Certification Report

Certified Reference Material

BAM-N012

Particle size of iron oxide nanocubes determined by transmission electron microscopy and small-angle X-ray scattering

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Coordinator:	Paul Mrkwitschka and Dr. Sarah-Luise Abram
Collaboration:	Dr. rer. nat. habil. Andreas F. Thünemann
Contact:	Dr. rer. nat. Harald Bresch Project coordinator TFP22 "nanoPlattform" Bundesanstalt für Materialforschung und -prüfung (BAM) Division 4.2 Richard-Willstätter-Str. 11 12489 Berlin, Germany Phone: +49 30 8104 3364 Fax: +49 30 8104 70000 E-mail: harald.bresch@bam.de

Summary

This report describes the preparation, analysis, and certification of the reference material BAM-N012, including all analytical aspects and the proof of homogeneity and stability.

BAM-N012 is a suspension of cubic iron oxide nanoparticles in toluene, certified for the following particle size parameters: i) median area equivalent circular diameter (ECD) and ii) median area equivalent square edge length (ESL). The ECD and ESL are determined as median values from the particle area measured by transmission electron microscopy. The certified values for ECD and ESL are shown in Table 1 together with their respective expanded combined uncertainty. Moreover, the mean edge length (EL), the particle number density (n), and the mass concentration (c) measured by small-angle X-ray scattering (SAXS) are given as informative values.

Table 1: Overview of determined values and their respective uncertainty.

	Certified value[III]	Uncertainty[IV]
ECD[I]	9.1 nm	0.8 nm
ESL[I]	8.1 nm	0.7 nm
	Informative values[V]	Uncertainty[IV]
EL[II]	7.9 nm	0.7 nm
n[II]	$0.12 \cdot 10^{-6}$ mol·l^{-1}	$0.05 \cdot 10^{-6}$ mol·l^{-1}
c[II]	0.22 g·L^{-1}	0.08 g·L^{-1}

I. *Median value obtained by transmission electron microscopy according to ISO 21363 and ISO 9276 (ECD only).*
II. *Mean value obtained by small-angle X-ray scattering according to ISO 17867 and ISO 23484.*
III. *The values and their uncertainty are traceable to the SI unit for length expressed as nm (Système International d'Unités).*
IV. *The uncertainty is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.*
V. *Informative values can be of interest to the user, but insufficient information is available to establish metrological traceability.*

The certified values are based on measurements performed at the starting point of the stability study on one transmission electron microscope which is traceably calibrated. They were confirmed by independent measurements within an interlaboratory comparison including four different electron microscopes which had to qualify by verifying their results with the CRM ERM-FD100.

BAM-N012 is delivered in glass bottles containing approximately 1 mL of the iron oxide nanoparticle suspension in toluene having an approximate iron concentration of 0.18 mg/mL.

BAM-N012 is intended to be used as a control sample for both precision and trueness estimation of particle size determination methods, in particular electron microscopy and small-angle X-ray scattering. Provided that the material is handled appropriately and stored at a temperature of (20 ± 3) °C in the dark, the validity of the certified values is:

i) one day after opening of the bottle and

ii) maximum one year after dispatch of the material to the customer.

Content

List of abbreviations

ANOVA	analysis of variance
c	mass concentration measured by SAXS
CRM	certified reference material
nanoRM	nanoscale reference material
EL	edge length measured by SAXS
df	degrees of freedom
ECD	area equivalent circular diameter
ESL	area equivalent square edge length
E_n	“normalized error“, a measure of data compatibility
F	Fisher statistics
F_{crit}	critical value of the Fisher distribution
ICP-OES	inductively coupled plasma optical emission spectroscopy
M	mean square
$M_{between}$	mean square between groups
M_{within}	mean square within group
MS	mean squared error
n	particle number density measured by SAXS
N*	number of bottles
n_0	number of replicate determinations
p	*p*-value
PTFE	Polytetrafluoroethylene
SAXS	small-angle X-ray scattering
SS	sum of squares
s	standard deviation
s_{bb}	between-unit standard deviation
s_r	repeatability standard deviation
TEM	transmission electron microscope/microscopy
TGA	thermogravimetric analysis
u_{bb}	uncertainty caused by inhomogeneity
u_{char}	uncertainty of the characterization method
u_{sts}	uncertainty caused by transport conditions
u_{lts}	uncertainty caused by instability
u_{rep}	uncertainty caused by repeatability
SEM	scanning electron microscope/microscopy
STEM-in-SEM	Scanning transmission electron microscopy in an SEM

1. Introduction and aim of the project

Nanomaterials are mostly provided with attractive size dependent physico-chemical properties that differ from those of the corresponding bulk materials mainly due to the considerably increased surface-to-volume ratio. Within the past decades, these special electrical, optical, magnetic, mechanical, or chemical properties have proven their great potential for applications in many industries and research areas like catalysis, optoelectronics, energy storage or biomedicine. Moreover, they can be found in common products like filters, lubricants, insulation, paints, or construction materials. However, the increased production and use of nanomaterials result in a higher chance of human exposure to nanoparticles and in their potential release into the environment. Rising concerns about health effects and eco-toxicologic impact have led to the regulation of the production and circulation of nanomaterials within the EU. Further progress in research and development as well as the regulatory requirements demand a reliable and accurate characterization of the size, shape, and size distribution of nanoparticles. To validate or calibrate the appropriate measurement techniques, nanoscale reference materials (nanoRMs), that reflect the great diversity of currently applied or investigated nanomaterials, are necessary.[1] However, most of the available nanoRMs are made of very few standard materials like gold, silica, polystyrene or titania and almost all have a spherical shape. The BAM-N012 presented here will expand this list of available nanoRMs with iron oxide as another industrially relevant nanomaterial, which is used in magnetic storage media, fluids, or inks, and many biosensing and medical applications, e.g. in immunoassays, as contrast agents in magnetic resonance imaging, for targeted drug or gene delivery, or hyperthermia cancer therapy.[2]

BAM-N012 is a stable dispersion of uniform iron oxide nanoparticles in toluene. The nanoparticles have a cubic shape and a nominal edge length of 8 nm. The particle size and size distribution are measured by two complementary standard techniques for particle size measurements: transmission electron microscopy (TEM), which can image single particles deposited on a substrate in vacuum and small-angle X-ray scattering (SAXS) as an ensemble technique measuring the dispersion. For both, no elaborate sample preparation of the provided dispersion is necessary. With a particle size below 10 nm and a medium average atomic number of iron in comparison to silicon or gold-based nanoparticles, the iron oxide nanocubes are challenging for high-resolution scanning electron microscopy (SEM), while still offering enough contrast for reliable TEM measurements. Furthermore, the nanoparticles have a sufficient scattering contrast for SAXS measurements, and their cubical shape and narrow size distribution are a dedicated case where the particle shape can be tested with the available models.

Thus, BAM-N012 qualifies as a test material to verify both precision and trueness of the size measurement with single particle methods like TEM, and ensemble methods like SAXS. For SEM this material is appropriate to test the limits of the instruments.

2. Preparation of the candidate material and samples for measurement

2.1 Synthesis, preparation, and packaging of the candidate material

The 8 nm iron oxide nanocubes were synthesized inhouse at BAM via a thermal decomposition method in high-boiling organic solvents using oleate as a capping ligand. After purification, the particles were suspended in toluene at a concentration of about 31.3 mg/mL and stored as a stock suspension at 4 °C. 10 mL of this stock suspension were later diluted to 1000 mL in a volumetric flask. An Eppendorf Multipette® was used to fill aliquots of approximately 1 mL to amber glass bottles closed with polypropylene screw caps containing a solvent resistant butyl/PTFE septum. The filled glass bottles were labelled according to their filling sequence and stored in a ventilated cabinet at room temperature.

2.2 TEM sample preparation

To prepare the samples for measurement a TEM grid (Formvar supported copper grid of size 200 mesh) was placed on a filter paper. The iron oxide nanoparticles were applied onto the TEM grid by drop casting 6 µL of the suspension with a piston-operated pipette using a 0.5 µL - 20 µL Eppendorf® tip. When a drop of the suspension remained on the grid, it was removed with another filter paper before transferring the sample into the TEM grid storage box. The samples were then dried for at least one day inside the box for grid storage at room temperature prior to measuring.

2.3 SAXS sample preparation

For the SAXS measurements, 100 µL of the BAM-N012 suspension were filled in a glass capillary with a diameter of 1 mm and measured immediately. As a general recommendation, at least 50 µL of the suspension should be used for a single analysis. Although this has not been tested here, it can be assumed that using smaller volumes than 50 µL for the measurements will give the same results. This assumption is indicated by an earlier study on certified reference materials of gold nanoparticles in which only 20 and 3 µL of suspension were used.[3]

3. Instrumentation and Measurands

The reference material candidate BAM-N012 is characterized by means of TEM and SAXS measurements which are both established methods for the determination of nanoparticle size descriptors.[4] Both methods were used for homogeneity and stability testing of the material. TEM is the most precise method to obtain size information of single particles and SAXS as an ensemble averaging method complements these results with a superior statistic considering the particle number (averaging over the order of 10^6 particles, i.e., all particles in the irradiated volume). The characterization was conducted with established procedures on calibrated instruments, however only the TEM results are directly traceable to the SI unit of length. The following two paragraphs describe in detail the equipment used, the measurement procedures, the data analysis as well as the chosen measurands.

3.1 Transmission electron microscopy

The TEM measurements were carried out on a JEM-2200FS (JEOL) instrument in brightfield mode operated at 200 kV and equipped with a Gatan CCD camera. The traceability of the instrument is discussed in Chapter 9. The acquired images are 2D projections of the iron oxide nanoparticles dried on the TEM grid, as described in Chapter 2.2. For each grid five positions were randomly chosen on the grid and three images were captured at each of them. The images were analyzed using the Fiji distribution of the software ImageJ.[5] After applying a median filter to the images, they were segmented using the ISOdata algorithm for thresholding.[6] The outlines of the detected particles were saved as regions of interest (ROI) and roughly 30% of the particles were manually adapted on overlays with the original image in case a particle was not correctly outlined. The corrected ROIs were saved as binary images that were analyzed for the particle area and the calculation of further particle size descriptors. The area equivalent circular diameter (ECD) was selected according to ISO 9276-6 as a general particle size descriptor that can be used for any particle regardless of its shape or roughness. Additionally, analogue to the ECD, an area equivalent square edge length (ESL) was calculated. This new type of parameter is not defined by ISO 9276-6, but is a relevant descriptor for BAM-N012, as it considers the cubic shape of the particles. Moreover, this descriptor allows direct comparison to the edge length, EL, as determined by SAXS (see Chapter 0). For each TEM grid, at least one image per grid position was randomly chosen to obtain results with a minimum particle count of at least 1000, i.e., exceeding the minimum of 500 recommended by ISO 21363. Representative particle size distributions (PSD) for both measurands, ECD and ESL, are shown in Figure 1. The PSD could not be fitted with a single Gaussian, lognormal or Weibull distribution as suggested in ISO 21363. Thus, a bimodal distribution, i.e., two Gaussian distributions, was used. For certification, only descriptive statistics are used to calculate the median of the measurands ECD and ESL. The minor mode of particles could not be identified by SAXS. Its size is around 6-7 nm for ECD and ESL

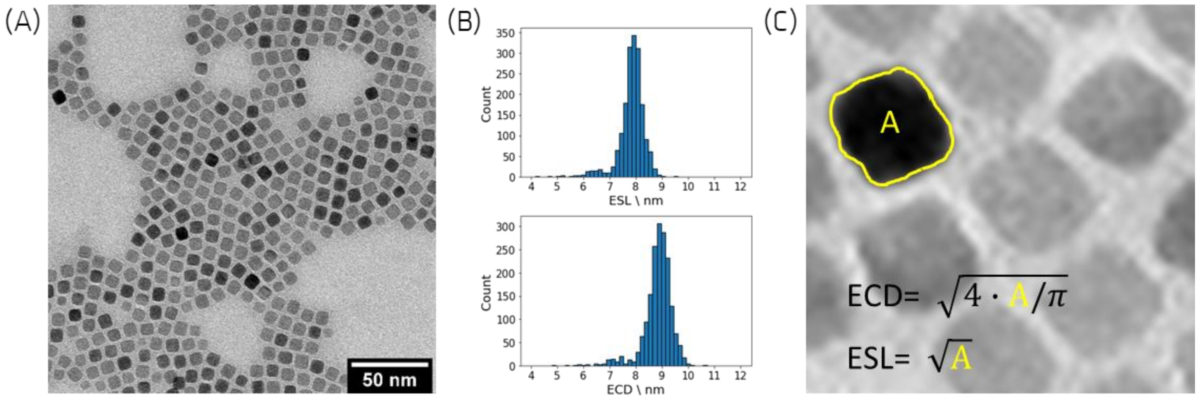

Figure 1: (A) Representative TEM image of BAM-N012 showing cubic particles. (B) Representative PSDs for ECD and ESL extracted from TEM micrographs. (C) Representation and calculation of the selected measurands ECD and ESL.

3.2 Small-angle X-ray scattering

SAXS measurements were carried out with a SAXSess instrument (Anton Paar) to analyze particle size, size distribution, particle number density and particle concentration. This instrument of a Kratky type is attached to a laboratory X-ray generator PW3830 (PANalytical) and was operated with a fine focus glass X-ray tube at 40 kV and 40 mA (Cu-K_α, λ =0.1542 nm). A focusing multi-layer optic and a block collimator provide a monochromatic primary beam with a low background as reported by Bergmann et al.[7] The used capillary allows to attain the same scattering volume for the scattering of i) sample, ii) solvent, and iii) empty capillary. The scattered intensity as a function of the scattering vector q was recorded for a measurement time of 120 s (accumulation of 12 measurements of 10 s each) with a Mythen detection system (Dectris AG). The intensity data were converted to absolute intensity data with the SAXSquant software (Anton Paar AG) as described by Orthaber et al.[8] The temperature of 21 °C was controlled with a TCS 120 sample holder (Anton Paar AG) with an accuracy of ±1 °C.

In conjunction with this reference material development, we conducted an international SAXS interlaboratory comparison as a feasibility study.[9] The material of the study was a suspension of spherical silver nanoparticles and the mean of the measurands particle size (diameter) d, the particle number density n, and the concentration c, were reliably determined. Therefore, we were guided for the nanocubes in the present report by the outcome of that interlaboratory comparison for the choice of the measurands, with the edge length EL, replacing the diameter as a measurand for particle size.

A model-driven small-angle X-ray scattering data evaluation was performed for the characterization of the size distribution and the concentration of BAM-N012 using the model of a lognormal size distribution of spherical particles following the international standard ISO 17867. Determination of the number concentration was performed according to ISO 23484.

In the analysis of iron oxide nanocubes, it has proven useful to apply the spherical model in SAXS data interpretation, as demonstrated by Disch et al.[10, 11] Cubes and spheres are both characterized by a single size parameter, which is the edge length for the cubes and the radius for the spheres. Therefore, a cube can be represented by an equivalent sphere of the same radius of gyration, R_g. The side length, EL, of a cube is related to its radius of gyration, R_g, by

$$EL = 2R_g$$

Equation 1

(see equation C.5 in[12]). The form factor of a cube is very similar to that of its isovolumic spherical counterpart as can be seen in a work on the scattering function of Platonic solids.[13] Experimentally relevant differences in the scattering of cubes and spheres appear only at relatively large q-values, more precisely, for the third and higher maxima of the scattering function. If there are fewer than three scattering maxima in the data, which is the case for BAM-N012, it is justified to utilize the scattering function of a sphere instead of the form factor of a cube. The former is reliably implemented and extensively tested in common SAXS curve fit programs and also used in the ISO standard. Curve fitting of the sphere model provides the radii of the equivalent sphere R_{sphere}. With the radius of gyration of a sphere being

$$R_{g,sphere} = \sqrt{\frac{3}{5}}\, R_{sphere}\ ,$$

Equation 2

the edge length of a cube is obtained as

$$EL = 2\sqrt{\frac{3}{5}}\, R_{sphere}$$

Equation 3

with the volume V_c of a single nanocube being

$$V_c = EL^3 = \frac{24}{5\sqrt[3]{\sqrt{5}}} R_{sphere}^3\ .$$

Equation 4

The detailed deduction of the measurands EL, n, and c from the model of the scattering intensity is given in Chapter 13 Annexes I. Application of this model is exemplarily shown in Figure 2 (left side) for one data set of the homogeneity study. The curve fit provides the parameters of the distribution of the nanoparticles edge length EL shown in Figure 2 (right side).

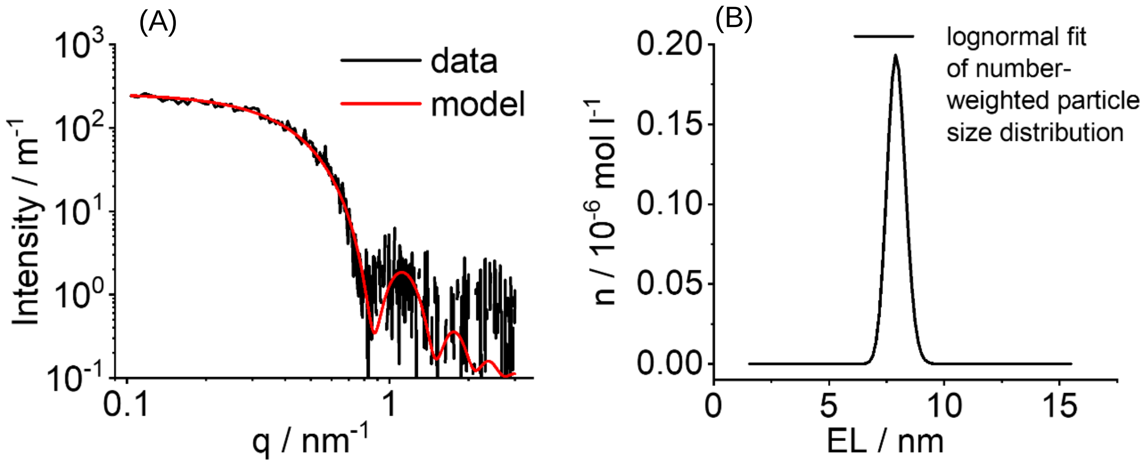

Figure 2: Analysis of the SAXS data by application of the model of spherical particles. Given are (A) the measured scattering data in terms of the scattering intensity as a function of the scattering vector q (black curve) and the best fit of the model (red curve) and (B) the corresponding distribution of the particles' edge length EL. The size distribution is number-weighted, and the distribution's shape is a lognormal function.

4. Homogeneity tests

The homogeneity of BAM-N012 was tested by TEM and SAXS for both certified and informative values. Therefore, TEM and SAXS measurements were performed on 10 individual bottles selected by random stratified sampling from the whole set of 1000 bottles following the recommendations of ISO Guide 35.

4.1 Evaluation of Homogeneity for TEM

For each of the randomly selected 10 bottles, two TEM grids were prepared as described in Chapter 2.2. The 20 grids were measured according to a simple randomized design within three consecutive days. One grid was measured four additional times (for a total of five) on day four to test the repeatability of the measurement. The median ECD was extracted for each grid as described in Chapter 3.1. Figure 3 shows the median ECD values for all 20 grids prepared from the 10 sampled bottles (black squares), the overall mean of these 20 values (8.92 nm, blue solid line), its standard deviation of ±0.12 nm (blue dotted line) and its double standard deviation of ±0.24 nm (red dotted line).

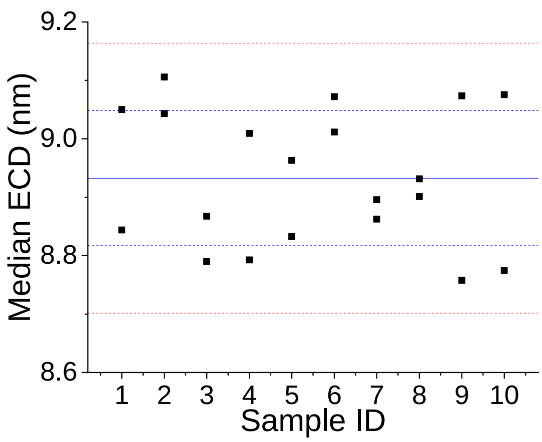

Figure 3: Median Area Equivalent Circular Diameter ECD for 10 bottles of BAM-N012 measured in duplicate by TEM. The blue solid line denotes the overall mean, the blue dotted line the standard deviation and the red dotted line the twofold standard deviation.

For the homogeneity study, only the median ECD is used for the calculations as both certified parameters (ECD and ESL) are derived from the same measured characteristic, i.e., the particle area. If the homogeneity of BAM-N012 can be proven for one of them, the other parameter is implicitly homogeneous. The initial evaluation of the values shown in Figure 3 gives no indication of obvious outliers or potential trends. The dataset was tested for a normal distribution type with the software Origin 2018G, using the Shapiro-Wilk, Kolmogorov-Smirnov and Anderson-Darling tests, with a significance level of 0.05. The null hypothesis of normality could not be rejected and thus it is concluded that the median ECD and median ESL values are close to normally distributed.

