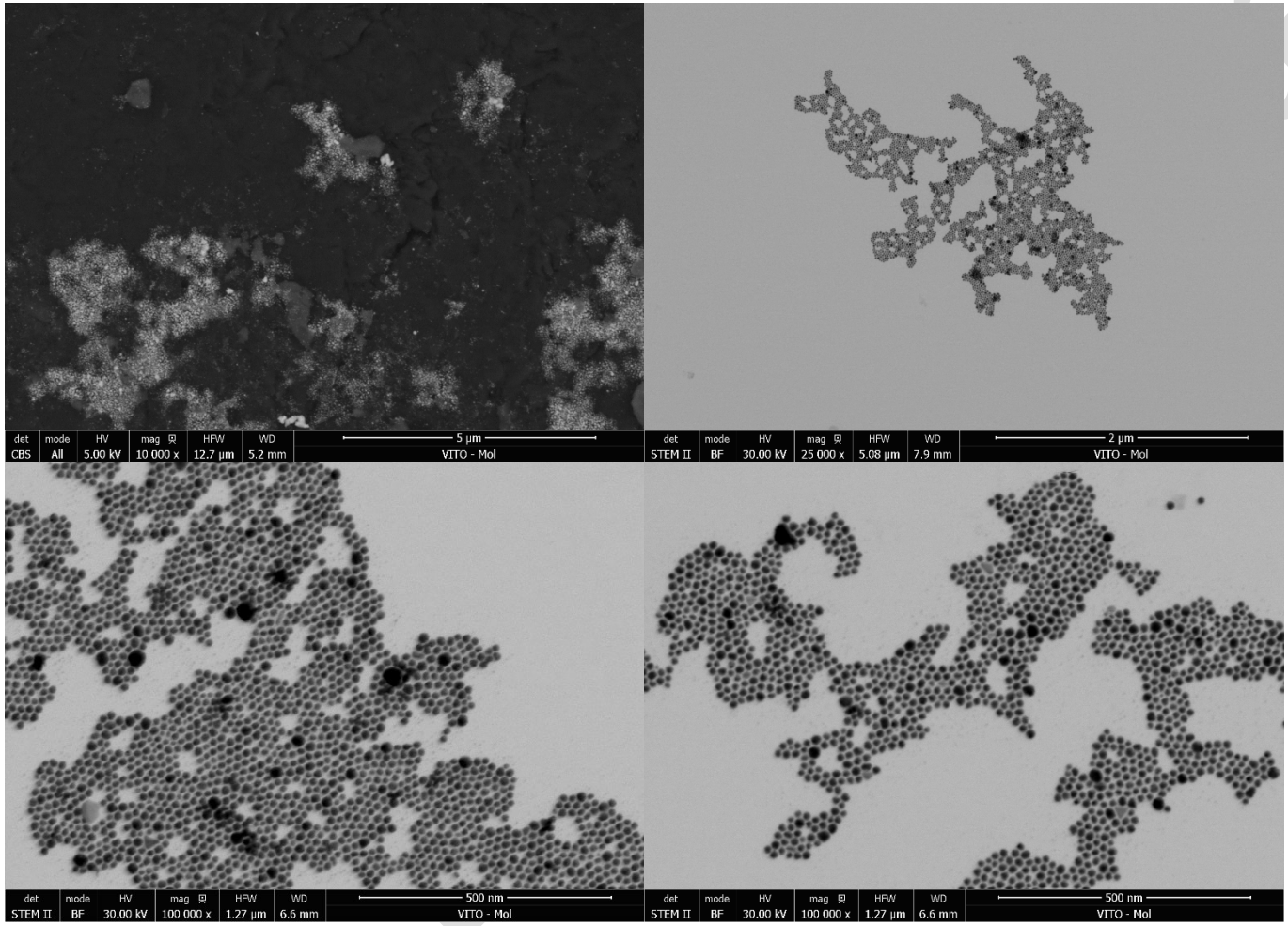


5 % w/v glucose 4 mg/l Ag micropowder suspension (15 minutes ultrasonication), time point 0 h.