To demonstrate that no analytical trends are present, the dataset was evaluated by Analysis of Variance (ANOVA) as shown in Figure 4. The hypothesis that three consecutive measurement days can be considered as a singular measurement run was evaluated through the scattering of values between and within the measurement days. Furthermore, the whole sequence of the measured TEM samples was analyzed by linear regression to show that no time dependent drift of the instrument is present.

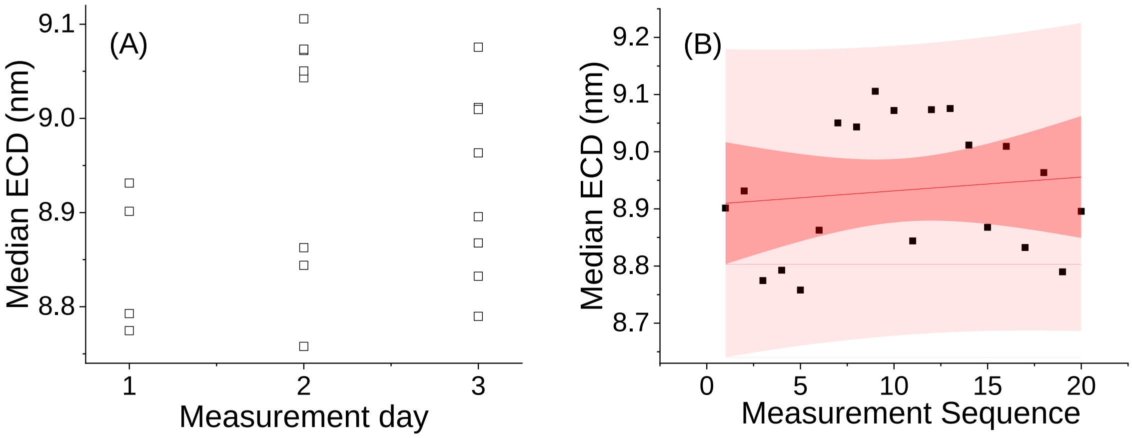

Figure 4: ANOVA of the analytical sequence sorted by measurement day (A) and sorted by measurement sequence (B). The red line shows the linear fit, the red area the confidence band, and the light red area the prognosis bands; NOTE: a single datapoint at measurement day 3 (left figure) was marked by a horizontal line for display considerations.

ANOVA uses a variance-based F-test of the group mean equality. Therein, the null hypothesis that all group means are equal cannot be rejected if the calculated F-value is below a tabulated critical value F_{crit}. As the F-values of the ANOVA are smaller than the F_{crit} and the *p*-values, which are inversely related to the F-values and additionally given as an indicator for significance, are significantly larger then 0.05, the null hypothesis that the three measurement days are equal cannot be rejected and no significant trend in the analytical sequence of the homogeneity test can be detected. Thus, the three measurement days can be treated as a singular measurement run. The results are summarized in Table 2.

Table 2: Summary of investigation for statistical trends by ANOVA: Measurement day and measurement sequence.

Measurand	slope	F	F_{crit}	p-value	Significant trend of the slope (95 % confidence level)
Median ECD /nm (by measurement day)	-	1.414	3.562	0.270	no
Median ECD / nm (by measurement sequence)	0.003	0.293	4.414	0.595	no

Further, the influence of the filling sequence on the measured values was tested by a linear regression with the values sorted according to the order of filling of the bottles and sorting into boxes (see Figure 5). As determined by ANOVA, the slope of the linear fit is not significantly different from zero. The F value is much lower than F_{crit} and the p-value of 0.508 is significantly larger than 0.05 as shown in Table 3. Therefore, no correlation between the filling sequence and the measured median ECD and ESL can be observed.

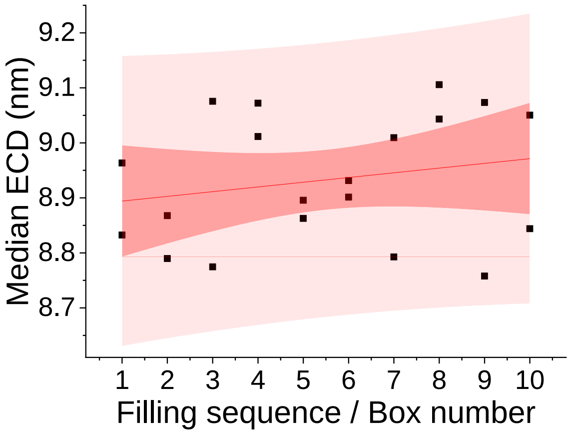

Figure 5: Evaluation of the bottles' filling sequence by linear regression. The red line represents the linear fit, the red area the confidence band and the light red area the prognosis bands.

Table 3: Summary of investigation for statistical trends by ANOVA: The bottles' filling sequence.

Measurand	slope	F	F_{crit}	p-value	Significant trend of the slope (95 % confidence level)
median ECD	0.006	0.456	4.41	0.508	no

According to ISO Guide 35, it is necessary to determine the homogeneity of a reference material with ANOVA. From the ANOVA, the within-bottles and between-bottles mean squares (M_{within} and $M_{between}$) of the measurands are obtained, and the between-bottle standard deviation (s_{bb}) can be estimated by

$$s_{bb} = \sqrt{max\,(\frac{M_{between} - M_{within}}{n_0}, 0)},$$

Equation 5

where n_0 describes the number of observations per group (2 for this study i.e., 2 grids per bottle). Furthermore, the repeatability standard deviation, s_r, can be estimated by

$$s_r = \sqrt{M_{within}}.$$

Equation 6

Due to the chosen measurement scheme with each bottle measured in duplicate, ISO Guide 35 recommends measuring one sample (in this case the same TEM grid) at least 5 times in total to verify the repeatability of the method. These results are shown in Figure 6.

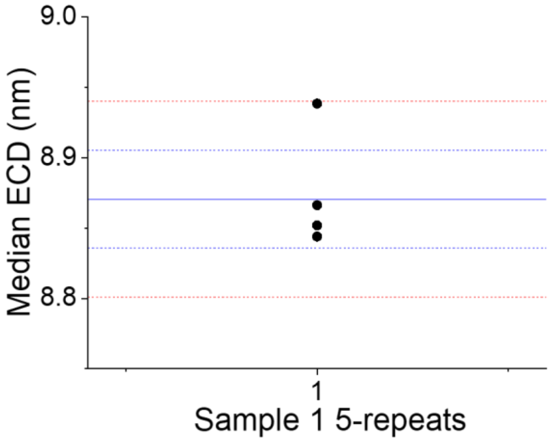

Figure 6: Additional measurements of one TEM grid from the homogeneity study to determine the repeatability uncertainty. The horizontal lines represent the statistics of the 5 repeated measurements, where the blue solid line denotes the overall mean (8.87 nm), the blue dotted lines the standard deviation (±0.04 nm) and the red dotted lines the twofold standard deviation.

From these additional measurements the standard deviation of the 5 repeated measurements was determined to be s_{r^*} = 0.04 nm. It should be noted that compared to the repeatability uncertainty obtained within the homogeneity study of s_r = 0.133 nm (see later Table 4), contributions from the sample preparation are not included. To account for all contributions to the repeatability uncertainty the homogeneity study with 20 datapoints will be used to estimate the repeatability uncertainty.

The results of the ANOVA and the standard deviations s_{bb} and s_r are given in Table 4 as S for "between bottles" and S for "within bottles", respectively. For the median ECD, s_{bb} is 0.0 nm and s_r is 0.133 nm. As the s_{bb} value is zero, between-bottle effects can be considered as negligible.

Table 4: ANOVA table for between-bottle homogeneity study of the median ECD with SS: sum of squares, df: degrees of freedom, MS: mean squares, S: estimated standard deviation.

Overall Mean (nm)	Overall s (nm)	Source of variation	SS (nm²)	*df*	MS (nm²)	S (nm)	*F*	F_{crit}	*p*-value
8.93	0.12	Between bottles	0.091	9	0.010	0.0	0.7	3.0	0.8
		Within bottles	0.177	10	0.0177	0.133			
		Total	0.267	19					

The data shown in Table 4 shows that the *F*-value is smaller than F_{crit} and the *p*-value of 0.794 is much larger than the typical threshold value of 0.05. Therefore, the *p*-value is not significant, and it can be concluded that the groups show no significant differences.
The between-bottle uncertainty can be derived from the between-bottle standard uncertainty as

$$u_{bb} = s_{bb},$$

Equation 7

where u_{bb} is an estimate of the uncertainty due to possible (undetected) inhomogeneity. In this study u_{bb} is 0 nm as shown in Table 4. Furthermore, the repeatability uncertainty is

$$u_{rep} = s_r$$

Equation 8

with u_{rep} denoting the uncertainty of the repeatability of a single measurement, including sample preparation. In this study u_{rep} is 0.133 nm.

4.2 Evaluation of Homogeneity by SAXS

Three samples were taken from the 10 selected bottles for three series of SAXS measurements. The resulting data curves were analyzed as described in Chapter 0.
The fitting model provides 30 values for each of the threeе measurands mean particle edge length EL, particle number density n and particle concentration c. An overview of means and standard deviations for all measurands of the 10 samples is provided in Figure 7. The overall means of the three properties are EL = (7.92 ± 0.19) nm with the relative uncertainties in brackets (±2%), n = (0.12 ± 0.01)·10^{-6} mol·L^{-1} (±10%) and c = 0.22 ± 0.02 g·L^{-1} (±7%). Red squares and error bars represent the mean of the 30 values, and one standard deviation of the mean, blue circles and error bars indicate the mean and twofold standard deviation.

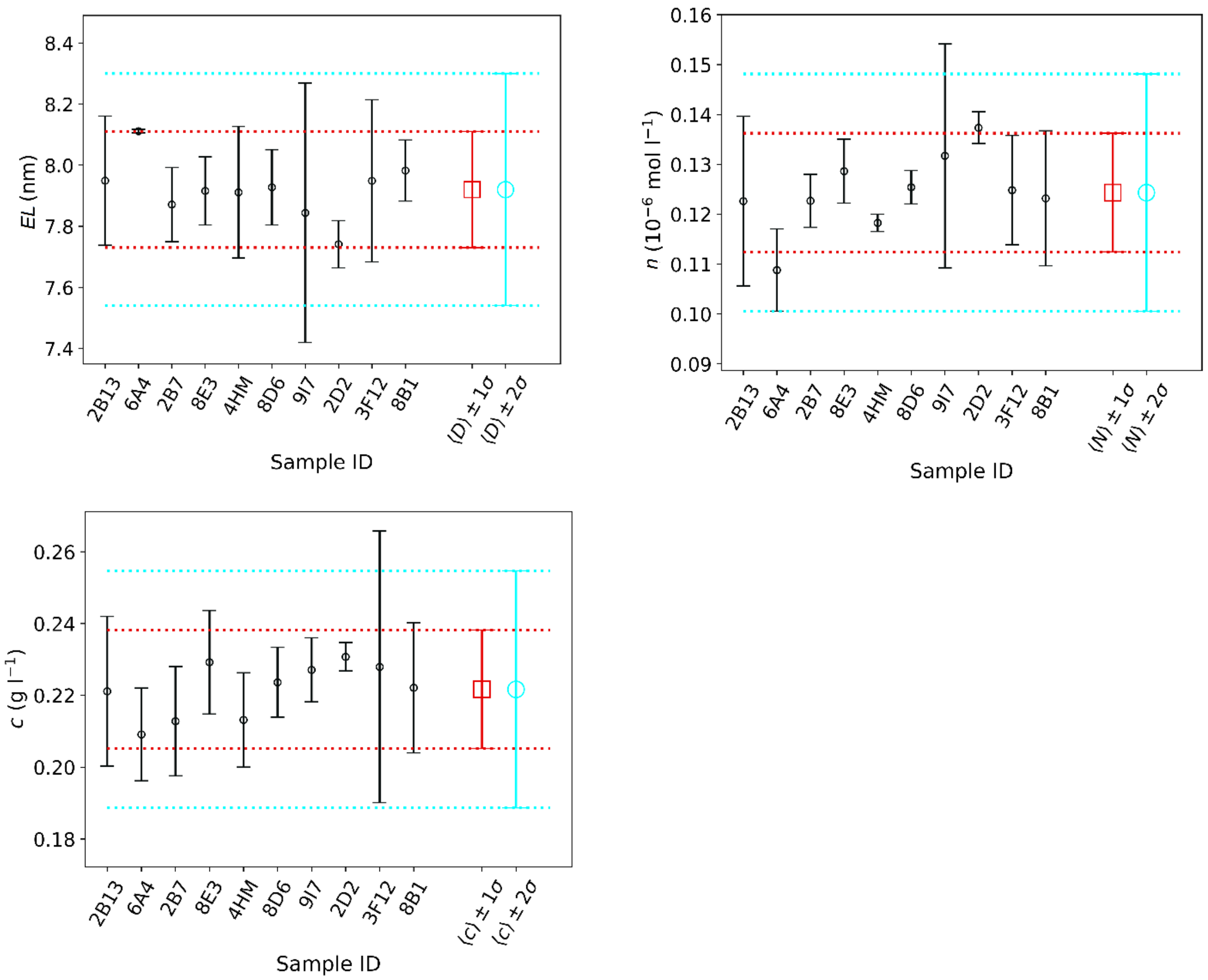

Figure 7: Homogeneity study on the mean edge length (EL), particle number density (n), and particle concentration (c). The overall mean is marked by a red square/ light-blue circle, the red dotted lines denote the standard deviation and the light-blue dotted lines the double standard deviation.

The ISO Guide 35 recommends initial evaluation of a homogeneity study with inspection of measurand trends and outliers. No obvious trend or outliers are visible for the measurands in Figure 7. Therefore, our hypothesis is that the values of the measurands are normally distributed. Three tests were utilized to try to reject this hypothesis on a significance level of 0.05. These tests are the Kolmogorov-Smirnov test, which is conservative, the Shapiro test and the Normal test, the latter combining skew and kurtosis measurements. The functions scipy.stats.kstest, scipy.stats.shapiro and scipy.stats.normaltest were used form the package scipy 1.6.3 (https://www.scipy.org/). The null hypothesis of normality cannot be rejected for EL, n and c based on all tests. These test results (with *p* always larger then 0.2) indicate that the distributions of the measurands are similar to normal distributions and an ANOVA can be applied. Next, an ANOVA was performed for the four measurands and s_{bb} and s_r were determined according to Equation 5 and Equation 6 with three observations per group (n_0 = 3). The results are summarized in

Table 5 to Table 7. The between-bottles and repeatability standard deviations are s_{bb} = 0.0 nm and s_r = 0.203 nm for EL, s_{bb} = 0.001 · 10^{-6} mol·L^{-1} and s_r = 0.012 · 10^{-6} mol·L^{-1} for n and finally s_{bb} = 0 and s_r = 0.018 g·L^{-1} for c.

Table 5: ANOVA table for between-bottle homogeneity study of EL.

Overall Mean (nm)	Overall s (nm)	Source of variation	SS	*df*	MS (nm)	S (nm)	*F*	F_{crit}	*p*-value
7.935	0.194	Between bottles	0.264	9	0.03	0	0.7	2.3	0.7
		Within bottles	0.827	20	0.04	0.203			
		Total	1.092	29					

Table 6: ANOVA table for between-bottle homogeneity study of n.

Overall Mean (·10^{-6} mol·L^{-1})	Overall s (·10^{-6} mol·L^{-1})	Source of variation	SS	*df*	MS (·10^{-6} mol·L^{-1})	*S* (·10^{-6} mol·L^{-1})	*F*	F_{crit}	*p*-value
0.125	0.012	Between bottles	0.001	9	0	0.001	1.1	2.3	0.4
		Within bottles	0.003	20	0	0.012			
		Total	0.004	29					

Table 7: ANOVA table for between-bottle homogeneity study of c.

Overall Mean (g·L^{-1})	Overall s (g·L^{-1})	Source of variation	SS	*df*	MS (g·L^{-1})	*S* (g·L^{-1})	*F*	F_{crit}	*p*-value
0.223	0.018	Between bottles	0.002	9	0	0	0.8	2.4	0.6
		Within bottles	0.007	20	0	0.018			
		Total	0.009	29					

The finding that the s_{bb}-values are very small or even 0 indicates that between-bottles effects for EL, n and c are negligible compared to repeatability uncertainty. Such a behavior could have been expected because all bottles were filled from the same stock suspension and the nanoparticles are too small to sediment. Moreover, the TEM study already suggested homogeneity of the particle size. A comparison of the data in Table 5 to Table 7 shows that the *F*-value is always smaller than F_{crit} and the *p*-values of 0.694 (EL), 0.439 (n) and 0.617 (c) given in the Table 5 to Table 7 are much larger than the typically utilized threshold value of 0.05. Therefore, the *p*-values are not significant, and it can be concluded that there is no significant variation between the groups.

To summarize, no evidence suggesting a rejection of the hypothesis that the material is sufficiently homogeneous was observed. The between-unit homogeneity contribution to the uncertainty is derived from the between-unit standard deviation according to Equation 7.

All data from the homogeneity study were accepted and pooled. Thus, from this homogeneity study, total mean values have been derived for the three measurands and estimates of the uncertainty u_{bb} due to possible (undetected) inhomogeneity. Next, the repeatability standard deviations were utilized as estimates for the uncertainty of the repeatability of a single measurement according to Equation 8.

5. Long-term stability (LTS)

BAM-N012 is assumed to be a stable suspension of iron oxide nanoparticles in toluene, but no prior study or proof of stability is known for this material system to date. Iron oxide as an inorganic material does not easily dissolve in toluene, and an additional protective coating with oleate further provides stability. Due to the extremely small nominal size of 8 nm the Brownian motion of the particles outranges gravitational forces and therefore sedimentation is unlikely to occur if prior agglomeration is non-existent. In the case that stability is expected, but no prior knowledge exists, proof must be established in accordance with ISO Guide 35. As critical influence factors, time and temperature are expected to have the highest impact on storage and transport stability of BAM-N012. Light irradiation of the sample can be disregarded with proper handling, as BAM-N012 is delivered in amber glass bottles and should additionally be stored in the dark. The long-term stability is evaluated for storage at room temperature because this allows easy handling and delivery, and it can help to avoid agglomeration processes at low thermal energy.[14] The whole collection of bottles is stored at room temperature and in regular intervals bottles are chosen randomly and investigated for stability by TEM and SAXS.

5.1 Evaluation of LTS by TEM

Stability measurements were performed after 6-, 10-, and 16-months storage of BAM-N012 at room temperature after bottling of the material and the measurements for the homogeneity study. The stability measurement after 6 months was chosen as the starting point for the stability study as it took place right after TEM maintenance with adjustments to critical systems such as the lens system and calibration measurement with the CRM MAG-I-Cal SN2145. Furthermore, the homogeneity study with SAXS and the starting point of the SAXS long-term stability study took place during that time. Two TEM grids were prepared from each of three randomly selected bottles for a total of 6 datapoints per point in time. Sample preparation, measurement and data evaluation were carried out according to Chapters 2.2 and 3.1. The behavior of the median ECD values over time is shown in Figure 8.

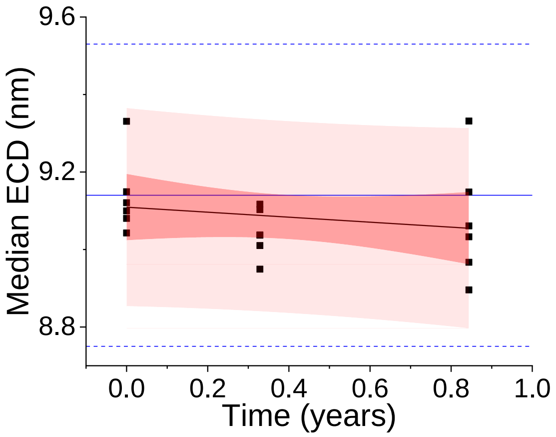

Figure 8: Evaluation of long-term stability: The initial stability starting point is equal to 6 months storage time at room temperature since the bottling of the material and homogeneity study. The blue solid line denotes the overall mean of 9.14 nm which is taken from the starting point of this stability study. The blue dotted lines represent the uncertainty u_{CRM} of 0.4 nm without the coverage factor. The red area marks the 95 % confidence bands and the light red area the prognosis bands.

After each point in time, the majority of datapoints are within the double standard deviation of the overall mean value at the starting point of the study. The overall means of each interval are shown in Table 8 and the ANOVA values for the linear fit are shown in Table 9.

Table 8: Comparison of overall mean values of the shown stability intervals.

Measurand	Starting point (nm)	4 months (nm)	10 months (nm)
Median ECD	9.14	9.04	9.07

Table 9: Summary of the TEM long-term stability linear regression fit evaluated by ANOVA.

Measurand	Slope / SD-slope (nm)/ (nm/year)	*F*	F_{crit}	*p*-value	u_{lts} (nm)	Significant trend of the slope (95 % confidence level)
Median ECD	-0.06 / 0.08	0.70	4.49	0.416	0.18	Not significant

Stability of BAM-N012 is further assessed with the E_n-criterion (according to BAM procedural instructions “AKRM-04” available on request) by

$$\frac{|x_a - x_e|}{\sqrt{u_{ref}^2 + u_{verf}^2}} \leq 2$$

Equation 9

where x_a denotes the values as obtained from the initial stability study, x_e is obtained from the 2 recent stability studies (after 4 and 10 months), u_{ref} is taken as the complete uncertainty of the certified reference material and u_{verf} is taken from the standard deviation of the latest stability study (10 months). The maximum difference of an individual measurement to the median ECD $\max|x_a - x_e|$ is 0.435 nm, with a measurement uncertainty of u_{verf}= 0.15 nm and a complete uncertainty u_{CRM}=u_{ref}=0.42 nm (see Chapter 7.1). This results in a value for E_n of 1.01, which is smaller than 2. Thus, the material passes the

E_n-criterion, and the material is stable within the given uncertainties (for details please refer to the Annexes).

The shelf life is estimated from the linear regression shown in Figure 8. According to ISO Guide 35 the uncertainty of the certified material U_{CRM} is adjusted for an uncertainty due to long-term storage u_{LTS}. For a shelf life of one year starting with the dispatch to the consumer, the time interval was calculated by adding the monitoring interval time and multiplying the result (2.3 years) with the standard deviation of the slope of the long-term storage study. This results in a contribution of long-term storage to the uncertainty of u_{LTS} = 2.0 %.

5.2 Evaluation of LTS by SAXS

The homogeneity study was carried out at the starting point of this stability study. The second stability measurement was performed after a period of three months, analyzing three samples without repetition. After 6 months, five samples were measured in triplicate and after 14 months the fourth stability study concluded with 5 samples measured in triplicate. The three measurands plotted over time are shown in Figure 9:

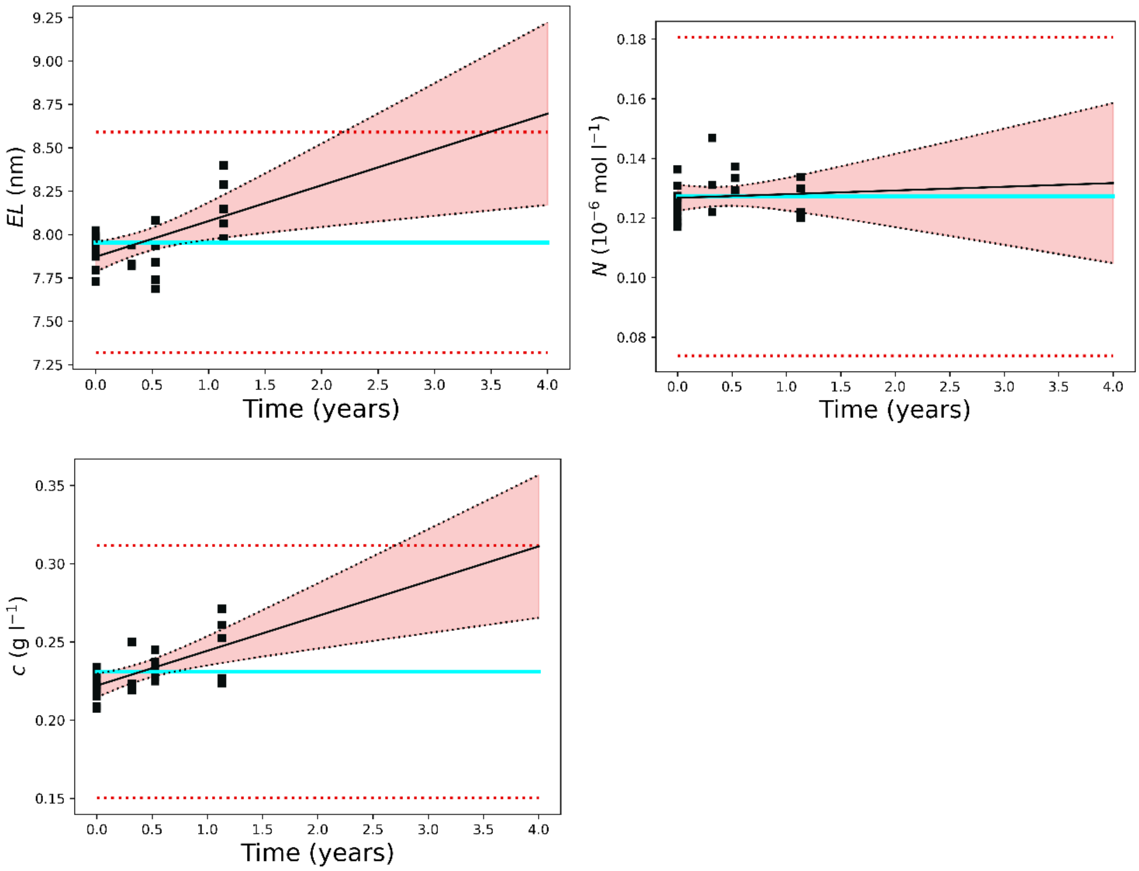

Figure 9: Stability study of the particle edge length (EL), particle number concentration (n), and weight concentration (c). Given are measurand values (black squares), mean of all measurand values (horizontal light blue line), expanded uncertainty of the measurand values (red dotted lines), linear regression lines (black solid lines) and their 95 % level of confidence (area between dashed black lines, displayed light red).

The data for the three measurands EL, n and c plotted over time was analyzed for statistical relevant trends showing that only the measurand n is stable within the observed time frame of 14 months. In contrast, the measurands EL and c showed a relevant trend at 95 % significance level. Analysis of the scattering data revealed beginning agglomeration of the particles, that might lead to the slight increase of the mean EL over time. As the concentration c is derived from the particles' size, its value must increase as well. However, the mean values for both EL and c are expected to stay within the expanded uncertainty up to 2.5 years. From this study the long-term stability uncertainty of EL, n and c are adjusted for the stability control interval time of 6 months plus one-year guaranteed stability starting with the dispatch to the consumer.

Table 10: Summary of the SAXS long-term stability test over 12 months.

Measurand	Slope	u_{lts}	Significant trend of the slope (95 % confidence level)
EL	0.24 nm/year	0.17 nm	significant
n	$4.01 \cdot 10^{-6}$ mol·L^{-1} /year	$0.008 \cdot 10^{-6}$ mol·L^{-1}	not significant
c	0.031 g·L^{-1} /year	0.016 g·L^{-1}	significant

6. Short term stability (STS)

6.1 Evaluation of STS for TEM

To simulate transport conditions, two bottles of BAM-N012 were stored for 4 weeks at -20 °C and 40 °C respectively. Additionally, two more bottles were stored for 24 weeks at 40 °C to see possible long-term effects of a higher storage temperature. All bottles were analyzed in duplicate in an isochronous study at the end of the 24 weeks period. This is schematically shown Figure 10.

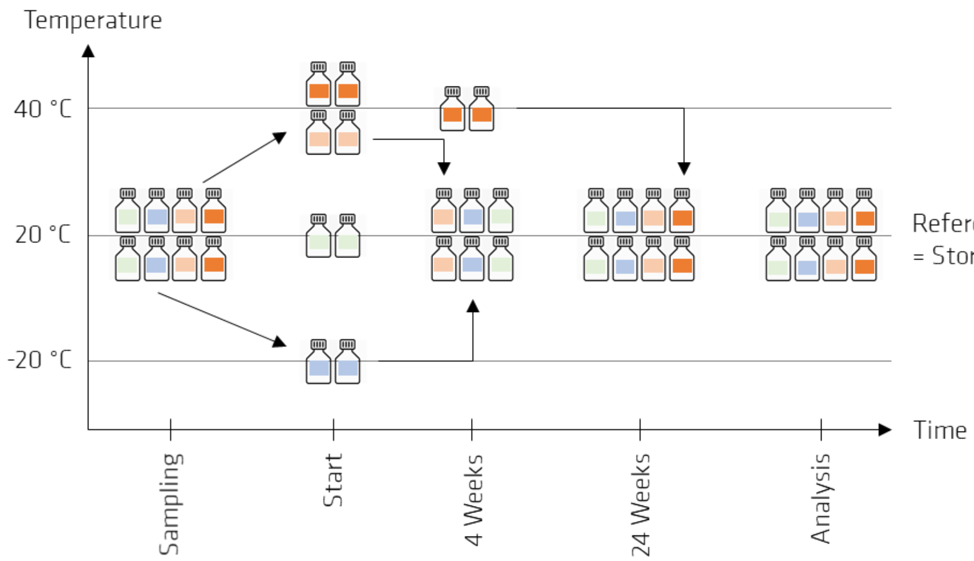

Figure 10: Scheme for the isochronous study showing the number of bottles at a certain storage temperature for each point in time. The action required at each point in time (bringing bottles to higher or lower storage temperature or back to reference

temperature of 20°C) is expressed by the arrow. All bottles are equal in the beginning; however, the color code should help to identify the treatment of the individual bottles.

The measurement was carried out with a randomized scheme over two days with one grid from each of the 8 bottles measured on each day. The data from the two measurement days are evaluated by ANOVA with the result that the two consecutive days can be considered as one measurement run.

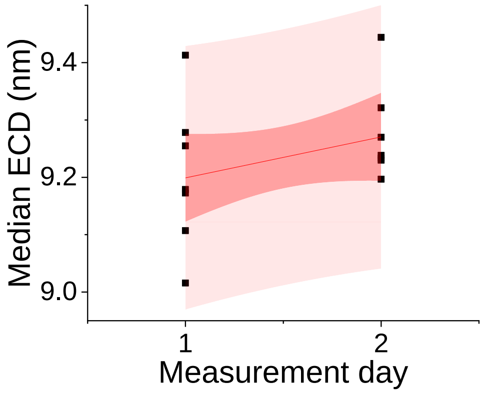

Figure 11: Median ECD plotted over measurement day. The red line shows the linear fit, the red area the confidence band and the light red area the prognosis bands.

The results for the evaluation of trends are summarized in Table 11.

Table 11: ANOVA values for the trend evaluation of the analytical sequence in two consecutive days.

Measurand	Slope (nm/week)	*F*	F_{crit}	*p*-value	Significant trend of the slope (95 % confidence level)
Median ECD (by measurement day)	0.07	2.0	4.6	0.2	Not significant

Figure 12 shows that the overall mean after storing the bottles at -20 °C and +40°C slightly increases. However, the ANOVA reveals that the slopes of the linear regressions do not deviate significantly from zero as shown in Table 12. Therefore, BAM-N012 can be considered stable if exposed to -20°C or up to +40 °C for shorter times during shipment.

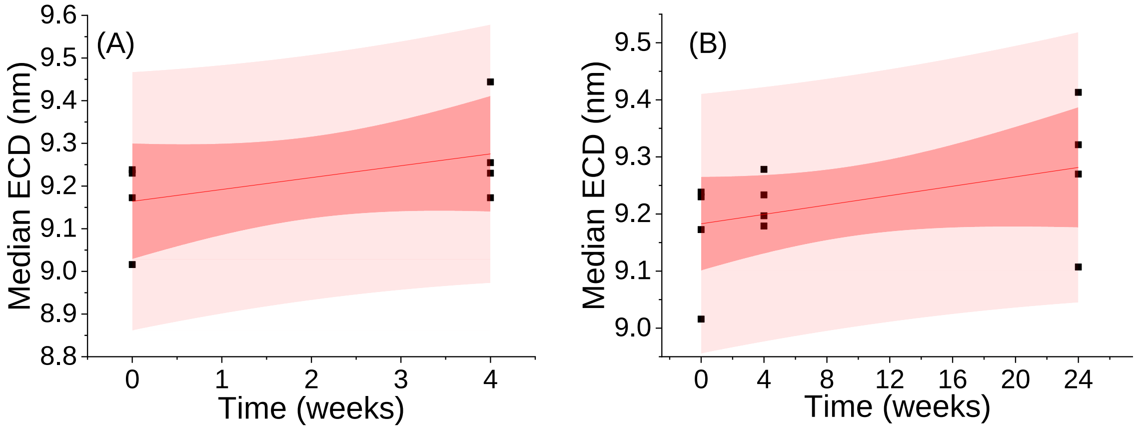

Figure 12: (A) Median ECD determined by the isochronous study after storage at -20 °C for 4 weeks. (B) Median ECD determined after storage at +40 °C for 4 and 24 weeks (right). The red lines show the linear fit, the red areas the confidence bands and the light red areas the prognosis bands.

No significant trend was found for both temperature regimes. The material should be stable for short-term dispatch to the customer. The results of the ANOVA of the linear regression are summarized in Table 12. The contribution of the short-term stability to the uncertainty budget u_{sts} was calculated by multiplying the standard deviation of the slopes of the linear regressions with a time interval of 4 weeks assumed as maximum duration of transport.

Table 12: ANOVA values for the trend evaluation after storage at -20 °C and +40 °C.

Measurand	Slope / SD-Slope (nm/week)	F	F_{crit}	u_{sts} (nm)	*p*-value	Significant trend of the slope (95 % confidence level)
Median ECD (Storage at -20°C)	0.03 / 0.02	2.0	6.0	0.08	0.2	Not significant
Median ECD (Storage at +40°C)	0.004 / 0.003	2.5	5.0	0.01	0.2	Not significant

6.2 Evaluation of STS for SAXS

The short-term stability was not investigated by SAXS as the results found in the respective TEM study (see Chapter 6.1) suggest stability of particle size during transport.

7. Uncertainty estimation

Various sources of uncertainty contribute to the overall uncertainty budget of each measurand. The potential uncertainty contributions are shown within an Ishikawa diagram in Figure 13.

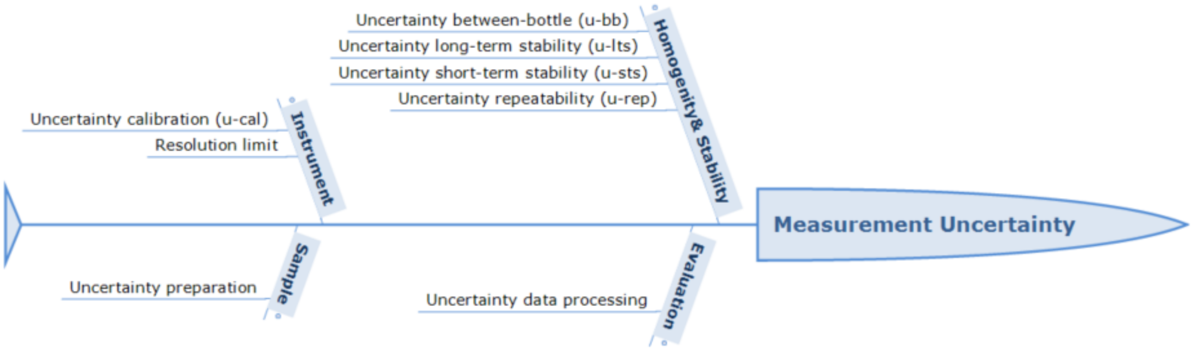

Figure 13: Ishikawa diagram with the main contributions to CRM measurement uncertainty. For specific information on TEM uncertainty see Table 13 and for SAXS see Table 14.

Potential inhomogeneities of the candidate material are investigated in the homogeneity study (see Chapter 4.1) and expressed by u_{bb}. The repeatability uncertainty as a measure of the analytical procedure's contribution to overall uncertainty was also assessed as part of the homogeneity study and is considered by u_{rep}. It includes sample preparation as well as the intrinsic uncertainties related to the measurement setup and instrument operation and will be considered in u_{char}. The uncertainty contribution of the analytical method, instrumentation and data processing used for the determination of the certified values is described in detail in Chapter 7.1 for TEM and Chapter 7.2 for SAXS and denoted as u_{char}. The uncertainty contribution related to the long-term storage of the material is derived from the long-term stability study as u_{lts} (see Chapters 5.1 and 5.2). Effects of temperature fluctuations during the transport of BAM-N012 to the customer are considered in u_{sts} derived from the short-term stability study in Chapter 6.1.

The root of the sum of the squares of these independent uncertainty contributions is the combined CRM uncertainty u_{CRM} which is multiplied by the coverage factor k = 2 for the expanded uncertainty U_{CRM} corresponding to a level of confidence of about 95 % in accordance with the ISO/IEC Guide 98-3[15] as shown in Equation 10.

$$U_{CRM} = \sqrt{u_{bb}^2 + u_{char}^2 + u_{lts}^2 + u_{sts}^2} \cdot k$$

Equation 10

7.1 TEM

The potential contributions to uncertainty from the TEM characterization were assessed and combined in u_{char}. Included in u_{char} are: i) the instrument calibration uncertainty (2.3 %) according to ISO 29301:2017 that is calculated as square root of the sum of the squares of the uncertainty of the detector (1 %) and the uncertainty of the calibration standard (2 %), ii) the repeatability uncertainty u_{rep} of 1.5 % (also containing the sample preparation; see Chapter 4.1), iii) the thresholding uncertainty, u_{thresh} resulting from the evaluation of the same dataset by two different operators which revealed a difference of 0.06 nm (0.7 %) and iv) the specific uncertainty from measuring with different instruments and therefore the reproducibility, u_{ILC}, is obtained from an interlaboratory comparison (see Table 16 and Chapter 8.1) and determined as half of the maximum difference between the maximum value of the participants to the certified value (0.2 nm, 2.2 %). These relative uncertainties are combined in Equation 11.

$$u_{char} = \sqrt{u_{cal}^2 + u_{rep}^2 + u_{thresh}^2 + u_{ILC}^2} = \sqrt{2.3^2 + 1.5^2 + 0.7^2 + 2.2^2} = 3.6\ \%$$

Equation 11

For the between-bottle uncertainty, u_{bb}, evaluated for the chosen measurands by TEM in Chapter 4.1, no relevant contribution was found. The uncertainty due to long-term storage, u_{lts}, is taken from the stability study in Chapter 5.1. For all measurands investigated by TEM the long-term stability contribution to the relative uncertainty was found to be insignificant (see Table 9). Uncertainty contributions associated with the transport conditions during dispatch, u_{sts} found by TEM were negligible (see Table 12).

Table 13 lists all uncertainty contributions for the TEM measurands ECD and ESL as well as the combined and expanded uncertainties.

Table 13: Uncertainty contributions for the TEM measurands median ECD and median ESL.

Parameter (unit)	Median ECD (nm)	Median ESL (nm)	Rel. Uncertainty ECD / ESL (%)
Value			
u_{bb}	0	0	0/0
u_{rep}	0.13	0.12	1.5/1.5
u_{cal}	0.21	0.19	2.3/2.3
u_{thresh}	0.06	0.06	0.7/0.7
u_{lts}	0.18	0.16	2.0/2.0
u_{sts}	0.08	0.07	0.9/0.9
u_{ILC}	0.2	0.18	2.2/2.2
u_{char}	-	-	3.6
u_{CRM}	0.39	0.34	4.2/4.2
U_{CRM}	0.78	0.68	8.4/8.4

7.2 SAXS

The uncertainties u_{RM} of the values obtained by SAXS are established in the following paragraph and calculated with Equation 11. Included are contributions from the repeatability uncertainty u_{rep} and the between-bottle uncertainty u_{bb} both taken from the homogeneity study (see Chapter 4.2). Furthermore, the long-term stability uncertainty u_{Lts} was acquired from the SAXS stability study (see Chapter 5.2).

U_{char} was determined from the results of an international SAXS interlaboratory comparison which includes instrument specific biases, the uncertainty regarding the data fit as well as the calibration uncertainty.[9] The results are summarized in Table 14 below.

Table 14: Uncertainty contributions for the SAXS measurands mean of EL, n and c.

Parameter (unit)	Mean EL (nm)	n ($\cdot 10^{-6}$ mol$\cdot$L^{-1})	c (g$\cdot$L^{-1})	Rel. Uncertainty EL/n/c (%)
Value				
u_{bb}	0	0.004	0	
u_{rep}	0.20	0.011	0.018	
u_{lts}	0.17	0.008	0.016	
u_{char}	0.17	0.021	0.030	
u_{RM}	0.31	0.025	0.04	4/21/18
U_{RM}	0.63	0.05	0.08	8/42/36

8. Property Values

BAM-N012 is provided with certified and informative values. Additional material information is provided at the end of this report. For a measurand to be qualified as a certified value it must be homogenous and stable. The results obtained with the instruments used for the certification study must be metrological traceable to SI units. Furthermore, the complete expanded uncertainty budget must be supplied. Measurands that were part of the certification study but have shown potential instabilities and insufficient information about traceability are given as informative values with their complete expanded uncertainty in Chapter 8.2.

8.1 Certified Values

The certified values for the median of the measurands ECD and ESL for TEM were taken from the initial stability study which is equal to 6 months of storage time at room temperature after bottling. For the TEM measurements 6 grids were evaluated and at least 1000 particles per grid were counted. From all grids' median values, the overall mean value is obtained. The results for the two measurands are summarized in Table 15.

Table 15 Certified values and expanded uncertainties for median ECD and median ESL.

	Median ECD (nm)	**Median ESL (nm)**
Certified value	9.1	8.1
Expanded uncertainty	0.8	0.7

The TEM measurements were further verified by a small interlaboratory comparison with three different TEMs and one SEM. Two TEM grids were prepared from one bottle of BAM-N012 for each instrument and accompanied by one grid prepared from the certified reference material ERM-FD100 for quality control. The raw data of the participants can be found in Annex_III.