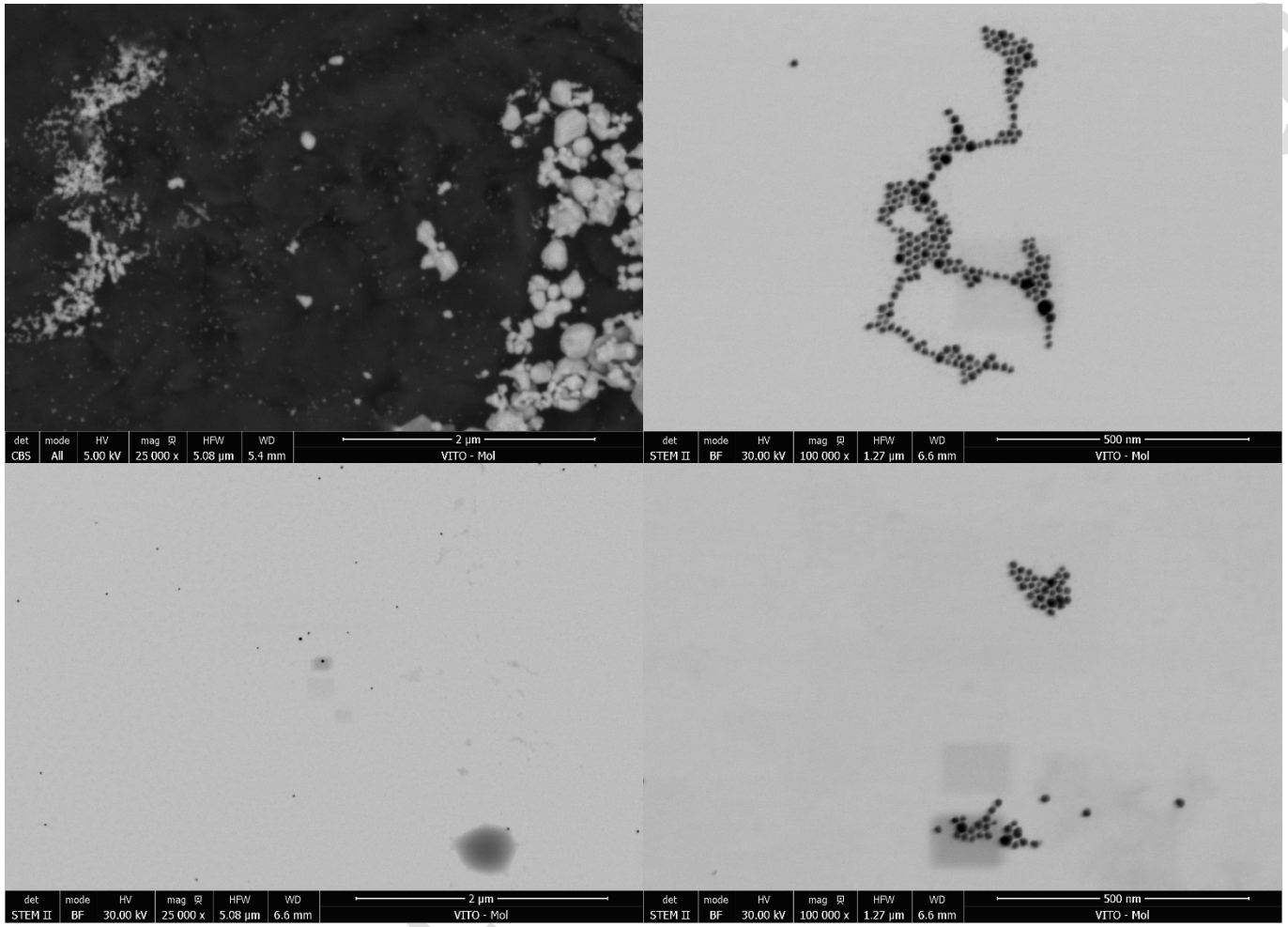




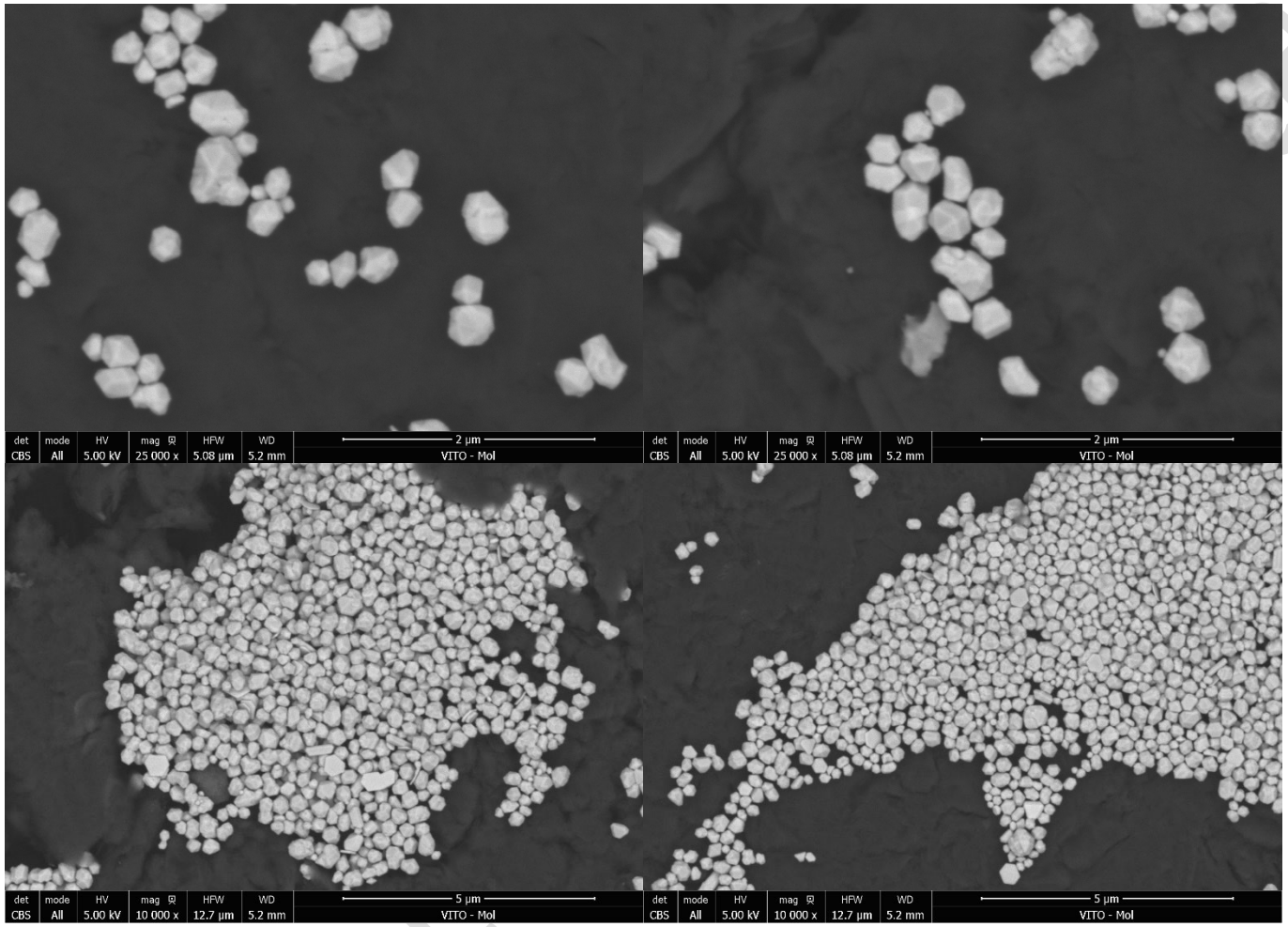
5 % w/v glucose 0,5 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 0 h.



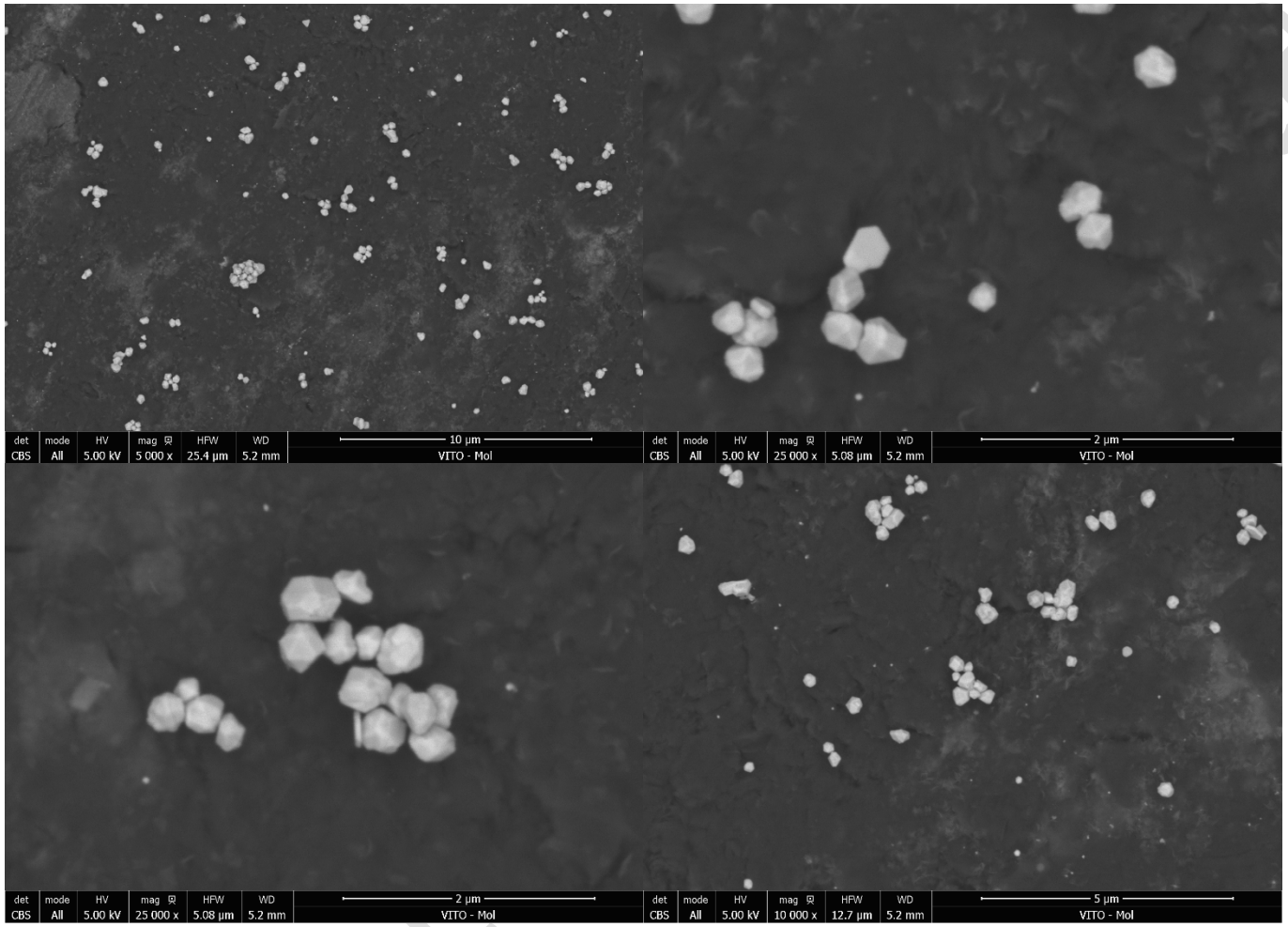
5 % w/v glucose 0,5 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 0 h.



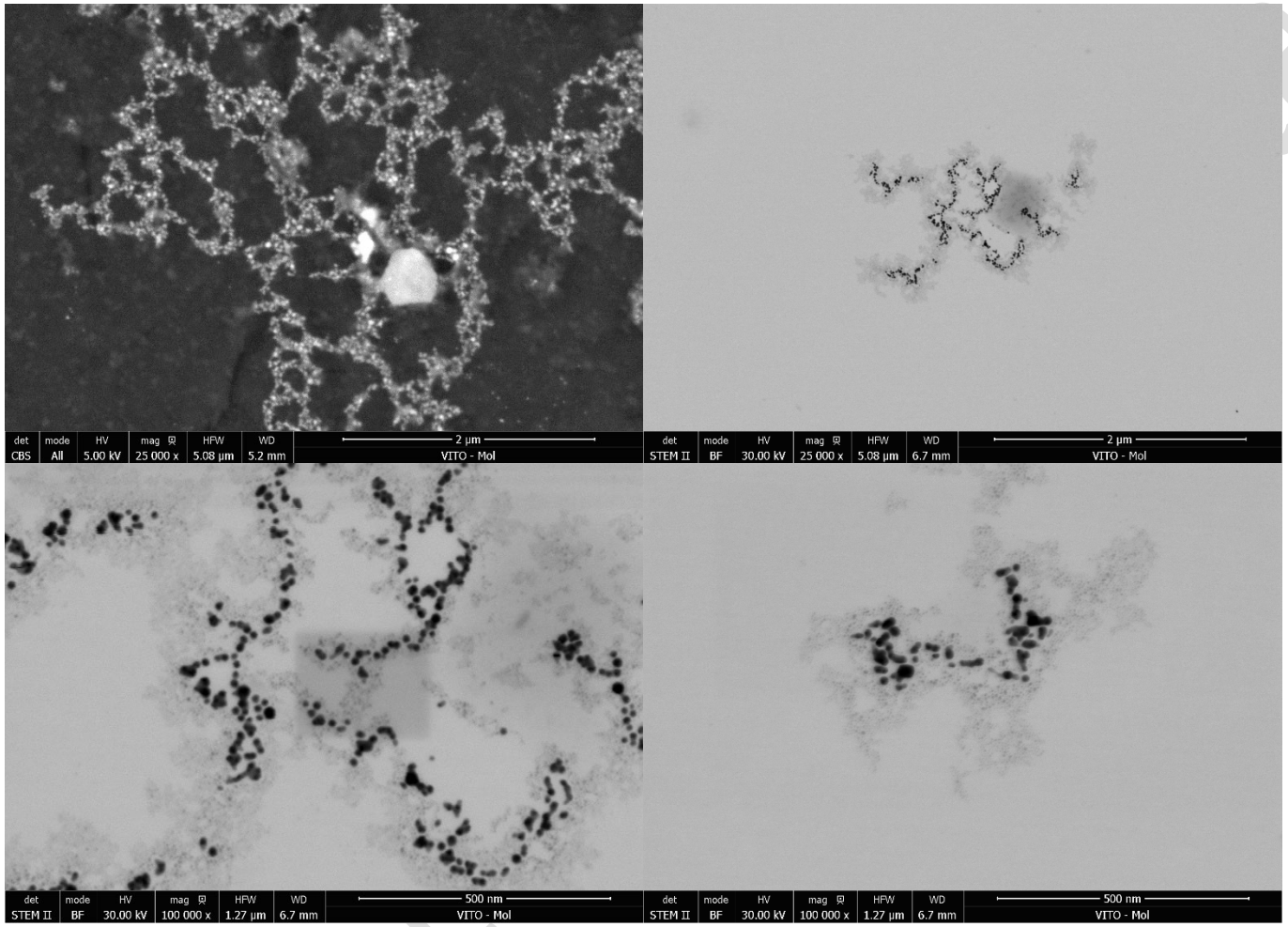
5 % w/v glucose 4 mg/l Ag micropowder suspension (5 minutes ultrasonication), time point 24 h.