For the certified reference material ERM-FD100 all 4 measured values agree and compared to the provided certified value of 19.4 nm are well within the given uncertainty. The values for BAM-N012 differ slightly between each instrument with a maximum difference of 0.4 nm and show a deviation of 0.1 nm – 0.4 nm compared to the certified value of 9.1 nm, which is within the expanded uncertainty of 0.8 nm. Overall, interlaboratory comparison confirms the certified ECD value. The individual results are shown in Table 16.

Table 16: Results of the interlaboratory comparison with the four participants TEM UE (BAM), TEM AH (BAM), TEM HU (Humboldt University Berlin) and SEM FB (BAM).

	Median ECD BAM-N012 (nm)	ECD ERM-FD100 mean (nm)
Certified Value	(9.1 ± 0.8)	19.4 ± 1.3*
EM 1	9.2	19.1
EM 2	9.5	19.1
EM 3	9.3	19.1
EM 4	9.3	19.4

*JRC ERM FD100 (modal diameter)

8.2 Informative values

This chapter provides values that did not meet the criteria for certification. The mean edge length EL, particle number density n and the particle concentration c are obtained from the SAXS homogeneity study. The property values for EL, n and c are arithmetic means of the values determined for 10 bottles in triplicates and can be found in Table 17.

To compare the results of the two methods (TEM and SAXS), the mean edge length determined with SAXS (EL) should ideally be compatible with the mean edge length of the area of an assumed perfect square (ESL) measured with TEM. It should be noted that the EL by SAXS is given as the mean of a logarithmic normal distribution whereas the certified ESL by TEM is the median calculated from single particle measurements. However, the mean ESL of 8.06 nm deviates only slightly from the certified median ESL of 8.13 nm. Thus, both values allow a valid comparison with the mean EL of 7.9 nm by SAXS. The slight deviation of only 0.16 or 0.23 nm is well within the respective uncertainties as given in Table 15. SAXS is based on a fundamentally different physical principle than TEM and allows the measurement of orders of magnitude larger numbers of particles. As the metrologically traceability to SI units is not conclusively clarified and beginning agglomeration leads to significant trends in the stability study, the mean EL, the particle number density n and concentration c will be provided as informative values with complete uncertainty budget (see Table 17).

Table 17: Property values for mean edge length EL, particle number density n and particle concentration c.

Quantity	EL (nm)	n ($\cdot 10^{-6}$ mol$\cdot$L^{-1})	c (g$\cdot$L^{-1})
Mean	7.9	0.12	0.22
Expanded absolute uncertainty	0.7	0.05	0.08

8.3 Additional material information

The stock suspension was used to determine a mass concentration by drying and weighing of aliquots before dilution to the final concentration of BAM-N012. Thermogravimetric analysis (TGA) of the dried solids showed a mass loss of about 22 mass-% that is allocated to the decomposition of the oleate capping ligand. Furthermore, inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to determine the iron concentration of the bottled suspension to be 0.18 mg/mL which would correspond to an assumed iron oxide (Fe_3O_4) concentration of 0.25 mg/mL which is slightly higher than the concentration c determined by SAXS. Additional to the characterization of the particle size descriptors by TEM and SAXS within the certification study, the crystalline nature of the particles was confirmed by X-ray diffraction (XRD) and electron diffraction (ED) with magnetite and/or maghemite as dominant phases which cannot be differentiated as shown in Figure 14. The Scherrer equation was used to calculate a crystallite size of 7.3 nm from the peaks of the X-ray diffraction pattern.

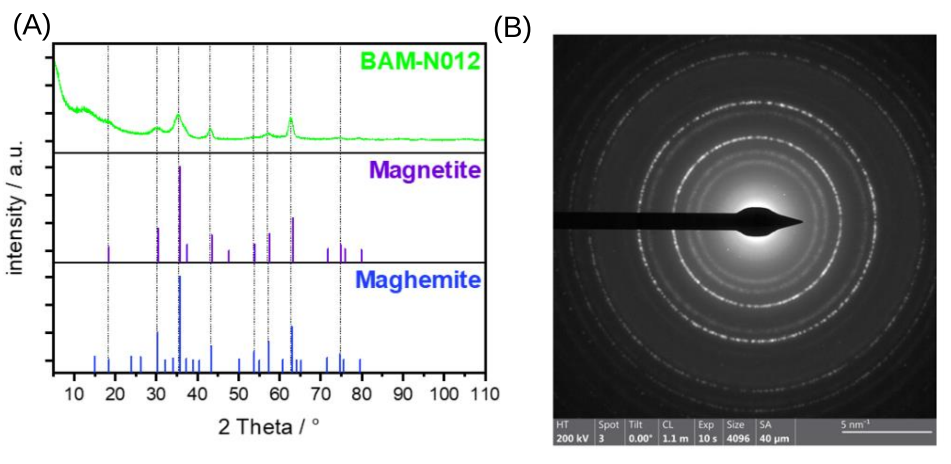

Figure 14: X-ray diffractogram (A) and electron diffraction pattern (B) of BAM-N012.

The hydrodynamic size of the particles was measured by dynamic light scattering (DLS) of three bottled units using the cumulants method for data interpretation and resulting in an intensity-weighted z-average of 16.7 nm, a volume-weighted mean of 14.3 nm, a number-weighted mean of 12.0 nm and a PDI of 0.07 (Zetasizer, Malvernpanalytical, see Figure 15 A). The stock suspension of the original batch was further analyzed by DLS using the frequency method to calculate a volume-weighted mean for the hydrodynamic diameter of 11.4 nm and a number-weighted mean of 9.9 nm (NanoFlex, Microtrac, see

Figure 15 B). The volume- and number-weighted distributions in Figure 15 are only approximations derived under the assumption of an ideal sphere.

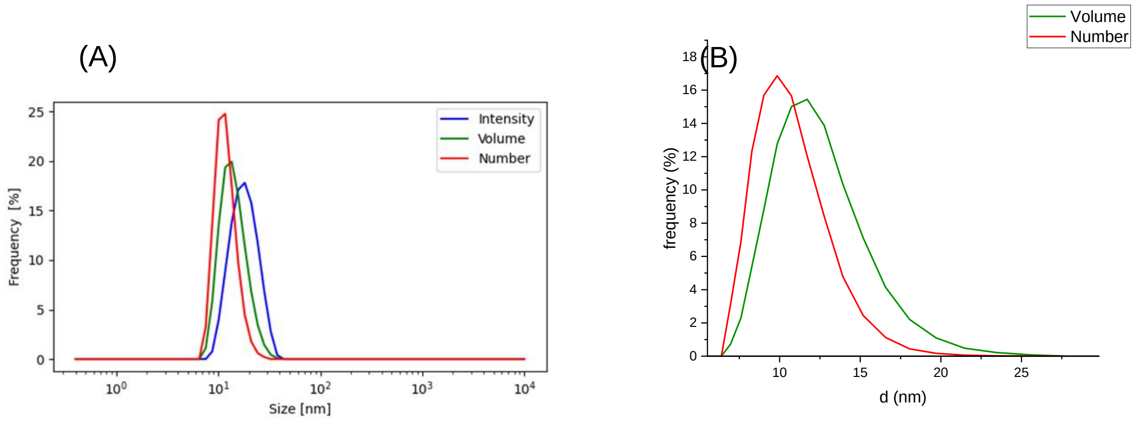

Figure 15: Intensity-, volume-, and number-weighted particle size distributions by DLS cumulant method (A). Volume- and number-based particle size distributions by DLS freqeuncy method (B).

The oxidation state of the iron oxide nanoparticles was analyzed by X-ray photoelectron spectroscopy (XPS). The sample was prepared by drop casting the suspension on a silicon wafer and then dried with nitrogen gas. The sample was cleaned by dipping it in a 50% toluene 50% ethanol mixture 3 times and drying with nitrogen gas each time. The silicon wafer was then measured with an Axis Ultra DLD photoelectron spectrometer (Kratos Analtical). XPS spectra were recorded using monochromatized aluminum Kα radiation for excitation, at a pressure of approximately 5 × 10-9 mbar. The electron emission angle was 0° and the source-to-analyzer angle was 60°. The binding energy scale of the instrument was calibrated following a Kratos Analytical procedure which uses ISO 15472 binding energy data. Spectra were taken by setting the instrument to the hybrid lens mode and the slot mode providing approximately a 300 × 700 μm^2 analysis area. Furthermore, the charge neutralizer was used. Survey spectra were recorded with a step size of 1 eV and a pass energy of 80 eV, high-resolution spectra were recorded with a step size of 0.1 eV and a pass energy of 20 eV. Quantification was performed with Unifit 2022 using Scofield factor, the inelastic mean free pathway and the transmission function for the normalization of the peak area. For peak fitting a sum Gaussian-Lorentzian function was used. As background a modified Tougaard background was used. Binding energies were measured at high-resolution with an uncertainty of +/- 0.2 eV (95% confidence interval). For quantification the relative uncertainty amounts to +/- 20% (95 % confidence interval). From the XPS analysis it is concluded that the iron oxide predominately consists of Fe_2O_3·, (magnetite). The XPS spectrum is shown in Figure 16 and the fit of one region of interest (702 – 746 eV) is shown in Figure 17

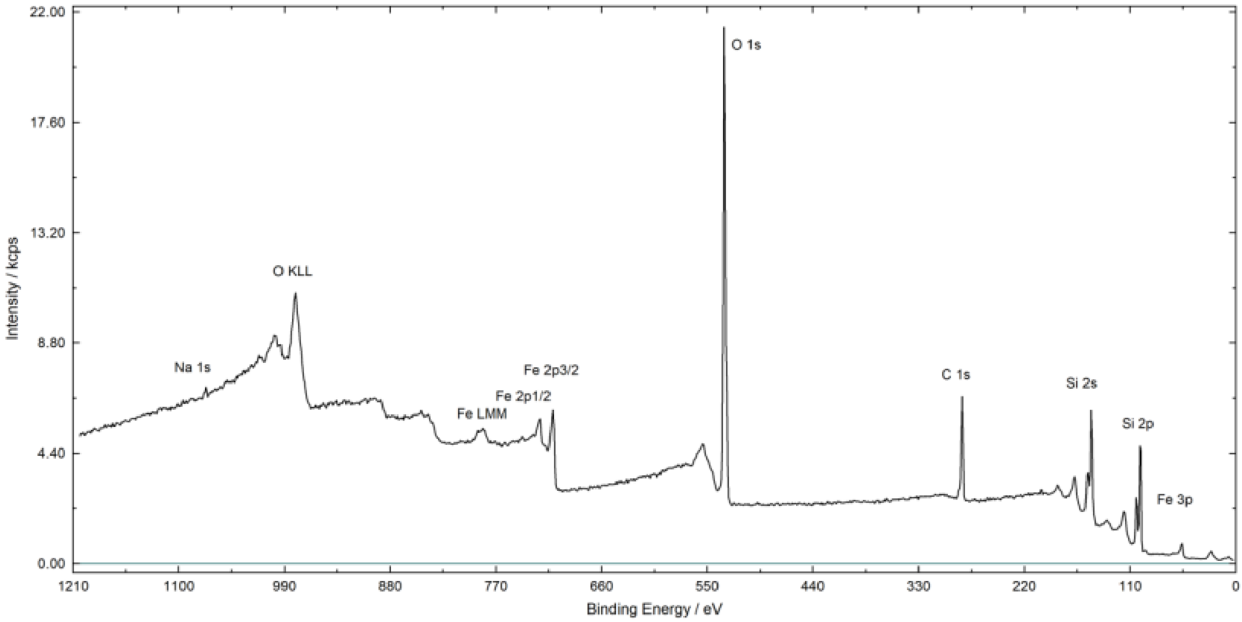

Figure 16: XPS spectrum of the BAM-N012 suspension dried on Si-wafer.

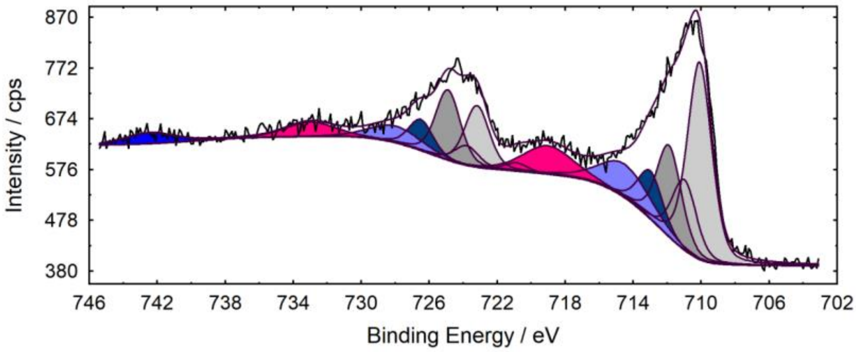

Figure 17: showing the peak fitting for the Fe 2p peaks in the ROI (Binding Energy 702 – 746 eV) from Figure 16 (see also Table 18 for Results)

Table 18: Results for the Fe 2p Peak fitting (shown in Figure 17):

Doublet name Fe2p	Peak height/ cps	Lorentzian	Position/ eV	FWHM/ eV	abs. Area/ cps·eV	rel. Area/ %
Pre Peak	0.003781 16.19	0.3 0.3	707.7 720.9	1.7 1.7	0.007623 33.13940	0 1.04
Fe2O3 Peak1	380.983 119.187	0.3 0.3	710.1 723.2	1.7 1.7	773.83 244.02	24.31 7.67
Fe2O3 Peak2	135.908 38.179	0.3 0.3	711 723.8	1.7 1.7	276.49 78.17	8.69 2.46
Fe2O3 Peak3	176.34 135.791	0.3 0.3	711.9 724.9	1.7 1.7	359.2 278.02	11.28 8.73
Fe2O3 Peak4	92.587 59.94	0.3 0.3	713 726.5	1.7 1.7	188.83 122.71	5.93 3.85
Surface Peak	64.433 32.088	0.3 0.3	714.5 728	3.5 3.5	267.27 133.75	8.4 4.2
Fe3+ 2p3/2 Satellite	54.824 28.379	0.3 0.3	719.0245 733	3.5 3.5	228.36 117.87	7.17 3.7
Satellite	20.973 0	0.3 0.3	742.4682 5.1086	3.5 3.5	81.73 0	2.57 0

9. Metrological traceability

The TEM JEM FS2200 used in the presented certification study is metrologically traceably calibrated to the SI. Calibration was done with MAG-I-CAL SN2145 (Technoorg Linda Co. Ltd.) certified for length calibration. This is done by verifying the calibration of the pixel size against the 111-lattice spacing of silicon. The uncertainty of the certified reference material s_{rm} was reported to be 2 % (see Annex_IV for further details).

The stability of the calibration and metrological traceability of the measurands are ensured through a BAM-internal standard operating procedure (StAA-16_TEM_Mag_Kalib_12_Fassung) based on ISO 29301:2017. Each TEM image, following best practices for operation and alignment, has a defined pixel size from which the length scale is extracted. The 2D projections of a particle can be analyzed by setting accurate thresholds for the transformation of greyscale images to binary masks. Size descriptors such as edge length of the cube, minimum Feret and many others are obtained by scientific image processing software packages such as ImageJ. TEM measurements are metrological traceable to the meter but are subjected to an expanded uncertainty of 8.4 % according to the reported uncertainty considerations U_{CRM} (see Chapter 7.1).

10. Information on the proper use of the RM

10.1 Recommended use

The intended purpose of BAM-N012 is to be used for quality control and verification of EM (TEM and SEM) and SAXS measurements.

10.2 Transport, storage, and handling

The stability of the certified size parameters allows the dispatch of the material at ambient temperature. The short-time exposure to different temperature regimes (both high and low) are accounted for in the expanded uncertainty budget. On receiving, it must be stored at (20 ± 3) °C in the dark.

10.3 Shelf life

Since the dispatch to the end user may occur at any time during the post-certification monitoring, the certified properties will be valid for 12 months beginning with the dispatch of the material from BAM. The uncertainty of the certified reference material includes the validity of 12 months as well as the monitoring interval time.

10.4 Safety information

The usual laboratory safety precautions must be applied. No hazardous effects are to be expected when the material is used under laboratory conditions. It is strongly recommended to handle and dispose the reference material in accordance with the guidelines for hazardous materials legally in force at the site of end use and disposal.

10.5 Legal notice

Neither BAM, its contractors nor any person acting on their behalf:

(a) make any warranty or representation, express or implied, that the use of any information, material, apparatus, method, or process disclosed in this document does not infringe any privately owned intellectual property rights; or

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11. Information on and purchase of the RM

Certified reference material BAM-N012 is supplied by

Bundesanstalt für Materialforschung und -prüfung (BAM)
Fachbereich 4.2 Materials and air pollutants
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany

Phone:	+49 (0)30 - 8104 2061
Fax:	+49 (0)30 – 8104 72061
E-Mail:	sales.crm@bam.de

Each vial of BAM-N012 will be distributed together with a detailed certificate containing the certified values and their uncertainties, the mean values and standard deviations and information on the analytical methods used.

Information on certified reference materials can be obtained from BAM:

https://www.bam.de

www.webshop.bam.de

12. Acknowledgements

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1 Model for interpretation of the data

A model-driven small-angle X-ray scattering data evaluation was performed for characterization of the size distribution and the concentration of the silver nanoparticles. The SAXS model of non-interacting spheres was utilized on the assumption of a constant internal density within the particles and that the particles are dispersed in a homogeneous continuous phase. This model is given as

$$I(q) = N_n(\rho_2 - \rho_1)^2 \int_0^\infty f(r) \left[\frac{4\pi}{3} r^3\right]^2 P(q,r) dr \tag{1}$$

where $I(q)$ is the scattering intensity, q is the scattering vector, N_n is the particle number density, ρ_2 is the scattering length density of the particles (iron oxide) and ρ_1 is the scattering length density of the continuous phase (toluene). Specifically, the ρ-values were calculated with the scattering length density calculation tool of the program SAXSfit [bressler_sasfit_2015]. Calculated values were ρ_2 = 4.040×10^{11} cm^{-2} for an iron oxide density (Fe_3O_4) of 5.17 g cm^{-3} and a copper K_α-energy of 8047.60 eV. An ρ_1 = 8.030×10^{10} cm^{-2} was calculated for toluene with a density of 0.87 g cm^{-3}. Hence, we employed a scattering length density difference of $\rho_2 - \rho_1$ = 3.237×10^{11} cm^{-2}. The scattering vector is defined as $q = 4\pi\lambda^{-1} \sin\theta$ with the wavelength of the x-ray beam $\lambda =$ 0.15418 nm and the scattering angle θ. The $f(r)$ is the particles' number-weighted radii distribution and $P(q,r)$ is their form factor with

$$P(q,r) = \left[3\frac{\sin(qr) - qr\cos(qr)}{(qr)^3}\right]^2 \tag{2}$$

In this study $f(r)$ is the lognormal distribution, which is recommended in the standard ISO 17867 [ISO_17867_2020] and was successfully employed earlier in an interlaboratory comparison for determination of particle size distribution with SAXS (see [pauw_nanoparticle_2017]). The lognormal size distribution is defined as

$$f(R) = \frac{1}{\sqrt{2\pi}\, wR} \exp\left[-\frac{\ln\left(R/R_{median}\right)^2}{2w^2}\right] \tag{3}$$

where R_{median} is the median of the radii and w defines the width of the distribution. It should be noted that w is the standard deviation of the natural logarithm of the radii distribution width (unit is 1), and must not be confused with the standard deviation of the radii distribution width itself (unit is nm). Since the shape of the lognormal distribution is defined by R_{median} and w, further parameters of interest can be derived thereof. The most frequently used derived parameters are mean, modal and standard deviation. The mean value of the radii is

$$R_{mean} = R_{median} \exp\left(w^2/2\right) \tag{4}$$

with an uncertainty of

$$u_{R_{mean}} = e^{w}\left(u^2_{R_{median}} + [R_{median} w\, u_w]^2\right)^{1/2} \tag{5}$$

where estimates of the uncertainties $u_{R_{mean}}$ and u_w were provided from the fit procedure. The standard deviation of the radii size distribution width is

$$\sigma_{lognormal} = \left[e^{2w^2} - e^{w^2}\right]^{1/2} R_{median} \tag{6}$$

with an uncertainty of

$$u_{\sigma_{lognormal}} = \left(\left[e^{2w^2} - e^{w^2}\right] u^2_{R_{median}} + \frac{e^{w^2}\left(1-2e^{w^2}\right)^2 R^2_{median} w^2}{e^{w^2}-1} u_w^2\right)^{1/2} \tag{7}$$

We employ $N_{fit} = N_n(\rho_2 - \rho_1)^2$ as scaling factor to avoid a numerically unfavorable usage of very large numbers of N_n and very small numbers of $(\rho_2 - \rho_1)^2$ in the curve fitting process. Together with the abbreviation $I_{fit} = \int_0^\infty f(r)\left[\frac{4\pi}{3}r^3\right]^2 P(q,r)dr$ we obtain

$$I(q) = N_{fit} I_{fit} \tag{8}$$

where I_{fit} represents the q-dependent term of the curve fitting procedure, providing the parameters R_{median} and w. The particles' diameter was calculated utilizing eq. (4) as

$$D = 2R_{mean} \tag{9}$$

with an uncertainty of

$$u_D = 2u_{R_{mean}} \tag{10}$$

The size distribution width of the diameter was derived form eq. (6) as

$$\sigma = 2\sigma_{lognormal} \tag{11}$$

with an uncertainty of

$$u_\sigma = 2u_{\sigma_{lognormal}} \tag{12}$$

1.1 Number concentration of particles

The units of absolute intensity $I(q)$, scattering vector q and the scattering length densities ρ_2 and ρ_1 had to be considered to convert the scaling factor N_{fit} to a particle concentration (in number of particles per cm^3). From the model employed, we get $N_{fit} = I(q) I_{fit}^{-1}$. The units of the measured intensity $[I(q)] = \text{m}^{-1}$ and the fit intensity $[I_{fit}] = \text{nm}^6$ provide $[N_{fit}] = [I]\,[I_{fit}]^{-1} = \text{m}^{-1}\text{nm}^{-6}$. Furthermore,

$$N_n = \frac{N_{fit}}{(\rho_2 - \rho_1)^2} \tag{13}$$

$$\tag{14}$$

where the unit for the square of the scattering length density difference is $\left[(\rho_2 - \rho_1)^2\right] = \text{cm}^{-4}$. Therefore, we get

$$[N_n] = \frac{[N_{fit}]}{[(\rho_2 - \rho_1)^2]} \tag{15}$$

$$= \frac{\text{m}^{-1}\text{nm}^{-6}}{\text{cm}^{-4}} \tag{16}$$

$$= 10^{40}\text{cm}^{-3} \tag{17}$$

This means that we have to multiply the calculated $N_{fit}/(\rho_2 - \rho_1)^2$-values by a factor of 10^{40} to obtain particle number densities N_n in units of cm^{-3}. The uncertainty of N_n depends on the uncertainties of N_{fit}, ρ_2 and ρ_1. The uncertainty of N_{fit} can be estimated as the uncertainty from the curve fit $u_{N_{fit}}$. In contrast, no reliable uncertainty estimates are available for ρ_2 and ρ_1. Therefore, the standard uncertainty, as calculated according to GUM [GUM_2008], is simply estimated as

$$u_{N_n} = \frac{u_{N_{fit}}}{(\rho_2 - \rho_1)^2} \tag{18}$$

In chemistry, molar concentrations are typically in use. Molar concentrations are obtained from particle number densities by

$$N = \frac{N_n}{N_A} \tag{19}$$

with an uncertainty of

$$u_N = \frac{u_{N_n}}{N_A} \tag{20}$$

The N_A is the Avogadro number defined as $N_A = 6.02214076 \times 10^{23}$ mol^{-1}. The units are

$$[N] = \frac{[N_n]}{[N_A]} \tag{21}$$

$$= \text{mol cm}^{-3} \tag{22}$$

$$= 10^6 \text{ mol l}^{-3} \tag{23}$$

Therefore, the values of N_n and u_{N_n} have to be multiplied by $10^6 N_A$ for conversion of number densities in units of cm^{-3} to molar concentration in units of mol l^{-1}.

1.2 Weight concentration

The particle concentration c in units of $[c]$ = g cm^{-3} is given by

$$c = N_n \rho \langle V \rangle \tag{24}$$

where N_n is the particle number density, ρ is the density of the particles and $\langle V \rangle$ is the mean particle volume. Uncertainties can be estimated for N_n and $\langle V \rangle$ from the curve fit parameters. No uncertainty is available for ρ, the density of iron oxide nanoparticles. Here the density of iron oxide as bulk materials of 5.17 g cm^{-3} was employed. The combined standard uncertainty, as calculated according to GUM [GUM_2008] is

$$u_{\text{con}} = \sqrt{u_1^2 + u_2^2} \tag{25}$$

The first contribution to u_c depends on the uncertainty of N_n and is

$$u_1 = \rho \langle V \rangle u_{N_n} \tag{26}$$

The second contribution to u_c is

$$u_2 = N_n \rho \, u_{\langle V \rangle} \tag{27}$$

and depends on the uncertainty of $\langle V \rangle$, which in turn depends on the particle size distribution. For spherical particles apply

$$\langle V \rangle = 4/3\pi \langle R^3 \rangle \tag{28}$$

and the radii distribution needs to be taken into account for correct calculation of $\langle V \rangle$. The mean volume of particles with a lognormal radii distribution is

$$\langle V \rangle = \frac{4\pi}{3} R_{\mathrm{median}}^3 \exp\left[\frac{9w^2}{2}\right] \tag{29}$$

Its combined standard uncertainty is

$$u_{\langle V \rangle} = \sqrt{u_{21}^2 + u_{22}^2} \tag{30}$$

with

$$u_{21}^2 = \left(4\pi R_{\mathrm{median}}^2 \exp\left[\frac{9w^2}{2}\right]\right)^2 u_{R_{\mathrm{median}}}^2 \tag{31}$$

$$u_{22}^2 = \left(12\pi w R_{\mathrm{median}}^3 \exp\left[\frac{9w^2}{2}\right]\right)^2 u_w^2 \tag{32}$$

Annex II Stability En-criterion

x_a	x_e_stab5		x_e(b2)-x_	x_e(b3)-x_	x_e4-x_a	x_e5-x_a	x_e6-x_a
9,080	9,3315		0,25133	0,0475	0,11328	0,18456	0,01935
9,331	9,03267		0,00063	0,2982	0,36398	0,43526	0,27005
9,042	8,96689		0,28901	0,00982	0,0756	0,14688	0,01833
9,149	8,89561		0,18265	0,11618	0,18196	0,25324	0,08803
9,100	9,06082		0,23185	0,06698	0,13276	0,20404	0,03883
9,120	9,14823		0,21103	0,0878	0,15358	0,22486	0,05965
		max	0,43526				
		min	0,00063				
		en-Krit	1,022722 ≤		2	WAHR	
cert_val	overall mean stab5						
9,137	9,07262						
0,151468 ≤		2	WAHR				

u_ref	u_verf/SD_stab5
4,40%	
0,402032	0,139632
0,161629	0,019497
SQRT(u_ref²+u_verf²)	
0,42559	

Legende

x_a: Anfangswert (Stabilität 1)

x_e: Endwert (stab#4,stab#5)

u_ref: complete uncertainty of certification value

u_Verf: standard deviation of stab#4, SD stab#5)

u_ref	u_verf/SD_stab4
0,402032	0,05396
SQRT(u_ref²+u_verf²)	
0,405637	

Equation:

$$\frac{|x_a - x_e|}{\sqrt{u_{ref}^2 + u_{verf}^2}} \leq 2$$

	x_e_stab4		x_e2-x_a	x_e3-x_a	x_e4-x_a	x_e5-x_a	x_e6-x_a
	9,12942		0,04925	0,04397	0,01443	0,0003	0,06372
	9,0362		0,20145	0,29467	0,26513	0,2504	0,31442
	9,06574		0,08693	0,00629	0,02325	0,03798	0,02604
	9,08047		0,01943	0,11265	0,08311	0,06838	0,1324
	9,17463		0,02977	0,06345	0,03391	0,01918	0,0832
	9,01645		0,00895	0,08427	0,05473	0,04	0,10402
		max	0,31442				
		min					
		en-Krit	0,775127 ≤		2	WAHR	
cert_val	overall mean stab4						
9,137	9,083818						
0,131312 ≤		2	WAHR				

Annex III ILC

Instrument	TEM	TEM		TEM		SEM	
Model	JEM-2200FS	TALOS-F200S		JEM-2200FS		Zeiss Supra 40	
Number of frames analyzed	4	5		14		2	
Number of particles analyzed	1472	1028		1021		788	
Accelerating voltage (kV)	200	200		200		20	
Frame size (nm x nm)	250,15 nm²	248 nm²		301,81 nm²		819,2 nm x 614,4 nm	
Pixel dimension (nm / pixel)	0,122	0,06		0,295		0,8	
Image analysis software	ImageJ	ImageJ		Bruker Esprit		ImageJ	
manual /automated counting	manual	manual		manual		manual	
Verification by	*CRM ERM FD100	*CRM ERM-FD100		*CRM ERM-FD100		*CRM ERM-FD100	
Median ECD	9,1 nm	9,5 nm		9,2 nm		9,3 nm	
Bam N012**	ERM FD100*	**	*	**	*	**	*
Values / nm		Values / nm		Values / nm		Values / nm	
9,27	15,3	9,46	20,0	9,08	15,9	9,03	18,8
9,27	21,1	9,37	26,8	8,70	19,7	7,82	20,4
8,97	25,1	9,55	15,3	6,11	19,7	0,80	18,9
7,02	17,5	9,56	20,7	9,47	16,2	10,45	14,0
9,54	17,6	10,26	16,1	9,29	17,5	8,32	11,3
9,52	16,8	9,10	20,6	9,45	16,6	9,25	16,9
9,33	13,2	9,59	20,7	9,74	21,0	0,92	16,6
9,58	16,8	9,91	17,1	9,19	24,4	8,66	19,5
8,90	22,4	9,50	14,3	9,19	16,4	8,47	15,6
9,54	18,0	9,76	19,5	9,60	20,3	8,98	15,5
9,20	15,0	9,45	20,3	9,57	15,6	8,22	15,4
9,71	20,4	7,33	19,2	7,18	11,7	8,37	17,4
9,47	22,0	9,85	20,2	9,65	20,7	10,05	18,2
9,33	18,0	9,59	20,7	8,88	19,3	10,37	15,8
9,29	24,1	9,99	19,8	9,50	17,9	10,01	19,2
8,54	21,7	9,37	19,2	9,49	20,2	11,17	18,4
9,33	17,4	10,22	16,4	8,85	22,2	8,37	20,6
9,31	16,5	10,00	21,7	9,60	16,1	8,61	16,6

9,30	18,1	10,23	18,6	9,38	14,0	9,64	19,9
8,71	17,9	9,44	18,6	10,08	19,5	1,09	13,0
8,98	18,1	8,86	18,7	9,64	17,9	9,68	19,9
9,23	19,9	9,42	13,5	9,90	16,7	10,60	17,6
8,20	17,4	10,34	20,8	9,98	18,6	0,94	15,9
8,97	21,3	10,54	20,8	9,32	17,4	9,64	16,1
9,21	20,3	9,76	16,4	9,42	17,2	9,89	18,0
9,71	15,8	10,13	18,9	8,74	9,9	8,61	19,1
9,58	18,4	8,09	18,3	9,06	13,8	8,71	18,6
9,25	19,7	9,86	18,4	9,09	13,7	10,09	18,4
9,10	21,3	9,66	18,1	8,07	22,8	8,07	21,6
7,72	20,6	10,09	19,4	9,20	17,5	9,16	23,8
9,14	17,2	7,82	14,5	9,90	19,9	9,25	20,9
8,95	18,7	9,58	21,9	7,62	20,9	11,45	18,0
9,28	20,0	9,32	16,2	9,31	20,3	9,72	19,8
8,92	15,5	9,51	18,7	9,67	17,2	8,80	22,5
9,01	20,7	9,10	16,4	9,35	12,7	9,16	19,9
8,94	23,1	9,24	18,5	9,04	20,5	10,45	18,6
9,83	17,9	9,18	20,4	9,30	19,0	0,94	23,7
8,83	15,4	9,56	23,2	8,67	15,4	0,69	19,3
8,94	18,2	7,09	22,5	8,72	21,9	9,34	25,1
8,85	23,2	9,70	17,9	7,14	18,0	0,86	14,7
8,78	21,4	9,58	15,6	9,25	16,9	9,93	17,0
9,17	17,0	9,59	20,9	8,94	17,6	8,37	23,5
8,72	20,2	10,01	15,6	9,00	21,2	10,01	17,4
9,81	20,5	9,88	19,0	6,90	17,1	0,94	20,1
9,68	19,4	9,83	25,7	8,92	15,4	9,38	20,4
9,18	17,6	8,82	17,1	9,15	16,7	10,41	17,6
8,97	20,4	10,14	17,5	8,06	23,3	9,55	23,9
9,55	22,8	4,04	14,4	8,70	14,7	11,20	22,2
9,17	20,5	9,50	23,2	9,23	21,6	0,93	20,2
9,12	18,4	8,27	19,8	8,65	20,8	8,07	19,8
9,40	17,5	8,87	20,6	9,69	14,5	8,84	15,3
8,69	18,9	9,14	16,9	9,16	16,5	9,16	17,3
9,34	17,4	9,59	21,5	9,28	17,5	9,81	17,2
9,29	21,4	9,37	21,0	8,73	15,0	8,80	17,8
8,99	24,7	7,68	17,1	9,66	17,7	11,17	18,3
8,96	20,2	10,04	19,8	9,07	15,7	9,76	25,1
9,17	20,6	10,07	22,0	9,30	18,4	9,85	22,7
9,31	21,4	9,01	20,3	8,99	17,9	9,34	21,1
9,30	17,6	10,20	19,8	8,66	16,0	10,13	21,7
9,00	21,3	10,21	15,2	9,57	13,7	10,09	20,4
8,64	20,0	6,28	18,7	13,00	19,8	9,38	21,3
9,07	17,7	9,18	20,2	9,70	14,7	8,32	21,3
9,12	24,1	10,05	18,6	9,27	19,9	9,25	20,5
8,33	22,7	9,41	19,8	7,80	14,2	0,94	18,1

9,81	18,1	10,55	19,5	8,88	17,8	10,01	18,7
9,28	17,7	9,32	16,9	6,23	16,4	0,94	15,3
9,86	18,2	9,64	16,8	9,08	16,5	0,92	18,6
9,92	20,1	9,05	20,3	9,21	17,4	8,52	23,5
9,40	22,1	9,22	20,6	6,65	17,8	10,37	19,0
8,57	19,0	10,07	22,5	9,24	18,9	0,93	19,7
9,23	19,9	9,24	17,7	9,57	13,9	9,76	20,2
8,72	19,9	9,96	21,3	8,11	18,9	10,13	21,2
10,29	20,2	9,14	17,2	8,57	15,9	8,32	24,1
9,18	21,5	9,18	15,9	9,73	19,1	11,42	20,3
8,77	19,4	8,59	13,9	8,89	19,6	9,12	17,4
8,93	19,5	9,12	24,9	9,16	19,2	10,01	17,6
9,61	17,4	7,90	13,2	9,18	17,4	10,79	24,6
10,13	16,9	8,75	15,7	8,27	15,3	10,98	20,9
9,25	18,6	9,84	16,0	8,72	14,6	10,01	24,6
8,94	21,8	9,82	20,7	9,51	18,7	9,12	19,9
9,41	19,9	9,56	18,6	9,45	12,1	9,76	14,9
9,05	22,8	9,19	18,0	8,95	24,3	10,25	19,8
9,45	21,9	9,53	20,3	9,28	19,2	10,83	18,1
9,61	18,6	9,97	22,2	9,50	22,3	10,64	19,7
8,72	16,8	9,09	12,2	9,33	15,8	9,89	20,4
8,91	18,1	10,14	21,9	9,81	22,6	11,31	23,0
9,63	20,4	9,19	22,5	9,47	18,2	9,64	19,7
9,11	16,6	8,96	16,9	9,05	13,8	8,80	15,8
6,69	18,9	9,97	17,9	9,30	17,0	9,34	15,6
9,10	15,5	9,72	21,9	5,86	18,6	9,38	20,2
9,92	19,7	10,07	16,0	10,30	17,0	9,03	16,7
9,15	18,8	9,79	13,3	9,29	19,7	9,25	17,6
9,63	20,5	9,30	21,2	9,59	21,3	9,89	15,5
10,00	23,9	9,41	14,3	9,11	20,9	10,45	21,7
8,88	20,0	9,61	28,6	9,15	17,0	9,97	21,8
8,85	25,4	10,42	14,9	9,29	19,6	10,60	18,0
9,11	18,8	9,10	18,3	9,38	11,3	9,34	15,6
9,23	24,9	7,42	18,5	8,80	18,8	10,79	16,2
9,13	21,9	9,75	20,2	9,21	11,8	10,49	20,2
9,10	21,6	9,50	16,4	9,73	15,5	9,68	25,3
9,51	21,0	9,65	14,2	9,07	16,9	9,85	19,2
9,58	20,1	10,22	23,7	9,43	11,8	10,21	19,8
8,84	19,4	9,12	20,0	9,46	18,9	9,16	16,1
9,67	19,5	10,48	11,5	7,06	18,1	9,68	17,7
9,15	19,5	10,12	23,3	9,17	20,7	9,81	18,7
8,90	24,8	8,77	18,4	9,13	14,9	10,60	26,1
9,62	16,5	9,70	18,9	9,37	14,9	10,76	21,7
8,82	14,2	5,80	17,8	9,38	18,0	9,12	17,6
9,72	17,9	8,95	15,2	9,95	21,4	0,93	24,6
9,92	26,2	8,65	17,7	9,44	17,8	8,07	21,9

8,28	16,7	10,16	15,4	9,64	17,8	9,81	21,3
9,94	16,2	9,11	22,3	9,79	20,9	9,81	25,4
9,14	18,2	9,37	18,5	8,86	20,1	10,09	16,6
9,74	18,9	9,53	21,1	9,30	19,8	10,17	15,1
8,86	18,5	9,31	18,9	9,47	18,1	8,98	23,3
9,15	19,0	10,35	20,9	9,63	16,6	9,07	18,2
9,49	15,8	9,38	18,1	8,51	14,7	0,89	17,2
9,10	24,9	10,16	16,8	9,76	19,5	8,07	17,4
9,21	23,0	10,26	18,1	9,35	21,6	10,37	20,0
9,41	16,2	9,03	24,0	9,53	20,7	10,21	19,8
9,00	21,1	9,12	18,0	8,93	16,1	0,86	15,7
9,46	15,7	9,28	25,2	9,63	18,7	9,68	22,4
8,70	19,8	9,22	17,8	9,02	15,2	9,68	13,3
9,18	25,8	9,26	25,2	9,02	22,5	10,13	24,6
9,28	20,1	10,13	15,8	9,09	19,1	9,51	16,2
8,89	17,8	9,70	18,7	9,43	21,7	10,91	19,5
8,73	14,8	9,87	21,1	8,88	15,4	9,93	22,7
9,67	21,8	9,64	20,2	10,28	17,8	9,16	20,7
9,79	17,3	9,18	13,4	9,39	19,7	10,21	24,4
8,49	17,8	6,33	17,5	8,20	20,2	9,97	19,5
9,40	21,6	9,44	22,0	9,61	16,7	10,60	18,7
9,24	20,3	7,88	22,5	8,57	22,3	10,01	19,0
9,37	26,4	10,00	20,5	9,94	18,1	9,81	14,6
9,06	17,1	10,11	21,1	8,76	18,7	8,42	18,3
8,92	18,8	8,74	20,4	8,74	21,5	9,34	16,8
9,62	20,1	9,88	20,4	9,22	16,3	9,72	16,9
9,08	19,3	9,51	22,3	8,71	15,5	0,93	20,6
9,42	16,3	9,50	17,9	8,59	21,9	10,01	21,7
9,54	15,2	9,10	18,7	10,11	19,0	9,07	22,7
9,35	16,4	9,32	17,3	9,58	20,5	9,03	17,9
9,86	18,0	10,14	15,7	8,47	20,0	11,24	21,6
9,09	20,0	9,37	15,1	9,22	13,7	9,34	19,5
9,53	13,8	9,63	13,9	9,84	15,0	0,94	19,8
8,81	15,8	9,04	25,8	9,22	14,7	9,03	15,4
8,75	16,6	9,30	20,2	9,47	17,4	9,07	17,6
9,30	17,9	9,23	18,5	10,22	16,0	9,89	24,5
10,35	15,8	8,95	18,0	9,64	16,7	11,06	16,7
9,13	20,5	9,25	23,7	7,90	15,8	11,17	20,5
9,40	19,0	9,34	16,9	9,34	17,1	9,93	16,6
8,55	21,8	9,99	19,4	10,03	18,0	9,34	17,3
9,28	20,8	9,45	19,0	9,37	17,6	9,85	15,8
9,40	17,6	8,92	12,2	9,69	20,5	10,87	19,7
8,87	24,8	9,11	15,1	9,23	18,9	8,71	20,4
9,24	14,4	9,60	16,6	9,80	17,5	8,32	19,8
9,39	20,0	9,13	23,2	9,27	19,4	9,89	16,9
9,13	18,4	9,59	19,5	9,87	23,4	12,24	21,5