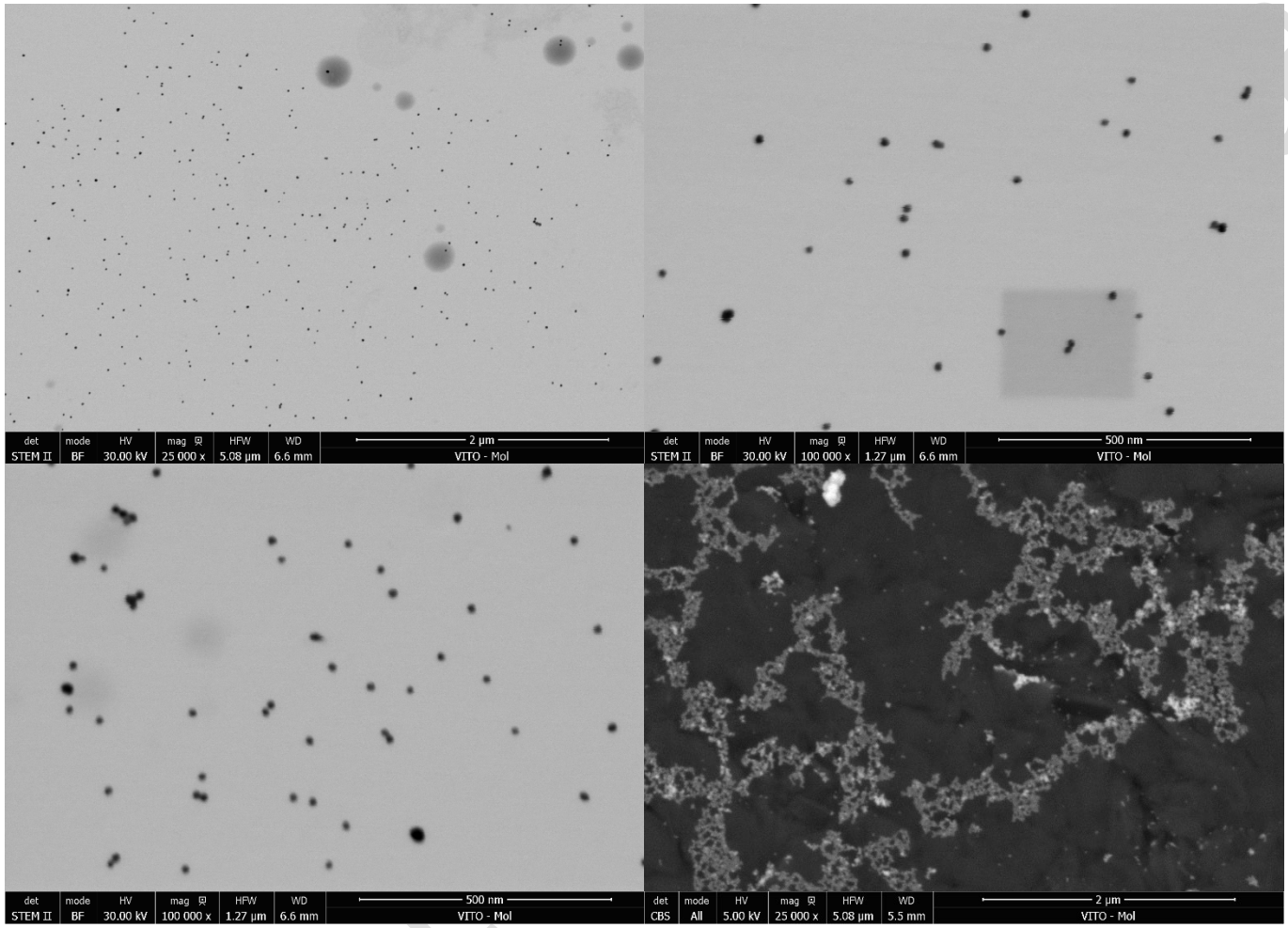
5 % w/v glucose 4 mg/l Ag micropowder suspension (15 minutes ultrasonication), time point 24 h.



5 % w/v glucose 0,5 mg/l Ag nanopowder suspension (5 minutes ultrasonication), time point 24 h.



5 % w/v glucose 0,5 mg/l Ag nanopowder suspension (15 minutes ultrasonication), time point 24 h.



ANNEX C : ZETA POTENTIAL

5 % glucose day 0 and day 1

Record	Type	Sample Name	Measurement Date and Time	T (−C)	ZP (mV)	Deviation	pb (ŧmcm/	ond (mS/cr	Attenuator	Count Rat	tive Volta	eta Potenti	Count Rate	red Currer	Zeta Runs
10	Zeta	ŧAg-4mg/ml-5'son-20'roeren-100x verd 1	maandag 17 februari 2020 12:04:02	25	-33,9	10	-2,654	0,00991	4	327024,6	150	-31,8	196,4	0,0219	22
11	Zeta	ŧAg-4mg/ml-5'son-20'roeren-100x verd 2	maandag 17 februari 2020 12:06:30	25	-34,8	5,29	-2,729	0,0102	4	833797,5	150	-32,9	500,7	0,0222	22
12	Zeta	ŧAg-4mg/ml-5'son-20'roeren-100x verd 3	maandag 17 februari 2020 12:08:39	25	-35,2	5,5	-2,762	0,0103	4	826483,1	150	-34,6	496,4	0,0225	22
13	Zeta	ŧAg-4mg/ml-15'son-20'roeren-100x verd 1	maandag 17 februari 2020 12:12:24	25	-34,2	5,37	-2,682	0,0104	5	102628,8	150	-19,1	145,4	0,0229	22
14	Zeta	ŧAg-4mg/ml-15'son-20'roeren-100x verd 2	maandag 17 februari 2020 12:14:53	25	-34,2	5,44	-2,684	0,0104	5	193595,8	150	-18,7	274,3	0,0229	22
15	Zeta	ŧAg-4mg/ml-15'son-20'roeren-100x verd 3	maandag 17 februari 2020 12:17:02	25	-33,7	5,6	-2,643	0,0104	5	111905,1	150	-18,3	158,5	0,022	22
28	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd 1	maandag 17 februari 2020 13:43:06	25	-3,41	4,39	-0,2676	0,658	5	83631,3	150	-1,42	118,5	1,5	22
29	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd 2	maandag 17 februari 2020 13:45:35	25	-4,2	5,31	-0,3289	0,685	5	66645,7	150	0,405	94,4	1,55	22
30	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd 3	maandag 17 februari 2020 13:47:44	25	-3,89	3,8	-0,3047	0,695	5	126943,7	150	-0,584	179,8	1,57	22
31	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd 1	maandag 17 februari 2020 13:51:04	25	-4,17	5,11	-0,3268	0,692	4	524933,8	150	-1,4	315,3	1,58	22
32	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd 2	maandag 17 februari 2020 13:53:32	25	-4,2	4,59	-0,3291	0,72	4	209119	150	-1,12	125,6	1,63	22
33	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd 3	maandag 17 februari 2020 13:55:41	25	-3,93	4,35	-0,3078	0,73	4	213132,1	150	-1,57	128	1,65	22
7	Zeta	ŧAg-4mg/ml-5'son-20'roer-100xverd-T24u 1	dinsdag 18 februari 2020 11:40:05	25	-35,6	5,16	-2,791	0,0101	4	324284,4	150	-29,6	194,8	0,0225	22
8	Zeta	ŧAg-4mg/ml-5'son-20'roer-100xverd-T24u 2	dinsdag 18 februari 2020 11:42:36	25	-35,9	5,67	-2,816	0,0103	4	458508,8	150	-28,5	275,4	0,0227	22
9	Zeta	ŧAg-4mg/ml-5'son-20'roer-100xverd-T24u 3	dinsdag 18 februari 2020 11:44:45	25	-35,3	5,42	-2,77	0,0107	4	462697,2	150	-30,8	277,9	0,0228	22
10	Zeta	ŧAg-4mg/ml-15'son-20'roer-100xverd-T24u 1	dinsdag 18 februari 2020 11:49:15	25	-34,8	4,84	-2,725	0,0101	4	875861,5	150	-30,4	526	0,0222	22
11	Zeta	ŧAg-4mg/ml-15'son-20'roer-100xverd-T24u 2	dinsdag 18 februari 2020 11:51:44	25	-34	5,02	-2,665	0,0103	4	585422	150	-31,5	351,6	0,0225	22
12	Zeta	ŧAg-4mg/ml-15'son-20'roer-100xverd-T24u 3	dinsdag 18 februari 2020 11:53:53	25	-33,8	5,82	-2,646	0,0102	4	694031	150	-32,3	416,8	0,0226	22
19	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd-T24u 1	dinsdag 18 februari 2020 13:06:31	25	-4,95	4,59	-0,3877	0,695	5	128847,9	150	0,00877	182,5	1,6	22
20	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd-T24u 2	dinsdag 18 februari 2020 13:09:02	25	-5,17	4,54	-0,4051	0,729	5	189639,7	150	-0,0877	268,6	1,66	22
21	Zeta	nAg-0.5mg/ml-5'son-20'roer-onverd-T24u 3	dinsdag 18 februari 2020 13:11:11	25	-4,93	4,08	-0,3863	0,742	5	199895,1	150	-0,407	283,2	1,68	22
22	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd-T24u 1	dinsdag 18 februari 2020 13:16:32	25	-4,48	3,44	-0,3514	0,704	5	313337,5	150	-0,46	443,9	1,61	22
23	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd-T24u 2	dinsdag 18 februari 2020 13:19:00	25	-4,75	4,12	-0,372	0,731	5	217024,3	150	-0,875	307,4	1,66	22
24	Zeta	nAg-0.5mg/ml-15'son-20'roer-onverd-T24u 3	dinsdag 18 februari 2020 13:21:09	25	-4,91	4,99	-0,3848	0,741	5	220039,3	150	-0,745	311,7	1,68	22

1 % methylcellulose day 0

Record	Type	Sample Name	Measurement Date and Time	T (−C)	ZP (mV)	Deviation	ob (ƒmcm/	ond (mS/cr	Attenuator	Count Rat	tive Volta	eta Potenti	Count Rate	ired Curren	Zeta Runs
10	Zeta	ƒAg 1.8 mg/ml-5'son-20'roer-30x verd 1	dinsdag 4 februari 2020 12:00:28	25	-0,783	3,63	-0,06136	0,00586	4	169086,8	150	-13,4	101,5	0,0115	40
11	Zeta	ƒAg 1.8 mg/ml-5'son-20'roer-30x verd 2	dinsdag 4 februari 2020 12:03:51	25	-0,715	3,87	-0,05602	0,0147	4	169147,7	150	-11,9	101,6	0,0121	50
12	Zeta	ƒAg 1.8 mg/ml-5'son-20'roer-30x verd 3	dinsdag 4 februari 2020 12:07:23	25	-0,518	3,32	-0,04063	0,0172	4	162721,8	150	-12,2	97,7	0,0126	50
16	Zeta	ƒAg_1.8mg/ml-24h-15'son-20'roer-30x verd 1	dinsdag 4 februari 2020 12:27:01	25	-1,15	4,45	-0,09004	0,0803	5	171517,5	139	-12,7	243	0,165	22
17	Zeta	ƒAg_1.8mg/ml-24h-15'son-20'roer-30x verd 2	dinsdag 4 februari 2020 12:29:32	25	-0,558	3,63	-0,04376	0,0577	5	170134,9	150	-12,4	241	0,151	50
18	Zeta	ƒAg_1.8mg/ml-24h-15'son-20'roer-30x verd 3	dinsdag 4 februari 2020 12:33:04	25	-0,809	3,32	-0,06341	0,0746	5	299919,5	149	-11,3	424,9	0,165	40
28	Zeta	ƒAg_50mg/ml_5'son-20'roer-750x verd 1	dinsdag 4 februari 2020 13:21:25	25	-1,66	4,31	-0,1301	0,00299	5	175556,1	150	-17,3	248,7	0,00371	28
29	Zeta	ƒAg_50mg/ml_5'son-20'roer-750x verd 2	dinsdag 4 februari 2020 13:24:14	25	-1,28	4,82	-0,1002	0,0018	5	75272,8	150	-16,6	106,6	0,00388	28
30	Zeta	ƒAg_50mg/ml_5'son-20'roer-750x verd 3	dinsdag 4 februari 2020 13:26:41	25	-0,757	3,9	-0,0593	0,00183	5	71359,7	150	-15,8	101,1	0,00397	50
34	Zeta	ƒAg-50mg/ml in MC-15'son-+20'roer-1000x verd 1	dinsdag 4 februari 2020 13:45:01	25	-0,606	4,95	-0,04747	0,0086	5	54617,4	150	-18,6	77,4	0,0184	26
35	Zeta	ƒAg-50mg/ml in MC-15'son-+20'roer-1000x verd 2	dinsdag 4 februari 2020 13:47:44	25	-0,882	4,3	-0,06912	0,00725	5	206092,8	150	-17,9	292	0,016	46
36	Zeta	ƒAg-50mg/ml in MC-15'son-+20'roer-1000x verd 3	dinsdag 4 februari 2020 13:51:04	25	-0,534	4,4	-0,04188	0,00604	5	93002	150	-18,1	131,7	0,0109	50
46	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 1	dinsdag 4 februari 2020 14:30:51	25	-0,568	3,81	-0,04452	0,356	5	200720,5	150	-0,406	284,3	0,791	50
47	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 2	dinsdag 4 februari 2020 14:34:45	25	-0,588	3,59	-0,04613	0,348	5	349958	150	-0,29	495,8	0,787	50
51	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 1	dinsdag 4 februari 2020 14:52:00	25	-0,57	3,6	-0,0447	0,329	5	250981,9	150	-0,184	355,5	0,75	50
52	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 2	dinsdag 4 februari 2020 14:55:58	25	-0,694	3,6	-0,05444	0,345	5	74356,8	150	-4,72E-04	105,3	0,771	50
53	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 3	dinsdag 4 februari 2020 14:59:30	25	-0,492	3,78	-0,0386	0,353	5	153689,5	150	-0,188	217,7	0,786	50
63	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 1	dinsdag 4 februari 2020 15:41:21	25	-1,52	3,24	-0,119	0,477	4	422681,8	150	-0,649	253,8	1,08	22
64	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 2	dinsdag 4 februari 2020 15:43:49	25	-1,56	3,67	-0,1219	0,494	4	428281,5	150	-0,839	257,2	1,12	47
65	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 3	dinsdag 4 februari 2020 15:47:13	25	-1,68	3,52	-0,132	0,5	4	428839,5	150	-0,54	257,5	1,13	33
66	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 1	dinsdag 4 februari 2020 15:50:25	25	-1,38	3,4	-0,108	0,454	4	169332,6	150	-0,487	101,7	1,05	46
67	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 2	dinsdag 4 februari 2020 15:54:04	25	-1,6	3,33	-0,1251	0,475	4	257273,4	150	-0,33	154,5	1,08	42
68	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 3	dinsdag 4 februari 2020 15:57:12	25	-1,66	3,45	-0,1302	0,48	4	322104,5	150	-0,286	193,4	1,09	50