9,28	20,7	9,29	9,3	9,74	21,7	9,76	18,2
8,96	24,0	9,57	19,9	9,26	20,9	10,17	20,0
8,74	17,3	10,25	18,4	9,52	21,3	8,61	17,2
8,69	17,5	9,89	14,7	9,20	17,7	9,47	19,6
9,53	19,3	10,13	17,0	8,95	18,4	9,64	12,7
8,94	14,1	9,68	16,3	9,63	22,1	9,55	16,7
9,25	14,2	10,08	15,2	9,75	16,4	1,03	18,6
8,86	19,3	9,18	15,3	8,69	12,6	9,38	14,9
9,81	18,5	9,21	16,5	9,53	17,6	0,92	14,9
7,41	19,8	10,40	10,5	8,82	20,0	9,72	18,2
10,19	15,2	10,29	17,6	9,50	19,3	11,20	18,0
8,82	19,4	10,01	22,1	10,33	20,2	10,41	16,6
9,49	19,2	10,36	20,4	8,98	19,1	8,22	19,3
9,45	22,6	10,84	21,6	9,36	17,0	10,41	17,5
9,17	19,5	10,67	21,2	9,52	19,3	9,16	16,7
8,63	18,6	10,53	16,2	9,64	21,0	9,85	17,0
9,85	20,9	10,34	16,4	9,08	19,3	0,93	18,6
9,33	18,1	8,73	15,6	8,86	17,7	9,76	19,0
9,32	22,3	10,46	13,4	9,89	19,4	10,05	20,6
9,32	16,5	10,71	16,5	9,24	18,6	8,94	18,8
9,17	18,6	10,45	16,4	9,27	21,0	9,47	18,9
8,76	18,5	10,91	17,0	10,20	17,5	0,94	20,3
8,55	16,0	9,70	21,2	9,76	17,3	0,94	14,9
9,13	20,5	10,17	16,3	9,44	18,5	7,97	17,7
9,08	21,8	8,95	20,0	9,39	19,1	0,89	16,2
9,43	17,5	10,44	21,5	8,96	16,2	8,94	16,4
8,96	22,0	9,59	21,8	9,44	22,9	10,01	16,3
8,67	23,7	9,67	19,4	9,20	21,1	8,07	12,8
9,28	21,6	9,37	18,0	9,49	17,9	8,27	14,5
9,28	21,1	10,14	19,5	9,10	17,5	8,71	11,1
9,29	17,6	9,50	21,8	9,20	17,5	9,55	17,0
8,81	17,0	10,15	18,7	9,14	21,4	8,84	20,6
9,34	22,6	10,09	19,0	9,30	15,4	9,34	20,6
9,47	18,0	9,96	24,7	9,72	15,6	0,94	18,1
9,24	20,9	10,37	17,8	9,42	20,2	9,72	17,2
9,26	17,6	10,50	18,6	9,75	18,1	10,83	14,3
8,67	14,6	9,05	20,3	8,98	14,5	10,49	20,0
9,51	17,6	8,81	21,4	9,29	25,5	10,13	23,2
9,38	14,5	9,99	15,9	9,32	18,1	9,68	19,9
9,53	17,6	9,80	16,6	9,71	17,8	9,47	12,5
9,15	16,9	8,88	20,1	9,17	15,6	9,07	14,6
9,77	19,6	9,39	20,0	5,19	22,7	11,24	22,4
8,99	23,9	9,65	19,3	9,08	19,3	7,87	21,2
9,15	15,3	9,43	16,7	9,23	13,3	10,01	19,5
9,55	19,1	9,47	21,0	9,12	18,9	8,75	26,1
8,86	17,7	6,93	17,6	9,29	21,0	9,07	24,1

8,80	18,9	9,41	17,6	9,38	18,7	9,76	23,0
9,11	16,8	9,49	20,7	9,94	17,5	9,34	17,8
7,09	17,5	9,62	22,7	6,83	18,1	9,93	17,6
9,19	19,2	8,58	21,4	9,62	19,1	8,98	20,0
9,24	20,6	9,68	22,0	9,12	16,8	9,93	18,7
10,42	17,2	9,50	21,5	9,55	16,3	8,37	17,7
10,34	19,1	9,81	21,8	9,70	16,4	7,92	18,7
8,73	16,1	9,42	19,4	9,76	22,6	8,32	19,4
8,97	19,2	9,18	20,3	8,58	23,5	10,09	18,5
9,06	18,0	9,73	19,5	8,98	15,7	8,94	19,4
9,35	18,6	9,61	18,4	9,86	16,4	10,68	28,9
8,69	15,8	9,86	22,5	9,81	18,5	10,41	17,7
9,95	18,2	9,62	17,8	9,77	18,4	10,37	20,0
8,90	19,4	8,73	27,3	8,90	16,2	10,79	19,4
9,04	19,9	9,62	18,6	9,99	21,7	10,37	24,0
8,78	20,2	9,17	16,7	10,04	17,9	9,25	25,5
8,84	20,7	9,16	21,3	9,47	13,9	9,72	20,3
9,27	12,6	10,17	17,0	9,57	18,2	9,12	21,2
8,07	17,4	9,52	16,9	9,37	18,0	0,93	18,2
8,83	21,5	8,76	21,5	9,05	19,3	8,71	22,1
9,13	21,9	9,31	16,9	10,07	17,6	9,85	29,8
10,30	16,1	9,57	22,4	8,93	18,8	10,87	16,1
8,83	18,7	8,54	20,6	9,73	20,2	9,89	16,6
8,85	15,7	9,19	19,3	9,30	17,3	9,12	21,7
9,34	17,9	9,52	23,0	9,94	19,1	8,07	20,9
8,81	18,6	8,92	18,1	9,50	21,1	9,51	20,6
9,76	16,7	9,18	25,8	9,35	18,4	9,60	25,3
9,53	18,9	9,61	15,9	9,84	16,0	10,01	21,2
9,98	15,3	9,51	18,5	9,64	20,5	9,07	19,2
8,97	18,6	9,07	15,5	9,68	22,4	9,07	20,2
9,17	20,1	5,62	15,9	9,72	21,6	10,33	16,1
10,01	18,2	9,69	17,4	8,82	23,8	10,49	17,3
9,27	19,4	9,54	22,1	9,84	20,5	9,72	14,7
9,04	18,1	9,03	22,1	9,56	24,3	10,21	15,4
9,14	18,5	9,49	19,5	9,37	20,7	8,32	16,1
9,15	19,2	8,00	15,8	9,51	21,7	9,51	16,3
8,54	20,0	9,30	24,4	9,28	18,0	8,66	20,9
9,27	19,8	9,22	15,3	9,59	19,8	0,77	16,7
9,71	18,9	10,55	17,6	9,46	21,6	9,03	18,7
9,00	20,3	9,25	17,9	9,62	20,1	8,94	15,9
8,68	18,6	9,81	18,7	9,21	13,4	8,66	18,0
9,25	20,6	9,32	15,4	9,67	19,0	0,94	15,5
9,38	23,1	8,76	23,3	6,06	18,3	8,80	18,2
9,00	20,2	9,43	20,4	9,85	19,7	7,97	11,8
8,60	15,8	9,86	20,6	9,78	21,2	6,87	16,3
9,30	20,9	9,64	20,6	9,73	17,4	5,26	15,5

9,30	20,1	10,04	22,0	9,20	18,4	9,85	17,7
8,84	20,3	9,46	23,4	9,01	20,9	10,49	18,0
9,15	20,2	9,37	21,7	9,19	17,6	8,98	23,6
8,06	20,3	9,77	17,7	9,41	18,1	8,52	18,0
7,40	18,6	10,01	16,0	9,61	14,8	7,82	19,2
8,87	15,6	9,42	18,6	8,99	20,2	9,16	19,9
8,42	14,2	9,54	22,5	9,35	19,1	6,82	21,4
8,64	19,0	9,44	17,1	9,84	14,8	9,64	21,4
9,59	19,0	9,52	23,5	9,61	18,6	7,61	26,5
9,60	18,7	9,50	18,0	9,11	17,7	6,99	20,4
8,76	20,0	9,11	19,8	9,63	18,4	9,55	21,1
8,62	19,2	9,37	19,3	7,29	17,9	9,16	21,5
9,15	20,7	9,00	19,6	10,11	19,5	9,72	18,8
8,94	18,2	9,33	20,4	9,72	21,4	8,42	18,4
8,80	19,5	9,73	19,7	10,24	21,0	9,07	18,1
9,15	21,5	9,75	16,4	8,20	17,6	0,89	18,9
7,41	18,8	9,45	18,8	9,86	14,0	9,76	23,5
9,44	20,1	9,17	18,0	9,07	22,1	9,51	17,7
9,07	21,6	9,44	18,6	10,13	17,4	10,05	18,2
9,15	18,0	9,70	24,4	9,71	21,6	9,89	19,8
8,65	18,8	9,42	27,1	9,02	20,4	0,86	20,5
8,93	19,8	9,15	22,4	9,51	22,0	9,81	20,7
9,06	18,8	9,41	19,3	9,47	16,6	8,61	16,9
9,57	22,4	9,25	19,3	9,59	19,8	10,01	20,2
7,40	18,5	9,89	19,5	9,86	15,8	10,21	18,2
8,69	19,3	9,74	16,4	10,10	21,5	8,47	30,1
9,21	20,6	9,24	20,0	10,20	20,2	8,52	18,0
9,76	22,9	8,89	18,7	9,41	13,9	9,64	19,2
9,84	19,8	9,13	23,2	9,96	20,3	11,45	25,9
8,47	19,7	8,62	19,0	8,81	17,9	7,87	18,3
8,67	21,4	9,22	17,0	9,21	19,1	7,77	25,6
9,84	20,2	9,68	22,3	8,95	16,1	7,22	18,7
9,80	19,7	9,92	21,8	9,20	22,1	9,47	19,3
9,05	20,0	9,12	21,4	9,44	15,3	9,38	24,3
9,33	15,0	8,74	19,0	9,32	22,5	9,16	25,3
9,39	16,6	10,25	21,2	9,12	20,7	9,85	21,4
8,82	15,1	9,35	15,9	9,12	21,5	9,47	22,4
9,12	19,5	9,52	16,1	9,00	19,5	7,87	21,7
8,87	16,4	9,02	19,8	9,51	16,1	0,86	21,3
9,71	19,0	9,63	19,8	7,26	14,9	9,38	23,8
8,63	18,2	10,15	14,4	9,13	20,6	9,55	24,9
6,69	23,5	9,27	18,8	9,63	15,8	9,60	20,5
8,94	20,1	9,23	15,2	9,63	18,7	0,92	21,6
8,89	20,2	9,79	19,7	9,34	13,7	8,80	23,7
9,33	20,3	8,15	20,5	8,24	21,6	9,25	13,4
8,42	17,5	9,99	17,5	8,99	17,6	8,75	16,5

9,80	23,4	8,59	19,7	9,33	13,8	9,60	16,3
9,39	20,4	9,86	19,1	8,99	14,7	8,42	18,4
9,15	16,9	9,79	15,2	9,06	18,2	9,12	19,4
8,80	18,6	7,97	21,3	9,35	19,5	8,37	25,6
8,79	20,7	10,38	19,7	9,89	23,3	7,44	19,4
8,70	22,5	10,32	17,9	5,74	14,3	9,89	23,6
9,29	18,8	10,05	17,0	9,34	18,5	9,47	18,0
9,04	23,4	10,41	26,4	8,98	17,3	9,03	18,0
9,44	24,1	10,94	19,9	9,03	22,5	9,16	17,9
9,00	18,9	10,44	16,8	6,60	18,3	8,22	20,8
9,06	24,2	9,83	17,1	9,74	15,5	10,33	19,9
9,40	17,2	7,45	20,2	9,57	19,4	8,94	17,5
9,61	16,9	9,13	22,1	9,99	13,4	9,93	22,8
9,08	14,9	9,40	21,3	9,19	14,7	9,34	20,3
8,51	16,4	9,88	26,1	8,92	20,8	10,37	27,7
8,67	18,4	10,03	17,1	9,01	20,6	8,22	20,0
9,63	19,1	9,44	20,4	9,23	18,3	9,81	23,1
9,19	20,9	10,00	14,7	10,00	16,6	9,55	24,0
8,65	14,7	9,15	18,3	9,06	19,4	8,94	27,1
9,33	22,5	9,25	19,8	9,25	14,8	10,17	21,8
8,95	17,7	9,07	19,0	9,02	22,7	0,92	16,1
8,66	20,9	9,16	22,9	9,50	20,7	8,32	18,7
8,83	23,1	10,25	16,4	7,69	27,2	8,17	23,5
9,75	16,7	10,15	20,9	9,05	12,6	8,66	26,0
8,60	20,7	9,30	20,3	6,81	16,9	0,86	23,6
8,62	16,8	7,72	14,6	8,68	17,7	9,81	23,0
8,53	17,6	9,63	23,3	9,70	18,0	8,17	21,9
8,79	16,4	9,52	14,5	9,22	17,7	8,17	18,9
8,86	20,2	7,60	19,9	9,40	17,4	0,92	22,1
8,45	18,1	8,85	19,7	8,91	17,2	6,82	22,7
8,90	17,2	10,20	20,4	10,08	17,0	5,34	20,1
7,57	20,6	9,49	19,6	9,25	16,3	8,07	21,1
8,79	16,6	9,30	15,5	8,69	16,3	8,47	19,3
7,83	19,7	9,08	19,9	8,90	18,4	8,37	20,4
8,74	20,9	9,65	18,5	8,73	17,2	8,75	23,8
8,82	21,6	9,29	16,5	9,57	18,0	9,97	23,1
9,48	21,9	9,43	16,7	9,36	14,2	7,66	18,6
8,32	20,0	9,33	23,1	10,18	17,6	7,77	15,6
9,45	19,5	9,25	17,4	9,76	19,6	10,25	26,0
8,66	20,2	9,14	19,9	7,54	16,4	9,85	18,9
8,33	17,0	9,78	21,9	9,21	21,1	0,92	19,3
8,88	20,6	9,47	17,0	9,52	18,8	0,94	19,6
9,04	17,5	9,70	18,1	9,38	20,4	8,47	17,3
8,99	21,0	9,23	16,1	9,60	16,6	8,42	16,7
9,29	15,7	9,84	15,5	9,45	16,5	8,66	20,2
7,80	19,7	9,92	21,7	9,46	15,1	9,03	22,3

9,09	18,2	9,76	18,0	9,50	20,0	8,37	21,8
9,53	18,4	9,90	21,0	9,57	20,3	8,94	21,3
8,80	20,5	8,72	17,2	8,96	15,3	0,86	18,4
9,70	17,2	9,34	16,9	9,61	17,5	9,89	22,5
8,65	19,0	9,13	20,8	9,39	17,3	7,11	19,4
9,95	15,0	9,62	14,4	9,44	20,8	8,52	21,4
8,96	16,0	9,88	21,7	9,27	19,1	6,87	17,8
8,97	20,4	9,28	19,4	8,75	18,8	9,07	20,1
9,23	17,5	9,11	16,9	9,13	21,0	8,84	20,9
8,85	17,5	9,12	17,0	9,98	23,1	7,55	17,3
8,69	19,6	9,06	21,2	9,85	19,6	8,47	21,3
7,87	19,5	9,38	18,6	8,28	16,5	0,86	18,2
8,69	20,9	8,49	24,5	10,38	16,1	9,55	19,3
9,67	17,4	8,96	16,9	8,84	16,4	9,85	18,4
8,64	21,0	9,61	28,2	9,21	16,2	9,34	21,0
6,95	21,9	9,80	22,4	10,10	17,4	7,55	15,7
9,14	22,4	9,15	20,5	9,67	19,5	7,97	22,3
9,57	23,9	9,50	18,8	9,12	21,0	9,51	20,0
9,73	20,9	8,50	21,1	9,11	19,0	9,60	17,6
9,66	21,1	9,33	17,4	9,23	18,8	0,86	18,9
8,58	15,5	9,44	20,4	9,49	15,3	8,80	19,8
8,75	21,3	9,34	24,3	9,78	17,1	9,64	23,2
9,58	19,5	9,48	22,8	8,60	19,5	0,89	19,7
8,51	17,1	9,39	19,5	9,96	18,2	10,72	20,2
9,26	19,6	9,05	16,8	9,85	18,5	0,92	20,8
7,67	20,1	9,55	4,6	9,72	17,2	9,03	19,8
9,14	20,1	8,36	18,3	9,35	22,8	11,06	22,6
9,50	23,2	9,60	24,0	9,08	19,4	10,37	16,6
8,75	20,1	9,72	22,6	9,52	19,3	7,66	19,5
8,94	19,7	9,69	18,3	9,23	16,9	9,81	18,7
8,98	19,2	9,31	18,8	9,94	19,0	9,89	21,1
9,05	21,9	8,60	16,6	9,43	19,5	10,13	19,8
9,23	18,8	8,89	19,0	9,68	18,5	9,76	19,7
8,95	17,3	9,70	21,0	9,30	20,0	8,71	20,4
9,48	14,7	9,36	20,5	8,94	22,3	9,72	20,3
7,66	19,0	9,15	17,1	8,96	20,6	9,47	17,8
9,26	18,7	9,99	18,2	6,91	15,9	8,32	19,5
8,63	17,3	9,71	16,5	8,41	17,7	8,07	24,0
9,13	16,4	9,77	22,5	9,80	20,2	8,32	18,2
8,82	19,2	7,49	23,3	9,06	22,4	9,38	22,9
9,09	18,9	9,38	20,0	8,07	16,7	8,98	21,9
9,76	22,9	9,49	17,4	9,47	18,3	9,03	18,5
8,72	20,1	9,09	20,3	9,13	19,5	10,45	19,1
8,64	20,2	9,54	18,1	8,76	19,4	9,72	20,8
8,43	20,5	9,53	20,7	9,12	20,2	0,86	21,7
8,61	19,6	7,72	20,9	10,02	18,7	10,33	19,1

9,02	22,0	9,62	19,5	9,81	18,1	9,97	22,1
8,61	15,0	9,01	28,3	9,50	22,8	9,55	23,4
8,65	20,1	10,38	19,7	6,42	18,0	9,76	21,8
9,24	17,7	8,98	18,9	8,68	20,7	10,13	19,7
8,05	17,1	9,37	17,5	8,96	20,7	7,05	22,0
9,26	17,0	9,02	17,0	8,14	17,3	7,05	17,6
8,42	18,1	8,83	16,8	9,17	14,6	9,16	25,9
8,96	17,1	7,90	19,9	9,29	20,1	0,94	24,5
9,09	18,4	8,64	23,2	9,10	16,7	10,45	22,7
9,24	23,2	9,20	24,5	9,21	13,6	9,89	18,8
9,28	18,6	9,37	19,8	8,66	19,5	8,42	21,9
8,34	17,4	10,34	14,9	9,85	23,1	8,52	22,0
8,83	21,1	10,14	17,9	8,83	18,2	9,85	17,0
9,06	17,8	8,19	19,1	9,25	17,7	0,89	20,8
8,96	17,8	9,70	19,5	10,06	17,0	9,34	14,0
8,62	18,1	9,85	23,0	8,88	20,6	8,98	16,6
9,04	19,4	10,07	21,2	8,53	18,4	9,55	12,4
8,57	21,1	9,66	22,6	9,15	16,6	9,07	19,0
8,86	21,2	8,07	20,6	9,21	16,8	8,80	18,1
9,05	18,3	10,61	21,5	9,34	18,5	10,01	17,4
9,57	18,3	6,84	19,8	9,09	15,2	10,01	15,4
9,59	15,8	9,54	17,9	9,15	21,3	0,86	19,7
9,45	15,8	11,60	17,7	9,45	17,6	10,41	17,8
9,17	16,7	10,43	11,9	8,90	18,8	10,45	20,0
8,46	20,4	10,91	15,4	8,62	22,0	9,55	14,6
9,50	15,6	9,81	17,4	9,09	21,1	0,94	17,8
9,26	17,9	9,71	17,7	9,60	18,0	0,92	15,1
9,04	18,8	10,22	17,9	9,57	19,2	10,09	16,0
9,49	20,1	9,94	21,6	8,98	17,7	8,98	15,4
9,29	18,6	10,10	19,4	9,38	23,5	7,44	17,6
8,36	17,8	10,45	16,5	9,06	21,0	6,38	12,0
9,16	20,3	9,05	17,3	8,60	25,0	9,55	14,0
9,12	19,5	10,14	23,0	9,36	15,7	0,86	18,7
9,91	14,7	7,97	17,2	9,15	21,1	0,80	17,8
9,50	22,0	9,93	16,1	9,06	18,2	6,99	15,1
9,60	17,1	9,49	17,5	9,48	21,4	7,87	15,3
8,94	19,5	7,39	20,7	9,47	19,5	4,69	18,4
8,88	21,3	8,97	16,8	9,03	17,8	10,45	15,6
8,88	19,7	10,57	24,0	9,46	19,7	8,94	16,6
9,18	17,7	9,55	17,6	9,34	18,2	8,80	19,6
8,79	21,0	9,21	20,0	9,42	20,3	8,61	16,6
9,80	15,4	9,35	21,4	9,81	22,5	0,94	16,1
10,26	18,0	9,63	18,3	9,12	18,9	1,03	17,5
9,20	15,4	9,79	17,3	8,47	18,9	10,33	16,6
8,66	14,6	9,59	17,7	9,55	18,4	9,89	17,8
9,07	17,3	7,10	15,5	9,04	16,6	9,34	21,6