1 % methylcellulose day 1

Record	Type	Sample Name	Measurement Date and Time	T (−C)	ZP (mV)	Deviation	ob (mcm/	ond (mS/cr	Attenuator	Count Rat	tive Volta	eta Potenti	Count Rate	ired Curren	Zeta Runs
10	Zeta	Ag 1.8 mg/ml-5'son-20'roer-30x verd 1	dinsdag 4 februari 2020 12:00:28	25	-0,783	3,63	-0,06136	0,00586	4	169086,8	150	-13,4	101,5	0,0115	40
11	Zeta	Ag 1.8 mg/ml-5'son-20'roer-30x verd 2	dinsdag 4 februari 2020 12:03:51	25	-0,715	3,87	-0,05602	0,0147	4	169147,7	150	-11,9	101,6	0,0121	50
12	Zeta	Ag 1.8 mg/ml-5'son-20'roer-30x verd 3	dinsdag 4 februari 2020 12:07:23	25	-0,518	3,32	-0,04063	0,0172	4	162721,8	150	-12,2	97,7	0,0126	50
16	Zeta	Ag_1.8mg/ml-24h-15'son-20'roer-30x verd 1	dinsdag 4 februari 2020 12:27:01	25	-1,15	4,45	-0,09004	0,0803	5	171517,5	139	-12,7	243	0,165	22
17	Zeta	Ag_1.8mg/ml-24h-15'son-20'roer-30x verd 2	dinsdag 4 februari 2020 12:29:32	25	-0,558	3,63	-0,04376	0,0577	5	170134,9	150	-12,4	241	0,151	50
18	Zeta	Ag_1.8mg/ml-24h-15'son-20'roer-30x verd 3	dinsdag 4 februari 2020 12:33:04	25	-0,809	3,32	-0,06341	0,0746	5	299919,5	149	-11,3	424,9	0,165	40
28	Zeta	Ag_50mg/ml_5'son-20'roer-750x verd 1	dinsdag 4 februari 2020 13:21:25	25	-1,66	4,31	-0,1301	0,00299	5	175556,1	150	-17,3	248,7	0,00371	28
29	Zeta	Ag_50mg/ml_5'son-20'roer-750x verd 2	dinsdag 4 februari 2020 13:24:14	25	-1,28	4,82	-0,1002	0,0018	5	75272,8	150	-16,6	106,6	0,00388	28
30	Zeta	Ag_50mg/ml_5'son-20'roer-750x verd 3	dinsdag 4 februari 2020 13:26:41	25	-0,757	3,9	-0,0593	0,00183	5	71359,7	150	-15,8	101,1	0,00397	50
34	Zeta	Ag-50mg/ml in MC-15'son-+20'roer-1000x verd 1	dinsdag 4 februari 2020 13:45:01	25	-0,606	4,95	-0,04747	0,0086	5	54617,4	150	-18,6	77,4	0,0184	26
35	Zeta	Ag-50mg/ml in MC-15'son-+20'roer-1000x verd 2	dinsdag 4 februari 2020 13:47:44	25	-0,882	4,3	-0,06912	0,00725	5	206092,8	150	-17,9	292	0,016	46
36	Zeta	Ag-50mg/ml in MC-15'son-+20'roer-1000x verd 3	dinsdag 4 februari 2020 13:51:04	25	-0,534	4,4	-0,04188	0,00604	5	93002	150	-18,1	131,7	0,0109	50
46	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 1	dinsdag 4 februari 2020 14:30:51	25	-0,568	3,81	-0,04452	0,356	5	200720,5	150	-0,406	284,3	0,791	50
47	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 2	dinsdag 4 februari 2020 14:34:45	25	-0,588	3,59	-0,04613	0,348	5	349958	150	-0,29	495,8	0,787	50
51	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 1	dinsdag 4 februari 2020 14:52:00	25	-0,57	3,6	-0,0447	0,329	5	250981,9	150	-0,184	355,5	0,75	50
52	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 2	dinsdag 4 februari 2020 14:55:58	25	-0,694	3,6	-0,05444	0,345	5	74356,8	150	-4,72E-04	105,3	0,771	50
53	Zeta	nAg-0.18mg/ml inMC-15'son-20'roer-onverd 3	dinsdag 4 februari 2020 14:59:30	25	-0,492	3,78	-0,0386	0,353	5	153689,5	150	-0,188	217,7	0,786	50
63	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 1	dinsdag 4 februari 2020 15:41:21	25	-1,52	3,24	-0,119	0,477	4	422681,8	150	-0,649	253,8	1,08	22
64	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 2	dinsdag 4 februari 2020 15:43:49	25	-1,56	3,67	-0,1219	0,494	4	428281,5	150	-0,839	257,2	1,12	47
65	Zeta	nAg-18mg/ml_5'son-20'roer-50x verd 3	dinsdag 4 februari 2020 15:47:13	25	-1,68	3,52	-0,132	0,5	4	428839,5	150	-0,54	257,5	1,13	33
66	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 1	dinsdag 4 februari 2020 15:50:25	25	-1,38	3,4	-0,108	0,454	4	169332,6	150	-0,487	101,7	1,05	46
67	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 2	dinsdag 4 februari 2020 15:54:04	25	-1,6	3,33	-0,1251	0,475	4	257273,4	150	-0,33	154,5	1,08	42
68	Zeta	nAg-18mg/ml_15'son-20'roer-50x verd 3	dinsdag 4 februari 2020 15:57:12	25	-1,66	3,45	-0,1302	0,48	4	322104,5	150	-0,286	193,4	1,09	50

1 % methylcellulose day 11

Record	Type	Sample Name	Measurement Date and Time	T (−C)	ZP (mV)	Deviation	ob (mcm/	ond (mS/cr	Attenuator	Count Rat	tive Volta	eta Potenti	Count Rate	ired Curren	Zeta Runs
7	Zeta	Ag_1.8mg/ml-5'son-20'roeren-30x verd-10d 1	donderdag 13 februari 2020 10:55:44	25	-2,45	3,62	-0,192	0,00289	4	139216,6	150	-15,5	83,6	0,00611	22
8	Zeta	Ag_1.8mg/ml-5'son-20'roeren-30x verd-10d 2	donderdag 13 februari 2020 10:58:16	25	-3,01	3,24	-0,2358	0,00323	4	82331,5	150	-15,5	49,4	0,00606	22
9	Zeta	Ag_1.8mg/ml-5'son-20'roeren-30x verd-10d 3	donderdag 13 februari 2020 11:00:25	25	-2,57	3,17	-0,2018	0,00736	4	70469,3	150	-16,9	42,3	0,00616	23
10	Zeta	Ag_1.8mg/ml-15'son-20'roeren-30x verd-10d 1	donderdag 13 februari 2020 11:03:23	25	-2,51	3,77	-0,1967	0,0101	4	330872	150	-22,1	198,7	0,00573	22
11	Zeta	Ag_1.8mg/ml-15'son-20'roeren-30x verd-10d 2	donderdag 13 februari 2020 11:05:52	25	-2,76	4,13	-0,2164	0,0252	4	236770,2	150	-21,5	142,2	0,0055	22
12	Zeta	Ag_1.8mg/ml-15'son-20'roeren-30x verd-10d 3	donderdag 13 februari 2020 11:08:01	25	-2,83	3,75	-0,2221	0,00263	4	264051,5	150	-21,7	158,6	0,0057	22
21	Zeta	Ag50mg/ml;5'son-20'roer-200xverd-10d 1	donderdag 13 februari 2020 11:52:55	25	-13,3	4,57	-1,044	0,00125	4	415032,7	150	-23,6	249,3	0,00267	22
22	Zeta	Ag50mg/ml;5'son-20'roer-200xverd-10d 2	donderdag 13 februari 2020 11:55:26	25	-13	5,22	-1,022	0,00134	4	479088,9	150	-23,9	287,7	0,00263	22
23	Zeta	Ag50mg/ml;5'son-20'roer-200xverd-10d 3	donderdag 13 februari 2020 11:57:35	25	-13,4	5,19	-1,048	0,00136	4	249714	150	-24,1	150	0,00266	22
24	Zeta	Ag50mg/ml;15'son-20'roer-200xverd-10d 1	donderdag 13 februari 2020 12:01:16	25	-11,9	4,21	-0,9319	0,00149	3	1051617	150	-30,7	128,9	0,00258	22
25	Zeta	Ag50mg/ml;15'son-20'roer-200xverd-10d 2	donderdag 13 februari 2020 12:03:44	25	-12	4,35	-0,9421	0,00314	3	1982198	150	-30,4	242,9	0,00263	22
26	Zeta	Ag50mg/ml;15'son-20'roer-200xverd-10d 3	donderdag 13 februari 2020 12:05:53	25	-12	4,43	-0,9391	0,00971	3	1617804	150	-29,8	198,2	0,0026	22
30	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 1	donderdag 13 februari 2020 13:09:39	25	-0,683	5,3	-0,05353	0,436	4	157327,8	131	-0,182	94,5	0,843	50
31	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 2	donderdag 13 februari 2020 13:13:33	25	-0,538	4,33	-0,04219	0,472	4	81590,2	140	-0,303	49	0,977	50
32	Zeta	nAg-0.18mg/ml-5'son-20'roer-onverd 3	donderdag 13 februari 2020 13:17:05	25	-0,609	4,92	-0,04772	0,502	4	96617	135	-0,152	58	1	50
36	Zeta	nAg_0.18mg/ml-15'son-20'roer-onverd 1	donderdag 13 februari 2020 13:33:42	25	-0,828	7,22	-0,06487	0,559	6	19339,1	124	-0,251	69,9	1,03	50
37	Zeta	nAg_0.18mg/ml-15'son-20'roer-onverd 2	donderdag 13 februari 2020 13:37:36	25	-0,729	7,44	-0,05712	0,373	6	20778,8	150	-0,0507	75,1	0,883	50
38	Zeta	nAg_0.18mg/ml-15'son-20'roer-onverd 3	donderdag 13 februari 2020 13:41:08	25	-0,509	8,13	-0,03994	0,335	6	33765,9	150	-0,117	122,1	0,774	50
47	Zeta	nAg-18mg/ml-5'son-20'roer-200xverd 1	donderdag 13 februari 2020 14:38:17	25	-2,37	4,45	-0,1861	0,117	4	105318	150	-0,778	63,3	0,263	32
48	Zeta	nAg-18mg/ml-5'son-20'roer-200xverd 2	donderdag 13 februari 2020 14:41:18	25	-2,31	3,8	-0,181	0,119	4	155580,2	150	0,0681	93,4	0,265	50
49	Zeta	nAg-18mg/ml-15'son-20'roer-50xverd 1	donderdag 13 februari 2020 15:06:41	25	-1,59	3,97	-0,1246	0,451	5	149207,7	150	-0,864	211,4	1,04	50
50	Zeta	nAg-18mg/ml-15'son-20'roer-50xverd 2	donderdag 13 februari 2020 15:10:32	25	-1,71	3,5	-0,1344	0,47	5	218904,1	150	-1,14	310,1	1,07	50
51	Zeta	nAg-18mg/ml-15'son-20'roer-50xverd 3	donderdag 13 februari 2020 15:14:04	25	-1,68	4,2	-0,1317	0,476	5	241141,9	150	-1,07	341,6	1,08	50

ANNEX D : SEDIMENTATION TESTING

1) Making of 50 mg/ml µAg suspension in 1% Methyl Cellulose 400 cp

=> Bring 300 ml of ultrapure water in a 1 liter HDPE wide-mouth round bottle with red cap VWR. The bottle was rinced with HNO3 and ultrapure water prior to use.

The diameter of the bottom of the round bottle is approximately 90 mm.

=> Weigh 4 g of Methyl Cellulose Methocel A4C VWR casnr: 9004-67-5 and add to the 300 ml of ultrapure water.

=> Dissolve the Methyl Cellulose (MC) by stirring the solution vigorously.

Stirring: try to obtain a vortex of half the dept of the suspension.
Stirring at 500 rpm for 2 hours using a cylidrical stirring bar with removable ring 75X12 mm VWR.
After 2 hours increase the stirring to 700 rpm, the solution gets more viscous and harder to stir due to the solution of the MC.
After 4 hours of stirring at 700 rpm the MC seems completely dissolved and a homogenous syrupy solution is obtained.
Leave the solution to rest overnight.

=> The next day, start stirring the 1% MC solution 60 minutes at 700 rpm before adding the µAg powder.

=> Weigh 20 g of µAg powder into a SCP Science Digitube PP 50 ml with clear screw cap.

=> Add 50 ml of ultrapure water to the µAg powder, cap the Digitube and vortex 60 sec. at 2500 rpm.

=> Place the Digitube with the µAg suspension into an ultrasonic bath for 30 minutes .

=> Vortex again 60 sec. at 2500 rpm.

=> Place the Digitube with the µAg suspension again in the ultrasonic bath for 30 minutes.

=> Vortex again 60 sec. at 2500 rpm.

=> Add the 50 ml µAg suspension to the 300 ml 1% MC 400 cp solution while stirring. Add the 50ml µAg suspension to the 1% MC 400cp suspension directly after the last vortex treatement. Do this carefully yet sturdy.
If there is to much time between the vortexing and the adding of the 50 ml µAg suspension, the suspension settles and sticks to the bottom of the 50 ml Digitube.

Increase stirring to 900 rpm. Try to obtain a vortex of half the dept of the suspension.

=> The Digitube that contained the 50 ml µAg suspension is then rinsed several times using in total 50 ml of ultrapure water. Add this 50 ml rinse water also to the final suspension.

After rinsing there is still some µAg powder, which can't be removed, against the walls of the Digitube.

After rinsing there is still some µAg powder, which can't be removed, against the walls of the Digitube.

=> The final suspension is now 400 ml and containing 1% of MC 400 rp and 50 mg/ml µAg powder.

=> The final suspension of 50 mg/ml µAg in 1% MC 400 cp was made in duplo. So we have an A and a B suspension.

=> Stir the final suspension 60 minutes at 900 rpm. Try to obtain a vortex of half the dept of the suspension

=> After the 60 minutes a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette a significant amount of 1% MC 400 cp 50 mg/ml µAg suspension sticks to the inside of the pipette tip and can't be removed.
Although the A and B suspension 1% MC 400 cp 50 mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 10 to 15% less than 1 ml of suspension A.

=> The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.

Although The A and B suspension 1% MC 400 cp 50 mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 10 to 15% less than 1 ml of suspension A.

=> After taking the subsamples at time = 0 the 1% MC 400 cp 50 mg/ml µAg suspensions are transferred to a tube rollar for 1 hour.

=> After one hour on the tube rollar the 1% MC 400 cp 50 mg/ml µAg suspensions are stirred at 900 rpm.

The 1% MC 400 cp 50 mg/ml µAg suspensions appear to be homogenous

=> After the 5 minutes of stirring at 900 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette a significant amount of 1% MC 400 cp 50 mg/ml µAg suspension sticks to the inside of the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.
Although the A and B suspension 1% MC 400 cp 50 mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 10 to 15% less than 1 ml of suspension A.

=> After 20 minutes of stirring at 900 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette a significant amount of 1% MC 400 cp 50 mg/ml µAg suspension sticks to the inside of the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.
Although the A and B suspension 1% MC 400 cp 50 mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 10 to 15% less than 1 ml of suspension A.

=> The 1% MC 400 cp 50 mg/ml µAg suspensions are stored in the fridge for 20 hours (overnight).

=> Remove the 1% MC 400 cp 50 mg/ml µAg suspensions from the fridge en stir them for 20 minutes at 900 rpm.

When the stirring starts the colour of the 1% MC 400 cp 50 mg/ml µAg suspensions gets darker suggesting a part of the µAg powder was settled down.
The surface of the B suspension 1% MC 400 cp 50 mg/l µAg was covered with airbubbles and foam.

=> After the 20 minutes of stirring at 900 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette a significant amount of 1% MC 400 cp 50 mg/ml µAg suspension sticks to the inside of the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.
Although the A and B suspension 1% MC 400 cp 50mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 5 to 10% less than 1 ml of suspension A.

=> The 1% MC 400 cp 50 mg/ml µAg suspensions are stored in the fridge for 3 days.

=> Remove the 1% MC 400 cp 50 mg/ml µAg suspensions from the fridge en stir them for 20 minutes at 900 rpm.

When the stirring starts the colour of the 1% MC 400 cp 50 mg/ml µAg suspensions gets darker suggesting a part of the µAg powder was settled down.
The surface of the B suspension 1% MC 400 cp 50 mg/l µAg was covered with airbubbles and foam.

=> After the 20 minutes of stirring at 900 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette a significant amount of 1% MC 400 cp 50 mg/ml µAg suspension sticks to the inside of the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.
Although the A and B suspension 1% MC 400 cp 50mg/ml µAg were made in an identical way it must be noted that 1 ml of suspension B weighs 5 to 10% less than 1 ml of suspension A.

=> 5 ml of nitric Acid 67-69% Optima Grade Fisher Chemicals is added to all the subsamples.
The tubes are coverd with a clear screw cap and placed into a SCP Science Digiprep MS heating block 120 minutes at 105°C.
After cooling down the samples are made up to 50 ml with ultrapure water, diluted further in Aqua regia and measured with ICP-AES.

2) Making of 50 mg/ml µAg suspension in 0.1% Methyl Cellulose 400 cp

=> Bring 300 ml of ultrapure water in a 1 liter HDPE wide-mouth round bottle with red cap VWR. The bottle was rinced with HNO3 and ultrapure water prior to use.

The diameter of the bottom of the round bottle is approximately 90 mm.

=> Weigh 0.4 g of Methyl Cellulose Methocel A4C VWR casnr: 9004-67-5 and add to the 300 ml of ultrapure water.

=> Dissolve the Methyl Cellulose (MC) by stirring the solution vigorously.

Stirring: try to obtain a vortex of half the dept of the suspension.
Stirring at 250 rpm for 2 hours using a cylidrical stirring bar with removable ring 75X12 mm VWR.
After 2 hours increase the stirring to 400 rpm.
After 4 hours of stirring at 400 rpm the MC seems completely dissolved and a homogenous solution is obtained.
Leave the solution to rest overnight.

=> The next day, start stirring the 0.1% MC solution 60 minutes at 400 rpm before adding the µAg powder.

=> Weigh 20 g of µAg powder into a SCP Science Digitube PP 50 ml with clear screw cap.

=> Add 50 ml of ultrapure water to the µAg powder, cap the Digitube and vortex 60 sec. at 2500 rpm.

=> Place the Digitube with the µAg suspension into an ultrasonic bath for 30 minutes .

=> Vortex again 60 sec. at 2500 rpm.

=> Place the Digitube with the µAg suspension again in the ultrasonic bath for 30 minutes.

=> Vortex again 60 sec. at 2500 rpm.

=> Add the 50 ml µAg suspension to the 300 ml 0.1% MC 400 cp solution while stirring. Add the 50ml µAg suspension to the 0.1% MC 400 cp suspension directly after the last vortex treatement. Do this carefully yet sturdy.
If there is to much time between the vortexing and the adding of the 50 ml µAg suspension, the suspension settles and sticks to the bottom of the 50 ml Digitube.

=> The Digitube that contained the 50 ml µAg suspension is then rinsed several times using in total 50 ml of ultrapure water. Add this 50ml rinse water also to the final suspension.

After rinsing there is still some µAg powder, which can't be removed, against the walls of the Digitube.

=> The final suspension is now 400 ml and containing 0.1% of MC 400 rp and 50 mg/ml µAg powder.

=> The final suspension of 50 mg/ml µAg in 0.1% MC 400 cp was also made in duplo. So we have an A and a B suspension.

=> Stir the final suspension 60 minutes at 400 rpm. Try to obtain a vortex of half the dept of the suspension

=> After the 60 minutes a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette an amount of 0.1% MC 400 cp 50 mg/ml µAg suspension remains inside the pipette tip and can't be removed.

=> The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.

=> After taking the subsamples at time = 0 the 0.1% MC 400 cp 50 mg/ml µAg suspensions are transferred to a tube rollar for 1 hour.

=> After one hour on the tube rollar the 0.1% MC 400 cp 50 mg/ml µAg suspensions are stirred at 400 rpm.

The 0.1% MC 400 cp 50mg/ml µAg suspensions appear to be homogenous.

=> After 5 minutes of stirring at 400 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette an amount of 0.1% MC 400 cp 50 mg/ml µAg suspension remains inside the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.