8,44	17,7	9,22	21,4	9,32	19,2	7,61	22,2
8,77	18,8	10,36	19,1	9,66	18,8	9,85	19,8
9,46	25,4	9,41	18,3	9,09	25,5	8,71	16,0
8,87	16,5	9,39	20,6	10,07	27,7	10,60	21,6
8,78	21,2	10,00	24,9	9,66	15,1	6,57	18,3
8,85	22,5	8,87	16,6	8,98	16,5	6,38	18,0
9,31	17,6	9,92	16,0	9,51	18,9	8,61	16,5
9,27	16,2	9,11	21,1	9,03	19,7	8,94	18,3
8,64	19,6	9,68	26,1	5,76	14,7	9,76	19,0
9,70	15,0	9,36	20,5	8,94	15,8	8,17	19,7
9,50	15,5	10,20	20,2	9,56	17,3	0,86	20,3
10,52	18,8	9,67	20,2	9,25	16,9	9,34	17,8
9,35	21,9	9,97	21,5	9,60	16,9	9,34	17,3
9,09	15,4	9,09	17,0	9,53	14,2	7,28	19,8
9,27	13,9	9,34	18,2	9,25	20,8	0,89	17,3
8,74	20,5	8,78	18,3	9,40	17,0	6,51	20,1
9,08	18,6	9,40	21,2	9,71	17,9	9,81	18,4
9,42	20,1	8,35	22,4	9,43	23,8	6,87	18,8
9,23	13,0	8,79	21,4	8,44	19,9	9,12	21,3
8,43	15,4	9,83	17,8	8,68	15,9	10,33	21,2
9,50	18,7	9,59	18,2	9,58	14,8	0,80	20,0
9,40	17,9	9,24	19,2	9,02	18,5	0,93	19,4
6,74	18,0	9,36	20,8	9,63	20,9	9,97	21,0
8,89	19,6	9,14	16,8	9,67	13,8	6,57	21,1
9,04	18,9	9,42	19,9	9,28	19,6	8,71	23,0
9,39	18,4	8,88	22,6	9,88	18,6	8,98	28,3
8,36	17,5	9,69	17,7	9,40	19,7	9,12	19,0
8,94	11,5	9,12	20,9	9,57	23,3	10,13	20,3
9,26	19,8	9,68	20,4	9,27	13,8	0,69	18,8
9,61	15,2	9,75	18,2	8,97	18,7	8,07	13,5
8,98	18,1	8,97	16,3	9,17	20,9	8,32	22,6
9,13	23,4	9,46	22,0	9,30	17,4	8,27	19,2
8,96	19,8	8,64	20,1	9,74	22,8	9,60	15,8
9,19	20,2	9,77	23,6	9,33	25,4	0,86	18,2
9,05	19,2	9,66	19,0	9,88	21,6	9,07	21,9
7,43	19,5	9,55	20,5	9,58	14,7	8,61	20,1
9,05	17,1	10,41	24,2	9,09	17,9	8,22	20,0
9,07	22,0	9,37	23,2	8,21	20,0	8,27	24,1
9,46	22,6	9,12	17,5	9,35	21,2	7,66	26,7
8,73	18,2	10,19	17,2	9,23	20,5	9,85	18,6
8,89	15,8	8,22	24,3	8,42	21,9	6,63	22,3
8,40	20,8	9,29	22,6	9,39	13,9	7,50	22,3
9,44	13,4	8,73	18,0	9,41	20,0	9,25	15,5
10,57	15,9	8,75	16,5	9,85	18,1	0,80	18,2
9,42	20,3	10,23	19,6	9,83	18,7	9,60	25,1
8,42	20,0	9,02	18,3	9,09	19,2	9,34	17,6

8,79	23,1	8,69	20,1	9,45	16,6	8,27	21,7
8,76	19,9	7,72	16,8	9,41	17,7	7,82	18,1
8,99	17,4	9,69	20,5	9,30	24,5	8,07	23,9
7,21	18,6	6,36	20,4	9,04	20,6	9,55	17,6
8,49	19,2	7,67	19,8	9,69	14,7	0,94	27,0
8,51	19,7	8,98	17,0	9,06	20,9	9,72	13,8
8,03	18,2	9,34	18,2	9,96	18,2	8,80	16,1
8,79	15,0	9,29	22,2	9,60	15,7	9,47	17,2
9,23	19,3	9,88	22,7	8,83	19,9	8,71	18,9
9,52	18,8	7,68	14,5	9,33	18,7	8,47	16,1
8,97	22,8	8,87	16,7	9,69	16,5	0,94	20,6
9,46	26,4	9,42	23,3	8,77	16,3	8,98	22,1
10,23	18,8	9,44	18,9	9,71	23,5	0,94	16,6
8,97	21,0	9,31	16,5	9,76	19,9	9,51	19,7
8,61	23,0	9,53	17,6	9,88	21,9	9,85	18,0
8,72	20,2	9,54	17,3	9,95	19,8	8,52	22,9
9,14	24,1	9,44	20,4	9,49	18,4	8,52	16,0
9,51	18,8	8,80	24,2	8,61	13,3	8,52	14,3
7,06	17,4	9,10	15,5	9,66	16,7	9,38	15,1
10,21	18,2	5,89	18,7	9,56	19,2	9,12	14,9
8,73	18,4	8,95	23,4	9,67	21,8	10,45	11,5
9,08	15,9	9,68	18,7	9,20	18,4	9,03	16,7
8,94	16,4	10,41	19,3	8,73	15,7	8,71	17,9
9,03	26,6	8,85	24,1	9,46	15,8	10,25	
8,95	17,4	9,54	22,3	9,14	16,1	10,41	
9,23	23,3	9,48	15,7	9,67	14,8	11,24	
6,16	23,3	9,51	15,6	9,00	14,7	9,07	
8,77	19,8	9,21	17,0	9,51	20,7	8,80	
9,04	16,9	8,98	21,7	8,84	14,9	8,37	
9,20	18,5	9,07	19,3	7,51	17,7	10,21	
9,85	25,5	8,99	25,0	8,85	20,5	8,71	
9,95	18,7	10,04	20,5	9,21	21,1	8,98	
9,97	19,9	9,85	19,6	9,43	17,2	9,38	
9,66	20,4	8,71	16,7	10,37	17,7	7,87	
9,12	16,6	9,68	24,0	9,12	20,6	9,25	
9,38	18,1	9,77	20,7	7,31	30,1	5,56	
8,35	17,3	10,24	15,9	8,81	17,1	10,13	
8,76	17,7	9,84	20,6	8,79	17,2	0,92	
9,87	18,9	8,62	17,3	9,46	20,5	8,80	
8,07	18,8	9,98	17,2	9,21	19,4	10,21	
9,38		9,78	16,3	9,53	19,4	8,98	
5,68		7,74	22,2	9,58	27,7	10,53	
8,88		9,26	18,0	9,54	18,5	10,60	
8,46		9,41	20,2	9,53	17,5	8,12	
8,96		7,18	18,6	9,45	18,6	9,03	
8,95		9,60	18,1	9,35	15,1	9,85	

10,01	10,27	22,1	9,87	17,0	10,05
8,82	9,18	18,3	9,44	20,6	10,33
9,22	9,14	20,6	9,43	21,6	9,07
9,11	8,86	16,8	8,62	21,6	9,93
9,68	9,05	14,4	9,65	19,7	7,66
9,26	8,70	20,9	9,58	23,3	8,80
9,28	9,84	21,0	8,62	19,6	8,66
8,99	9,31	14,3	9,59	21,3	0,89
9,75	9,59	21,1	9,59	16,6	9,16
9,85	9,25	17,7	9,29	19,2	9,38
9,35	10,30	18,8	9,32	18,7	9,64
9,20	10,10	18,8	8,59	16,9	8,66
8,90	8,76	16,7	9,67	14,5	8,75
8,99	9,05	13,5	9,06	17,0	9,93
9,23	8,90	19,5	9,51	23,7	1,03
7,25	9,58	16,3	9,71	13,8	9,34
9,43	9,78	26,3	8,72	17,3	9,07
9,11	9,75	15,8	9,19	19,0	9,55
8,51	9,72	23,9	9,13	17,9	0,93
9,44	10,01	22,3	10,18	19,8	9,51
9,39	8,93	21,9	9,17	17,5	9,68
9,09	9,69	21,5	9,89	17,6	8,37
7,93	8,80	21,4	9,06	19,2	10,01
9,09	8,18	15,5	9,59	19,7	8,52
9,02	9,52	19,2	9,33	21,8	9,38
8,58	10,35	18,3	9,27	15,8	8,42
8,89	9,24	16,9	9,65	19,2	8,94
8,72	9,93	21,9	9,03	22,6	8,47
9,30	9,75	17,4	9,98	19,7	9,64
8,89	9,23	17,8	9,15	18,9	10,41
9,91	9,84	17,2	7,47	20,0	10,05
9,06	9,33	19,6	9,17	12,3	8,66
9,08	8,88	19,6	8,02	17,1	8,32
6,48	9,43	23,2	9,73	15,5	9,64
9,50	9,83	21,9	8,18	21,4	10,13
9,22	9,29	17,9	8,58	19,8	9,64
6,64	9,43	22,7	9,56	16,3	9,76
9,93	9,22	16,7	9,32	16,4	9,51
9,29	9,31	20,0	9,01	25,5	9,03
9,30	9,33	17,9	9,59	21,4	6,32
9,78	9,41	20,7	9,61	22,4	10,01
9,38	9,66	22,2	9,34	21,1	9,60
8,99	10,26	26,6	10,23	14,9	0,94
9,39	9,87	18,1	10,09	19,0	9,93
9,72	9,80	22,5	10,17	19,8	9,34
9,77	10,22	20,4	9,47	15,8	8,66

8,84	10,32	21,7	9,44	15,5	9,81
9,08	8,60	25,3	9,54	19,1	9,72
9,24	10,73	22,5	9,40	21,3	9,38
9,11	7,12	18,9	9,08	18,8	9,55
8,59	8,31	18,4	9,41	17,4	9,34
8,53	10,24	18,6	10,14	13,9	10,45
9,25	10,19	24,4	9,23	16,8	9,47
8,33	9,85	18,9	9,47	19,7	9,55
9,07	10,57	17,7	9,52	20,4	9,25
8,84	6,98	21,5	9,69	18,3	0,92
8,89	7,30	22,7	9,62	16,6	8,75
9,41	9,98	23,0	7,31	21,2	9,68
8,70	9,32	17,5	9,43	17,5	9,68
8,77	10,28	24,4	9,20	17,8	10,33
8,25	10,09	21,2	9,01	14,3	8,22
8,39	10,33	22,4	9,87	18,6	9,51
8,84	10,29	19,6	9,18	18,4	9,68
9,22	7,66	19,7	9,81	19,8	9,47
9,18	10,06	19,2	9,43	20,3	10,25
8,78	9,58	16,3	8,94	18,0	9,60
10,25	9,61	19,2	8,77	17,1	9,34
8,80	10,56	16,2	9,51	16,6	9,81
9,43	9,89	12,7	9,77	17,6	8,71
9,73	11,72	13,2	9,28	16,7	10,87
9,70	10,50	17,1	9,55	21,4	10,72
8,68	9,60	19,2	9,21	16,9	0,86
9,40	8,31	18,4	9,07	19,5	8,71
8,74	9,76	24,3	9,23	21,7	8,32
8,69	8,14	26,0	8,92	13,3	10,09
8,61	7,54	17,2	10,04	22,6	8,94
8,80	10,16	20,7	9,59	18,2	7,44
9,04	10,30	22,5	9,46	21,5	8,71
10,11	10,12	20,6	9,10	14,5	0,92
7,80	10,05	19,0	9,22	20,3	9,12
9,30	10,42	19,9	10,49	20,4	9,68
9,35	10,03	19,8	8,37	10,0	10,37
9,43	6,81	11,5	9,58	14,8	9,60
8,47	9,83	14,2	8,75	18,2	8,27
8,96	10,56	20,6	9,12	15,5	9,97
8,91	11,18	20,0	9,29	15,8	9,03
8,97	10,29	21,4	8,99	17,8	10,13
9,62	8,92	18,5	6,84	19,8	9,64
9,14	11,32	18,4	9,03	16,4	8,12
8,68	9,83	20,5	9,46	18,3	8,42
8,69	9,70	14,3	9,06	19,6	9,60
9,00	10,42	21,6	9,32	22,6	10,05

9,22	10,18	16,4	9,76	19,6	9,16
8,12	10,25	22,2	9,31	20,5	0,94
9,17	9,56	18,3	9,65	21,5	8,84
9,03	9,83	15,4	8,29	22,8	0,73
8,66	9,65	20,5	9,65	17,1	9,64
9,24	10,37	22,7	10,22	18,1	0,89
9,09	10,61	18,6	9,73	18,7	9,68
8,46	10,77	19,7	9,99	21,6	9,89
8,88	10,45	18,2	9,70	17,9	8,84
8,73	5,88	16,3	9,26	20,6	8,37
9,29	10,43	15,5	7,72	17,2	8,94
8,29	10,60	19,1	9,24	19,3	10,68
9,67	9,96	22,8	9,03	18,4	1,03
8,69	9,96	18,0	9,20	18,6	9,07
8,52	7,65	17,9	9,71	17,5	8,84
10,05	10,56	20,8	6,41	19,4	8,94
9,16	8,48	21,5	7,26	13,3	9,72
9,37	10,53	19,6	8,00	20,5	9,47
9,12	8,48	20,3	8,94	20,7	0,92
9,53	11,26	24,4	9,15	15,9	8,84
9,25	10,15	22,0	9,76	20,7	9,38
9,70	9,83	19,6	10,26	21,6	10,21
9,28	9,16	18,7	9,03	16,9	9,16
10,15	10,80	19,9	9,30	17,4	8,47
8,85	9,68	19,3	8,65	17,9	9,72
8,62	10,70	16,9	9,61	21,0	9,93
6,69	9,66	21,1	9,25	21,3	0,92
9,21	10,26	26,4	9,87	16,3	9,81
9,33	10,36	18,9	8,75	18,1	9,68
9,11	10,14	20,5	8,57	21,9	9,55
9,31	10,40	17,9	9,29	18,3	9,16
8,60	9,78	20,3	8,77	22,3	9,34
8,77	9,83	18,6	9,30	16,9	9,12
9,42	10,26	22,8	9,93	19,4	9,55
7,69	10,18	20,7	8,50	15,7	9,16
9,28	9,59	17,8	9,20	18,1	1,03
8,96	9,91	24,2	7,42	19,3	9,60
8,38	10,07	18,2	9,73	21,7	8,22
8,79	9,85	17,5	9,64	17,9	9,60
9,77	10,03	20,5	9,12	14,4	9,47
9,77	10,17	20,5	9,27	20,6	7,61
9,51	8,71	16,6	8,02	16,8	7,77
8,47	9,60	19,6	9,47	17,5	8,71
10,12	9,88	18,5	9,79	23,7	9,38
9,38	9,45	13,5	9,62	21,2	8,61
9,60	7,98	20,9	10,20	15,4	10,17

9,48	10,01	22,6	9,09	19,6	9,81
9,35	9,38	23,5	9,69	20,6	8,12
8,68	10,04	19,9	8,26	18,0	9,47
8,60	8,65	18,5	6,77	19,6	10,76
8,54	9,23	13,5	8,60	21,4	0,86
9,91	7,88	21,0	9,44	22,1	10,17
8,86	9,55	16,6	9,17	19,8	9,34
8,87	9,05	24,3	8,76	13,1	9,51
8,85	8,95	20,9	9,40	16,6	10,21
8,96	9,38	19,4	9,44	19,0	10,13
9,25	10,25	17,1	10,02	13,5	9,25
9,52	10,33	21,2	8,65	20,1	0,94
9,22	10,18	17,7	9,65	18,9	10,13
9,35	9,93	19,8	8,70	19,3	8,94
6,82	9,83	18,0	9,93	18,4	0,94
9,11	9,33	17,1	9,39	17,8	9,76
9,27	9,48	17,1	9,99	14,8	8,27
9,61	9,49	17,9	8,77	19,6	9,55
8,62	9,37	18,9	6,96	21,4	9,16
9,96	9,36	14,2	9,50	16,3	8,80
9,50	8,87	17,1	9,57	15,8	9,72
8,78	8,87	19,4	9,68	20,5	10,17
8,77	9,76	15,3	9,49	19,8	8,47
8,75	8,39	13,9	10,31	17,5	9,12
9,46	9,54	21,7	9,03	18,6	8,80
9,06	9,94	20,5	9,09	17,9	9,16
9,00	9,09	19,4	7,46	19,1	10,25
8,52	8,84	16,7	9,60	18,8	8,12
7,25	8,82	17,9	8,88	14,2	9,03
9,16	9,11	19,3	9,69	17,5	9,93
8,68	8,92	23,2	9,04	16,0	8,75
9,15	9,36	16,5	9,45	14,8	8,80
8,93	9,25	20,5	9,58	14,4	8,98
9,01	9,35	14,3	9,03	20,8	8,84
7,02	9,05	18,6	8,80	18,9	9,64
8,40	9,85	17,9	8,94	12,2	10,17
9,51	8,38	18,3	9,09	19,2	9,03
9,04	9,83	20,7	10,16	18,9	1,13
9,89	9,79	16,1	7,87	12,8	0,92
8,39	9,54	20,7	8,66	18,8	7,44
9,43	8,93	17,2	8,50	14,8	10,01
9,44	9,50	17,5	9,25	19,5	0,89
8,77	10,04	20,4	7,30	19,1	11,27
9,15	9,02	22,8	9,09	21,4	0,86
8,90	10,31	14,8	9,40	16,6	8,47
9,63	9,50	14,1	9,99	11,3	9,81

8,99	8,77	16,2	9,39	19,5	8,75
9,39	9,15	21,6	9,07	18,3	9,81
9,28	9,75	20,4	9,71	20,4	6,12
6,72	10,06	13,7	9,39	18,4	9,76
9,07	8,73	15,6	9,08	18,8	0,92
9,00	9,65	17,6	8,81	17,6	1,03
8,46	8,59	20,1	8,30	15,4	7,11
9,10	9,75	15,9	9,35	19,4	9,97
8,17	9,14	15,4	9,32	15,8	9,47
9,07	9,61	17,4	9,05	20,5	8,42
9,65	9,11	18,9	8,95	20,8	9,25
9,28	9,68	17,1	9,02	16,9	10,37
8,65	9,46	21,1	10,00	17,8	0,92
9,27	10,40	26,0	9,25	15,2	9,81
8,24	9,98	21,6	8,92	18,3	10,17
9,35	8,90	14,8	9,51	21,4	0,94
8,85	8,59	18,0	9,49	13,9	9,97
9,53	9,40	19,1	9,40	21,7	0,94
8,69	10,06	28,5	8,91	18,0	9,47
8,92	9,88	18,8	9,21	15,6	7,22
8,63	9,53	17,4	8,83	20,5	0,92
9,84	9,28	20,4	6,80	17,9	9,25
9,87	9,40	16,8	9,70	16,1	9,38
7,77	8,96	22,1	9,46	16,6	9,34
5,94	9,13	17,1	9,52	23,1	9,97
9,60	10,13	17,2	9,70	14,2	8,98
8,88	9,39	21,8	9,48	16,2	9,76
9,72	9,70	21,4	9,09	19,9	9,51
8,72	9,17	19,1	9,37	20,9	10,72
9,25	9,74	21,8	9,48	15,6	9,25
8,73	9,31	22,8	8,79	19,6	9,47
8,95	9,96	14,2	8,17	19,5	0,93
9,38	9,13	28,2	9,52	17,2	0,93
9,02	7,99	20,0	9,14	18,2	8,80
9,19	9,87	19,8	8,68	18,2	8,22
9,82	9,40	17,4	8,96	20,0	0,93
9,67	9,03	17,5	9,22	14,1	9,76
9,62	8,65	18,2	9,86	13,7	8,98
9,06	10,12	18,8	9,14	21,0	9,89
9,46	9,72	20,6	9,34	20,7	9,72
8,62	9,01	18,6	9,01	17,0	9,76
9,48	8,82	19,7	9,24	18,1	10,64
9,73	6,53	14,9	8,45	16,8	0,86
9,24	8,60	15,7	9,30	19,8	9,60
9,44	8,89	19,1	9,30	17,7	10,37
9,69	9,18	19,9	8,87	18,6	8,94

9,01	9,72	20,8	9,58	15,4	10,13
9,66	7,35	18,7	8,96	20,8	10,37
9,08	8,67	16,5	9,01	16,1	8,84
9,42	8,82	20,4	9,31	14,9	9,72
9,50	9,14	19,4	9,54	16,8	9,07
8,82	8,37	15,2	8,62	16,2	9,07
9,70	7,73	23,6	9,61	18,2	6,99
9,23	9,25	18,7	9,21	18,5	8,47
8,94	9,86	16,8	9,53	19,5	10,05
7,15	9,65	19,5	9,29	19,9	9,55
9,18	7,55	19,0	8,99	16,1	9,25
8,99	9,32	19,7	9,17	12,8	8,27
9,42	9,41	20,0	9,14	17,2	10,17
8,84	9,16	19,0	8,88	17,4	7,77
9,12	9,55	25,5	8,63	19,1	9,03
8,88	9,52	22,2	8,87	17,8	10,13
5,79	10,05	17,7	9,03	19,1	0,94
9,22	9,01	19,0	8,85	21,9	9,81
8,94	9,67	19,6	9,54	19,4	8,75
9,60	9,52	20,0	8,73	22,4	0,89
9,48	6,47	21,6	9,21	17,5	8,32
9,32	9,31	20,8	9,16	19,3	0,86
9,62	9,25	23,0	9,36	16,9	8,66
8,85	9,57	17,8	9,77	20,5	7,97
9,03	9,68	20,4	8,96	18,7	6,76
9,51	9,40	18,1	8,78	17,3	8,22
9,47	9,75	26,0	9,18	17,0	8,75
9,79	9,32	19,6	8,69	17,7	9,85
9,03	9,96	18,5	9,22	14,6	9,76
9,00	9,59	17,9	8,55	17,8	10,21
9,60	9,47	15,4	9,65	19,7	9,34
9,62	9,46	16,0	9,79	18,9	9,85
9,02	9,38	10,1	9,39	17,8	0,92
9,57	9,61	18,0	8,86	19,4	8,61
9,60	10,31	20,8	9,66	20,3	
8,92	9,14	15,0	9,24	16,8	
9,30	9,31	20,0	8,64	14,6	
9,57	8,74	19,7	8,97	16,2	
9,31	9,47	20,3	8,33	19,8	
9,71	10,00	16,5	9,49	22,7	
6,18	6,84	20,2	9,82	18,1	
9,41	9,26	20,8	9,57	23,6	
9,16	9,38	22,0	9,39	17,8	
8,90	10,14	20,7	8,77	15,9	
10,04	9,54	20,5	9,38	18,2	
9,16	9,83	23,5	6,99	18,9	