=> After 20 minutes of stirring at 400 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette an amount of 0.1% MC 400 cp 50 mg/ml µAg suspension remains inside the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.

=> The 0.1% MC 400 cp 50 mg/ml µAg suspensions are stored in the fridge for 20 hours (overnight)

=> Remove the 0.1% MC 400 cp 50 mg/ml µAg suspensions from the fridge en stir them for 20 minutes at 400 rpm.

When the stirring starts the colour of the 0.1% MC 400 cp 50 mg/ml µAg suspensions gets darker suggesting a part of the µAg powder was settled down.

=> After 20 minutes of stirring at 400 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.

The subsample is taken just on the edge of the vortex.
Let the pipette tip fill slowly with the suspension.
The outside of pipette tip is wiped very carefully with a tissue.
The pipette is emtied very slowly into the 50 ml PP Digitube.
After the emptying of the pipette an amount of 0.1% MC 400 cp 50 mg/ml µAg suspension remains inside the pipette tip and can't be removed.
The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.

=> The 0.1% MC 400 cp 50 mg/ml µAg suspensions are stored in the fridge for 3 days.

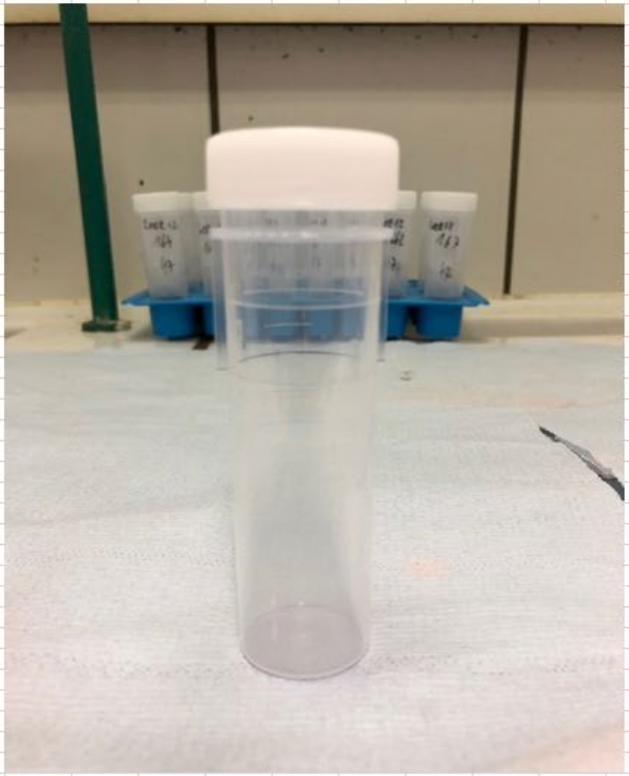
=> Remove the 0.1% MC 400 cp 50 mg/ml µAg suspensions from the fridge en stir them for 20 minutes at 400 rpm.

When the stirring starts the colour of the 0.1% MC 400 cp 50 mg/ml µAg suspensions gets darker suggesting a part of the µAg powder was settled down.

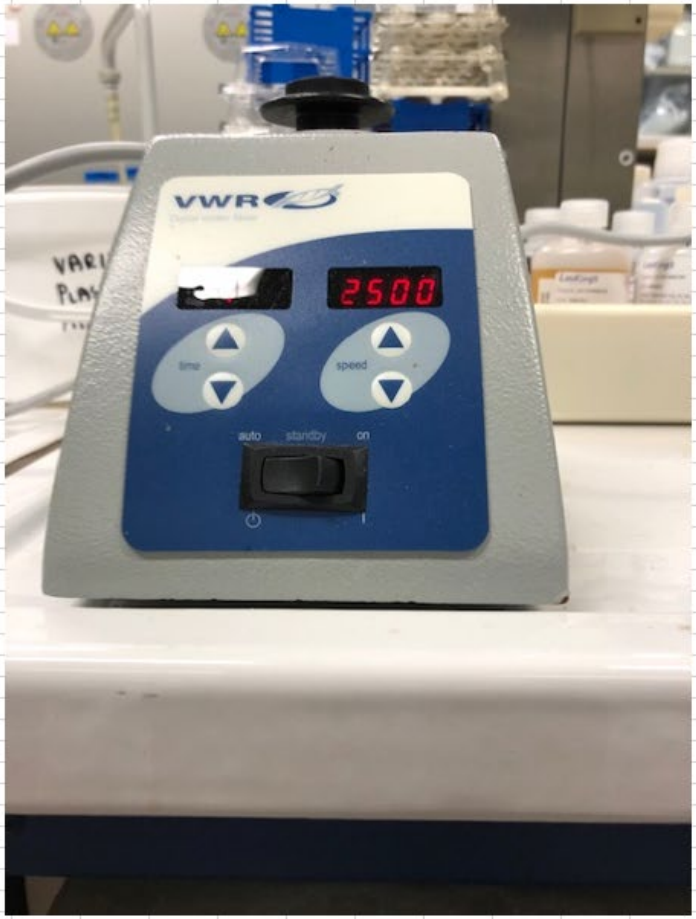
=>	After 20 minutes of stirring at 400 rpm a subsample of 1 ml with an automatic pipet (eppendorf research plus 100-1000 µl) is taken for total Ag determination. The subsample is taken in duplo.
	The subsample is taken just on the edge of the vortex.
	Let the pipette tip fill slowly with the suspension.
	The outside of pipette tip is wiped very carefully with a tissue.
	The pipette is emtied very slowly into the 50 ml PP Digitube.
	After the emptying of the pipette an amount of 0.1% MC 400 cp 50 mg/ml µAg suspension remains inside the pipette tip and can't be removed.
	The subsamples are brought into a SCP Science Digitube PP 50 ml. These tubes are weighted before and after adding the 1 ml subsample.
=>	5 ml of nitric Acid 67-69% Optima Grade Fisher Chemicals is added to all the subsamples.
	The tubes are coverd with a clear screw cap and placed into a SCP Science Digiprep MS heating block 120 minutes at 105°C.
	After cooling down the samples are made up to 50 ml with ultrapure water, diluted further in Aqua regia and measured with ICP-AES.

Cylidrical stirring bar with removable ring 75X12 mm VWR

1 liter HDPE wide-mouth round bottle with red cap VWR



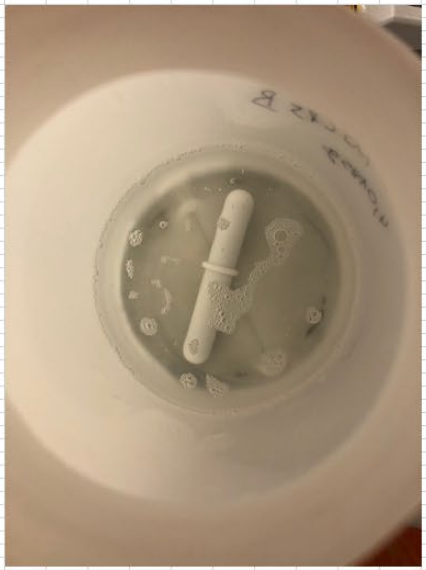
SCP Science Digitube 50 ml PP with clear screw cap



VWR digital vortex mixer.

Stirring Setup

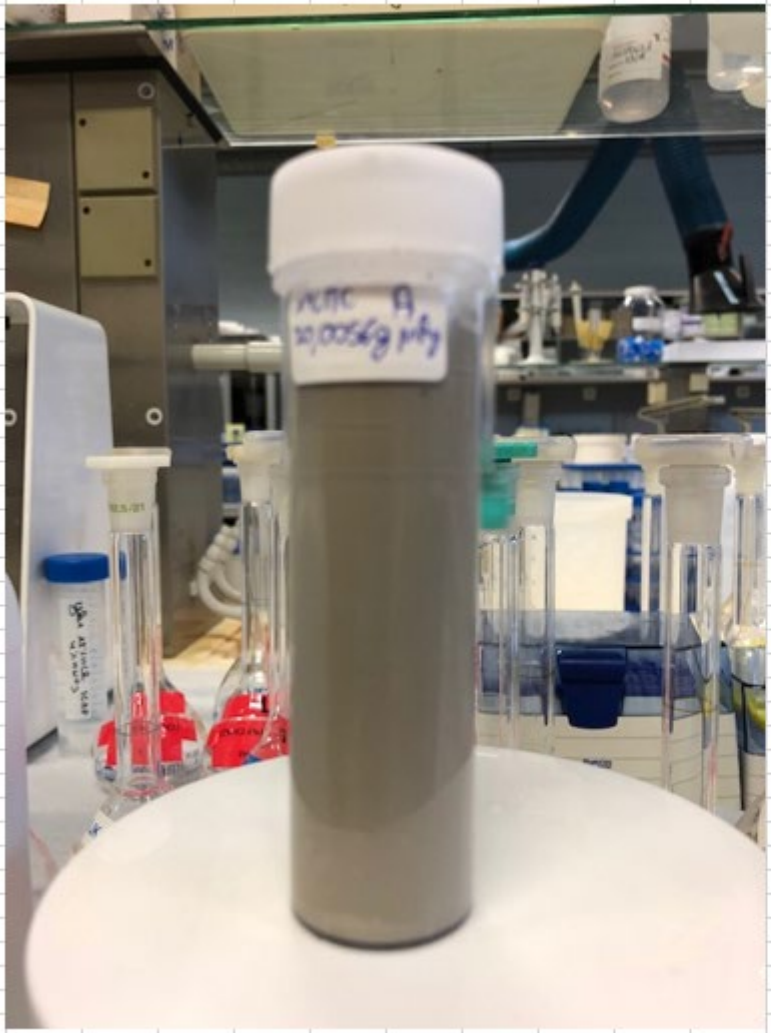
1% MC 400 cp A suspension after 2 hours at 500 rpm, 4 hours at 700 rpm and resting overnight.