9,74	9,32	14,4	8,97	18,2
8,98	6,94	18,5	9,04	18,7
9,14	9,86	19,8	9,69	15,3
9,60	9,40	18,6	9,16	15,1
9,21	10,17	20,2	8,79	21,5
9,44	9,80	22,2	9,17	20,5
9,46	9,54	12,9	9,86	20,9
9,10	9,44	20,3	9,46	21,0
9,52	10,08	21,6	9,45	16,4
9,30	9,41	22,7	9,36	18,4
7,36	9,49	21,9	7,43	18,4
9,58	9,30	20,6	9,19	20,8
8,94	9,39	16,5	9,41	16,3
9,42	9,91	17,7	8,24	17,2
9,25	9,95	16,5	8,83	25,0
9,17	9,71	21,3	9,38	17,4
9,14	9,32	18,5	7,20	19,9
9,50	9,15	18,1	8,78	16,8
8,33	10,07	26,3	8,47	13,9
8,90	9,38	18,8	8,81	15,3
10,13	9,30	17,1	9,64	16,7
8,80	9,73	26,1	9,41	12,8
9,32	9,35	20,3	8,45	15,4
8,99	9,89	21,2	9,49	14,4
9,05	9,33	17,4	8,58	17,9
8,64	10,14	17,1	9,26	17,4
9,30	9,94	16,3	9,48	20,1
9,14	8,26	16,8	9,21	18,7
9,67	9,21	16,4	9,25	17,5
9,23	9,78	24,1	9,31	18,5
7,43	8,91	19,1	9,96	11,8
9,85	9,29	20,6	8,86	18,4
9,66	9,25	18,7	8,56	18,6
9,02	9,46	21,9	9,38	20,5
9,74	9,88	19,9	9,35	14,5
9,82	10,54	14,2	6,86	19,6
9,77	10,18	19,7	9,76	17,7
9,73	8,84	14,4	7,24	21,4
9,29	9,46	18,9	8,76	15,9
9,52	9,71	24,5	8,72	19,6
9,27	8,43	16,7	9,16	17,4
9,78	10,01	17,8	9,29	21,1
9,18	9,05	21,4	9,65	17,1
8,86	8,84	18,3	9,66	15,4
8,54	9,07	17,4	8,44	15,6
9,76	10,38	18,8	9,37	23,3

9,38	10,00	19,0	9,88	17,1
9,36	10,13	19,4	9,60	22,2
9,67	10,96	20,9	9,66	15,7
9,61	9,52	18,0	9,25	22,0
9,28	9,18	18,3	9,26	18,9
10,08	10,30	15,4	6,34	21,1
9,07	10,83	16,1	8,78	20,1
7,64	9,92	15,0	9,36	20,0
8,58	9,87	21,1	9,26	16,9
9,02	5,22	19,8	9,56	18,1
9,44	9,74	17,6	9,14	16,9
8,66	9,73	18,4	9,28	16,0
9,09	10,06	25,4	9,14	21,4
9,87	9,09	13,7	9,22	18,1
9,33	9,30	19,6	9,29	17,4
9,58	9,13	18,2	8,89	20,7
8,66	9,34	19,7	9,06	18,1
8,59	9,16	18,8	9,29	22,9
7,29	9,30	15,7	9,26	15,7
8,47	9,60	18,7	9,31	16,9
9,43	9,99	22,2	9,46	16,0
6,84	9,24	19,1	8,60	17,1
10,01	9,39	18,6	8,89	20,8
9,34	9,68	11,8	9,89	19,6
8,43	9,85	21,4	7,57	15,2
9,94	10,77	22,1	8,99	18,7
9,31	9,65	22,4	9,80	17,2
8,81	10,26	18,9	9,43	19,7
8,75	7,13	17,8	9,41	23,5
9,05	10,44	19,7	9,15	18,0
9,08	9,00	16,6	9,86	14,2
9,41	10,61	24,2	10,07	20,1
9,30	11,68	20,9	9,36	24,0
9,21	10,29	18,9	9,53	15,9
9,40	10,03	17,8	9,75	21,9
9,06	10,16	20,5	9,48	18,3
8,77	10,30	20,3	9,99	14,7
9,42	10,75	16,4	9,53	21,8
9,07	10,21	22,0	9,06	18,2
9,30	9,83	14,7	9,49	20,0
9,43	8,55	16,3	8,93	16,1
8,04	9,65	16,4	9,54	15,2
9,15	9,88	12,6	9,60	19,8
9,31	10,80	14,5	8,98	18,1
8,83	9,56	16,4	9,47	18,6
8,86	10,54	19,2	9,53	20,1

9,07		10,80	19,8	8,87	16,2	
9,05		10,56	17,4	10,29	17,8	
8,99		8,41	19,9	9,52	23,3	
9,84		10,60	28,7	9,32	15,9	
9,15		10,27	14,5	6,72	18,9	
8,94		10,71	23,2	7,99	19,8	
9,44		9,50	14,8	9,42	19,9	
8,49		8,99	15,7	9,20	19,3	
9,41		9,72	22,7	9,69	20,0	
8,94		9,89	15,9	8,93	18,0	
9,43		9,20	21,2	9,57	15,8	
9,52		9,60	20,7	9,19	18,3	
9,93		9,43	20,0	9,17	19,7	
8,99		10,50	16,7	8,49	18,6	
9,73		9,23	15,3	8,85	14,5	
9,94		9,57	15,7	9,17	20,1	
8,92		10,40	14,9	9,09	14,4	
9,28		10,29	19,5	9,32	23,7	
9,30		10,72	21,3	8,22	19,8	
8,99		7,29	15,6	8,59	14,6	
9,44		10,79	19,2	8,95	18,7	
8,60		9,95	13,7	9,08	20,3	
8,99		10,79	17,3	8,49	19,1	
9,29		10,27	19,6	9,15	15,3	
8,90		9,96	18,7	9,18	18,8	
9,25		9,74	16,0	9,47	18,9	
9,22		9,15	19,4	9,96	15,0	
9,03		9,39	16,0	8,63	19,0	
9,63		9,24	18,7	9,09	16,8	
9,54		10,03	18,2	8,82	15,8	
8,97		9,53	19,1	9,49	18,5	
8,44		9,51	17,9	9,87	19,3	
8,88		9,85	18,8	9,29	20,9	
8,48		9,31	18,4	9,31	18,9	
9,22		10,94	14,1	9,61	16,6	
9,08		10,36	18,4	9,05	17,7	
8,31		10,28	16,4	8,77	18,3	
9,82		9,59	20,6	6,79	16,6	
9,41		9,45	14,4	9,69	22,8	
8,95		9,30	21,5	9,23	15,3	
9,29		9,27	17,6	9,48	19,3	
8,39		9,43	17,8	8,91	16,3	
9,48		9,49	17,3	8,68	19,2	
8,80		9,93	12,6	6,73	17,0	
10,32		9,45	19,0	9,51	14,4	
9,57		10,51	17,1	10,11	18,2	

10,16	10,50	22,7	8,78	15,5	
9,23	10,10	19,6	9,35	18,1	
8,76	10,09	15,0	9,10	22,2	
9,40	10,45	17,4	9,80	19,9	
9,46	7,97	20,0	9,34	15,4	
9,24	9,72	20,2	9,56	18,0	
9,53	9,51	18,5	8,90	15,5	
9,42	9,68	9,9	9,59	23,6	
9,38	8,93	18,6	9,84	17,0	
7,33	9,57	16,3	9,23	17,9	
9,64	9,13	16,5	10,11	19,5	
9,19	10,17	19,9	8,10	22,8	
5,85	10,10	10,7	9,16	16,3	
9,58	9,16	16,4	8,98	12,9	
9,34	9,48	16,0	8,46	19,6	
9,71	9,10	21,7	9,13	20,6	
9,08	8,19	17,7	8,70	16,7	
9,13	9,49	19,5	9,24	17,6	
8,67	10,50	24,1	9,60	18,0	
9,41	9,29	21,0	9,43	13,7	
10,25	9,01	18,5	9,72	21,1	
9,37	10,29	13,4	9,95	19,7	
9,04	8,82	29,0	9,32	22,6	
8,67	9,63	19,0	9,26	21,8	
9,58	8,86	18,1	8,69	17,1	
9,12	10,12	24,4	7,67	17,4	
9,15	9,00	19,6	9,75	19,3	
9,73	9,25	17,4	8,53	18,8	
9,02	8,09	19,5	8,95	18,7	
9,36	10,94	15,2	8,35	23,0	
8,83	9,50	18,7	9,16	21,3	
9,00	10,32	18,5	9,55	17,6	
8,90	9,85	13,3	8,83	16,9	
8,76	9,48	18,9	9,68	16,7	
9,88	10,02	21,3	9,72	18,2	
9,15	10,14	40,3	8,59	18,7	
5,51	7,21	15,9	9,70	19,1	
9,21	9,42	18,8	9,31	21,0	
9,59	9,05	19,1	9,69	18,7	
9,23	9,71	17,0	9,69	15,7	
8,93	8,99	16,8	9,25	22,0	
9,12	10,75	19,0	9,33	17,1	
9,07	10,10	16,4	9,03	18,4	
7,08	10,50	18,0	9,05	21,7	
7,18	9,78	17,0	9,23	21,7	
8,66	10,87	24,2	9,91	20,9	

9,23	10,21	18,3	9,47	24,2
8,94	9,95	17,2	9,46	18,6
8,98	10,04	19,9	9,43	18,8
8,62	9,15	18,2	9,29	16,5
8,98	10,35	27,7	9,90	19,0
8,79	10,10	20,9	9,30	19,0
9,19	10,70	22,9	9,94	18,1
10,01	10,76	19,0	9,26	19,5
8,86	10,62	17,2	9,28	12,1
8,86	10,94	24,8	9,79	21,9
9,00	9,36	12,3	9,01	17,9
9,42	9,90		9,83	17,5
7,67	9,64		9,27	17,5
9,84	9,66		9,56	19,0
8,84	8,45		9,18	16,2
9,28	9,43		10,01	21,5
9,75	9,30		8,66	19,0
9,46	10,51		9,49	15,0
8,41	10,38		9,48	17,1
9,28	9,60		9,80	23,6
9,58	10,61		9,23	19,0
8,98	10,02		9,65	16,4
8,91	8,51		9,41	20,6
9,44	9,95		9,95	16,4
8,91	9,91		9,64	19,6
9,76	9,98		9,57	18,7
8,86	10,46		8,91	17,5
8,57	7,98		9,19	18,8
9,21	10,02		8,69	20,7
9,23	11,59		9,40	21,1
8,53	9,83		8,87	20,2
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9,31	9,99		8,99	17,7
8,95	9,96		9,26	18,6
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7,07	9,96		9,89	20,5
5,09	9,90		9,55	17,5
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7,64	10,00			19,7
9,08	9,67			17,9
7,73	10,33			18,6
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8,83	20,4		
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8,88	20,3		
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TECHNOORG LINDA

MAG*I*CAL® Reference Standard for TEM

Reference Standard **Serial No.:** 2145 **Date:** 24/JUNE/2020

Certificate of Calibration

The MAG*I*CAL® calibration reference standard is a cross-sectional TEM sample of a silicon-based semiconductor multilayer. It consists of five sets of five ~10 nm thick $Si_{0.81}Ge_{0.19}$ alloy layers, alternating with ~10 nm thick pure silicon layers. The device-quality epitaxial layers were grown by Molecular Beam Epitaxy (MBE) as strained layers on a single crystal silicon <001> substrate. All calibrated values incorporate the strain affects.

The four sets of alternating layers (superlattices) provide dark and light contrast in the TEM, and were directly calibrated by high resolution transmission electron microscopy (HREM) in reference to the {111} lattice spacing of silicon, as measured on the single crystal silicon substrate. This spacing is known to be 0.313 560 11 (8) nm [1]. The uncertainty in measurement across each of the full superlattices is less than one atomic layer at both the top and bottom interfaces: Δt (superlattice) < 0.5%.

The variation in layer thicknesses across the wafer material used for the current series of MAG*I*CAL® calibration reference standards was measured by double crystal x-ray diffraction (DCXRD) mapping as < 1%. The estimated uncertainty in all sets of calibrated values (other than the individual thin SiGe and Si layers) assumes a normal distribution, and is the combination of all uncertainties added in quadrature. The **overall uncertainty** on the calibrated values listed on the 'Layer Thickness Values' sheet is:

$$\Delta t < 1\ \%$$

Multiplying the overall uncertainty by a coverage factor of k=2 gives a confidence level of approximately 95%. An user can fine-tune the calibrated values and decrease the uncertainty of their individual calibration reference standard by verifying the calibrated values with reference to the Si {111} lattice spacing in the substrate.

Replace the MAG*I*CAL® Calibration Standard three years after the date on this Certificate of Calibration, or earlier if contaminated. One year limited warranty on defects in manufacture. Replacement or refund provided by the distributor.

[1] CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Florida 33431

Andras Szigethy

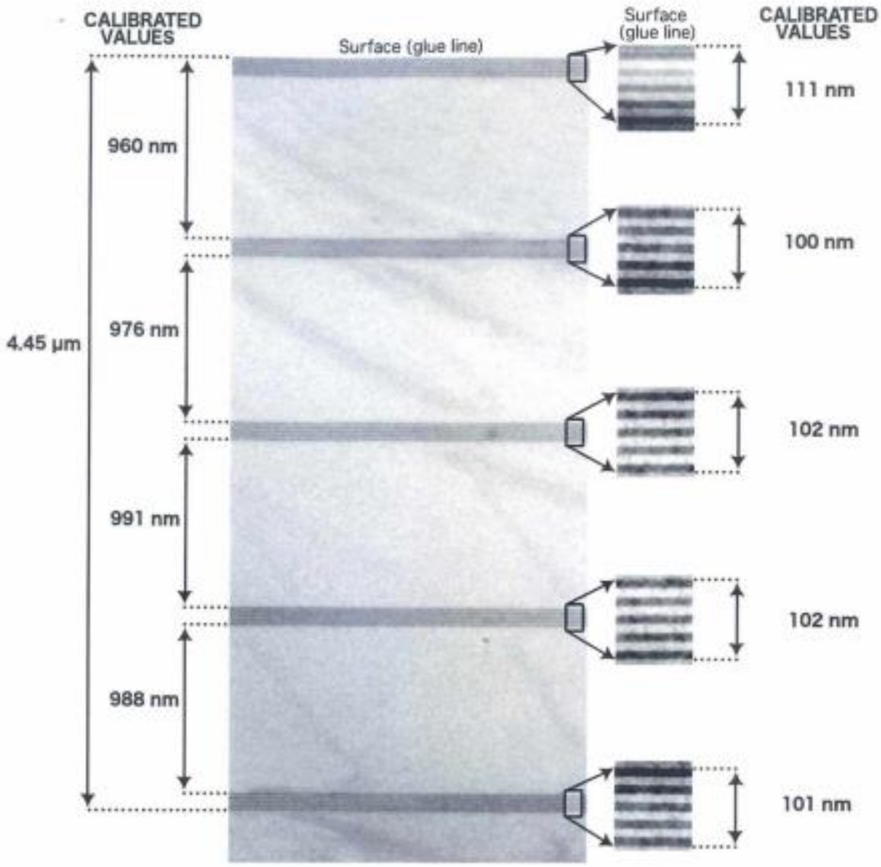
Technoorg-Linda Co. Ltd., Ipari Park u. 10, H-1044 Budapest, HUNGARY, www.technoorg.hu
Tel: 36-1-4790608, Fax: 36-1-3224089, Email: info@technoorg.hu 4/16

MAG*I*CAL® Reference Standard for TEM

Layer Thickness Values

Rev. 5

Calibrated values = ± 2%



To improve accuracy when making measurements with CALIBRATED VALUES, always measure across the largest distance visible on MAG*I*CAL® Calibration Standard. For example, at high magnification, always measure across a complete superlattice (the five darker bands and four lighter bands in bright field image). At lower magnifications, measure across one or several of the 0.xx µm layers, remembering to add in the thickness of any intervening superlattices.

For accurate measurements of structures of 100 nm or less, the TEM must be calibrated against the (111) lattice spacing of Si available on the MAG*I*CAL calibration standard.

Technoorg-Linda Co. Ltd., Ipari Park u. 10, H-1044 Budapest, HUNGARY, www.technoorg.hu
Tel: 36-1-4790608, Fax: 36-1-3224089, Email: info@technoorg.hu

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14. Literature

1. Stefaniak, A. B.; Hackley, V. A.; Roebben, G.; Ehara, K.; Hankin, S.; Postek, M. T.; Lynch, I.; Fu, W.-E.; Linsinger, T. P. J.; Thünemann, A. F., Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities. *Nanotoxicology* **2013,** *7* (8), 1325-1337.
2. Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R. N., Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications. *Chemical Reviews* **2008,** *108* (6), 2064-2110.
3. Bienert, R.; Emmerling, F.; Thünemann, A. F., The size distribution of 'gold standard' nanoparticles. *Analytical and Bioanalytical Chemistry* **2009,** *395* (6), 1651.
4. Gleber, G.; Cibik, L.; Haas, S.; Hoell, A.; Müller, P.; Krumrey, M., Traceable size determination of PMMA nanoparticles based on Small Angle X-ray Scattering (SAXS). *Journal of Physics: Conference Series* **2010,** *247*, 012027.
5. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.-Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A., Fiji: an open-source platform for biological-image analysis. *Nature Methods* **2012,** *9* (7), 676-682.
6. Ridler, T. W.; Calvard, S., Picture Thresholding Using an Iterative Selection Method. *IEEE Transactions on Systems, Man, and Cybernetics* **1978,** *8* (8), 630-632.
7. Bergmann, A.; Orthaber, D.; Scherf, G.; Glatter, O., Improvement of SAXS measurements on Kratky slit systems by Gobel mirrors and imaging-plate detectors. *Journal of Applied Crystallography* **2000,** *33* (3 Part 2), 869-875.
8. Orthaber, D.; Bergmann, A.; Glatter, O., SAXS experiments on absolute scale with Kratky systems using water as a secondary standard. *Journal of Applied Crystallography* **2000,** *33* (2), 218-225.
9. Pauw, B. R.; Kastner, C.; Thunemann, A. F., Nanoparticle size distribution quantification: results of a small-angle X-ray scattering inter-laboratory comparison. *Journal of Applied Crystallography* **2017,** *50* (5), 1280-1288.
10. Disch, S.; Wetterskog, E.; Hermann, R. P.; Salazar-Alvarez, G.; Busch, P.; Brückel, T.; Bergström, L.; Kamali, S., Shape Induced Symmetry in Self-Assembled Mesocrystals of Iron Oxide Nanocubes. *Nano Letters* **2011,** *11* (4), 1651-1656.
11. Disch, S.; Wetterskog, E.; Hermann, R. P.; Wiedenmann, A.; Vainio, U.; Salazar-Alvarez, G.; Bergström, L.; Brückel, T., Quantitative spatial magnetization distribution in iron oxide nanocubes and nanospheres by polarized small-angle neutron scattering. *New Journal of Physics* **2012,** *14* (1), 013025.
12. Dekker, F.; Kuipers, B. W. M.; Petukhov, A. V.; Tuinier, R.; Philipse, A. P., Scattering from colloidal cubic silica shells: Part I, particle form factors and optical contrast variation. *Journal of Colloid and Interface Science* **2020,** *571*, 419-428.
13. Li, X.; Shew, C.-Y.; He, L.; Meilleur, F.; Myles, D. A. A.; Liu, E.; Zhang, Y.; Smith, G. S.; Herwig, K. W.; Pynn, R.; Chen, W.-R., Scattering functions of Platonic solids. *Journal of Applied Crystallography* **2011,** *44* (3), 545-557.
14. Kestens, V.; Gerganova, T.; Roebben, G.; Held, A., A new certified reference material for size and shape analysis of nanorods using electron microscopy. *Analytical and Bioanalytical Chemistry* **2021,** *413* (1), 141-157.
15. ISO Guide 98-3:2008 Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995) 2008.