1% MC 400 cp B suspension after 2 hours at 500 rpm, 4 hours at 700 rpm and resting overnight.

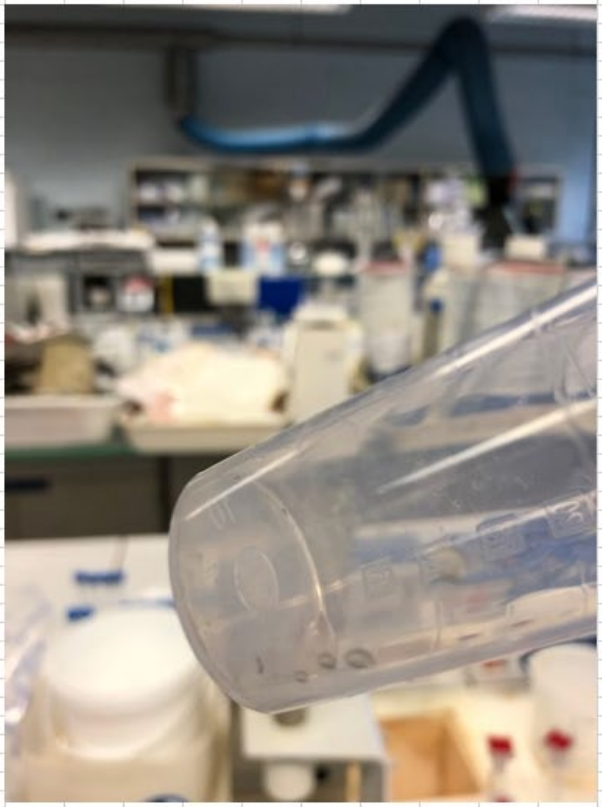
0.1% MC 400 cp A suspension after 2 hours at 250, 4 hours at 400 rpm and resting overnight.

0.1% MC 400 cp B suspension after 2 hours at 250, 4 hours at 400 rpm and resting overnight.

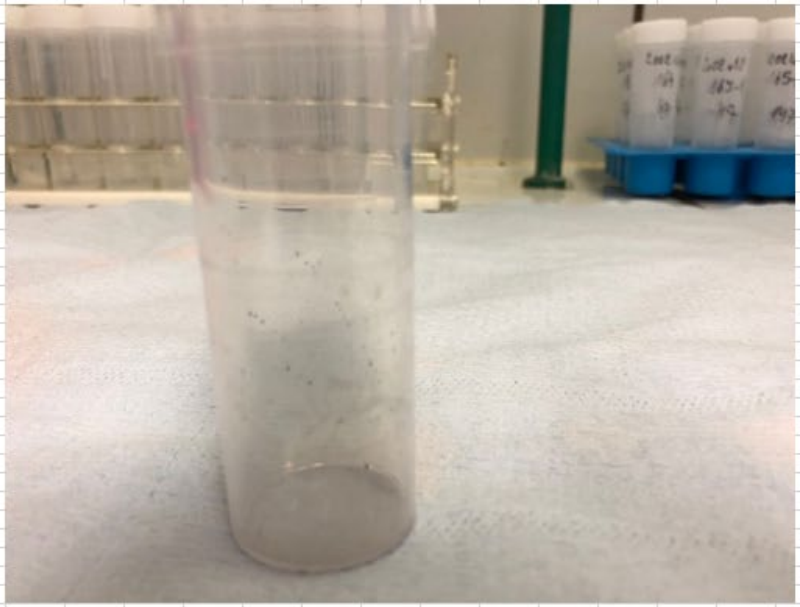


20 g µAg powder in 50 ml ultrapure water after final vortex treatment.

20 g µAg powder in 50 ml ultrapure water after final vortex treatment.

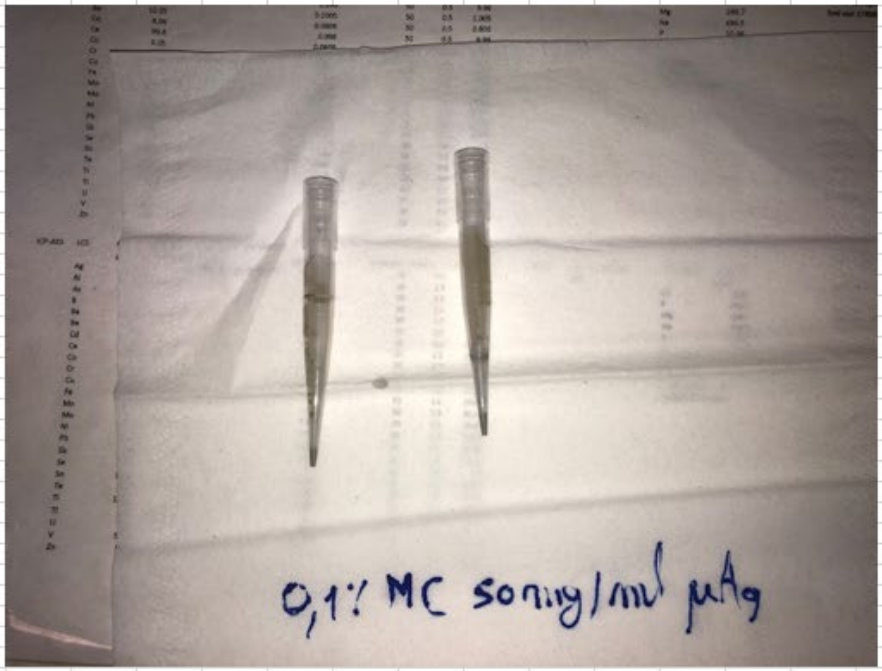


SCP Science Digitube 50 ml PP with clear screw cap which contained the 20 g µAg powder in 50 ml ultrapure water after rinsing with the additional 50 ml ultrapure water



SCP Science Digitube 50 ml PP with clear screw cap which contained the 20 g µAg powder in 50 ml ultrapure water after rinsing with the additional 50 ml ultrapure water

Inside of the pipette tip after taking subsample 1ml 1% MC 400cp 50 mg/ml µAg suspension

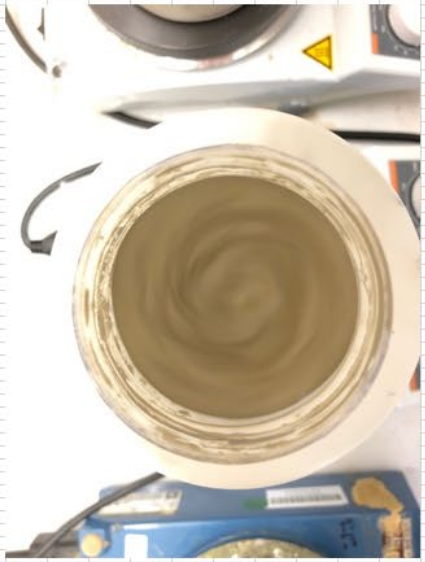


Inside of the pipette tip after taking subsample 1ml 0.1% MC 400cp 50 mg/ml µAg suspension

Tube roller

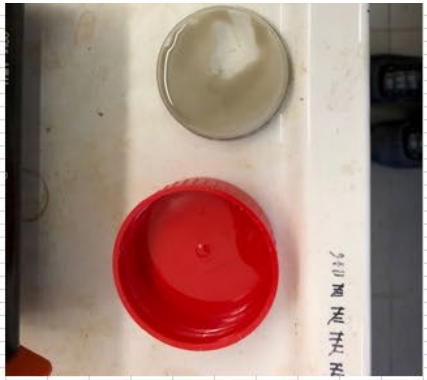
1% MC 400 cp 50 mg/ml pAg Azurpenrian after 1 hour on the tube roller and 5 minutes stirring at 900 rpm

1% MC 400 cp 50 mg/ml pAg Bzurpenrian after 1 hour on the tube roller and 5 minutes stirring at 900 rpm



0.1% MC 400 cp 50 mg/ml pAg Azurpenrian after 1 hour on the tube roller and 5 minutes stirring at 400 rpm

0.1% MC 400 cp 50 mg/ml pAg Bzurpenrian after 1 hour on the tube roller and 5 minutes stirring at 400 rpm



The lid of the 1% MC 400 cp 50 mg/ml pAg Azurpeorian after 1 hour on the tube roller and 5 minutes stirring at 900 rpm

1% MC 400 cp 50 mg/ml pAg Azurpeorian after 20 hourr in the fridge.

1% MC 400 cp 50 mg/ml pAg Bzurpeorian after 20 hourr in the fridge.

0.1% MC 400 cp 50 mg/ml pAg Azurpeorian after 20 hourr in the fridge.

0.1% MC 400 cp 50 mg/ml pAg Bzurpeorian after 20 hourr in the fridge.