Report

Reference Material

BAM-P202

Polystyrene
(powder)

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Summary

This report describes preparation and analysis of the reference material BAM-P202.

The following particle sizes and standard deviations have been determined:

PSD	Particle size[1] **D** in µm	Twofold standard deviation[2] **$2 \cdot s$** in µm	Expanded uncertainty[3] U in µm
D10	91	9	13
D50	206	27	29
D90	311	28	28

[1] Mean value of 10 measurements with 1 g BAM-P202 by laser diffraction under dry dispersion (HELOS/BR + RODOS/L + ASPIROS, Sympatec, Germany, ISO 13320:2009 certified).

[2] Twofold value of standard deviation of the accepted data set mean.

[3] Estimated expanded uncertainty $U = k \cdot u_c$ with a coverage factor $k = 2$, corresponding to a level of confidence of approximately 95%, calculated according to ISO Guide 35. The combined uncertainty u_c includes the standard uncertainty due to characterization, the contribution of variation between bottles and the long-term stability contribution.

This report contains detailed information on the preparation of the reference material as well as on homogeneity and stability investigations and on the analytical methods used for accompanied analysis. The values for particle size distribution are based on the results of 30 measurements of the reference material.

Content

List of abbreviations

(if not explained elsewhere)

ANOVA	analysis of variance
D10	D10 value describes the particle diameter of the measured volume below which 10.3% of the particles fall below
D50	D50 value describes the particle diameter of the measured volume below which 50.3% of the particles fall below
D90	D90 value describes the particle diameter of the measured volume below which 90.3% of the particles fall below
$M_{between}$	mean of squared deviations between bottles
M_{within}	mean of squared deviations within one bottle
n	number of replicate measurements per bottle
N	number of bottles selected
PS	polystyrene
PSD	particle size distribution
RM	reference material
rel.	relative
r.H.	relative humidity
s	standard deviation
s_{bb}	standard uncertainty due to between-bottle (in)homogeneity
s_r / s_w	standard deviation of repeatability within the bottles
T_g	glass transition temperature
U	expanded standard uncertainty of a property value
u_c	combined standard uncertainty of a property value
u_{char}	standard uncertainty due to characterization
u_{hom}	standard uncertainty due to (in)homogeneity
u_{lts}	standard uncertainty due to long-term (in)stability
$\bar{x}$	mean property value of material
$\bar{x}_{hom}$	mean property value of material for homogeneity test
$\bar{x}_{stab}$	mean property value of material for stability test

1. Introduction

Polystyrene (PS) is one of the most abundant polymers used in industry and hence, relevant for microplastic analysis. Polystyrene can be easily and cheaply purchased as round particles. But this shape will not be near to reality, where polystyrene macroplastic will be decomposed by environmental weathering into small microplastic particles with sharp edges.
The idea is to offer a reference material close to reality for the validation of sampling, sample preparation and detection of microplastics. At the same time, it can be used for the evaluation of effects in the field of ecotoxicology, human toxicology, pollutant transport and agglomeration behaviour related to microplastics. The maximum particle size shall be < 500 µm.

The evaluation of this new RM has been carried out based on BAM Guidelines for the Development and Production of BAM Reference Materials [1], ISO 17034 [2] and relevant ISO Guides [3], [4].

2. Material

PS-granulate (PS 158 K) was kindly provided by PlasticEurope. The granules were cryogenic ground in a cryomill. Subsequently 285 units with 1 g PS powder each were filled in amber glass bottles. The metal lids have a seal consisting of silicone and polytetrafluorethylene.

2.1. Material characterization

The RM was characterised by recording the infrared spectra by Fourier transform infrared spectroscopy (Fig. 1), determining the glass transition temperature by differential scanning calorimetry (Fig. 2), and by imaging the particle morphology by scanning electron microscopy (Fig. 3).

Fourier transform infrared spectroscopy (FTIR) was performed in transmission mode by a Nicolet Nexus 6700 FTIR spectrometer (Thermo Scientific, USA), whereas the RM BAM-P202 was measured embedded in KBr pills six times. Obtained spectra were identified by characteristic peak positions and additionally compared with the Hummel Polymer Sample Library from Thermo Scientific™ and the results confirm that the material is composed of PS (Fig. 1).

Differential scanning calorimetry (DSC) was carried out three times by a DSC 7020 (Seiko, THASS, Germany; Last calibration date 26th of March 2019) with a constant heating rate of 10 °C/minute. Measurements revealed a glass transition temperature of 104.68 °C (Fig. 2 and Table 1), which is in accordance with PS.

RM particles were analysed with a Zeiss EVO MA 10 scanning electron microscope (SEM). The powders were fixed on adhesive double-coated carbon conductive tabs and gold sputtered. Secondary electron mode (SE) with an acceleration voltage of 10 kV was used to obtain images. SEM examination confirmed that material preparation to achieve near to reality decomposed shapes of BAM-P202 was successful. RM particles exhibit sharp edges, rougher surfaces and are varying in size (Fig. 3).

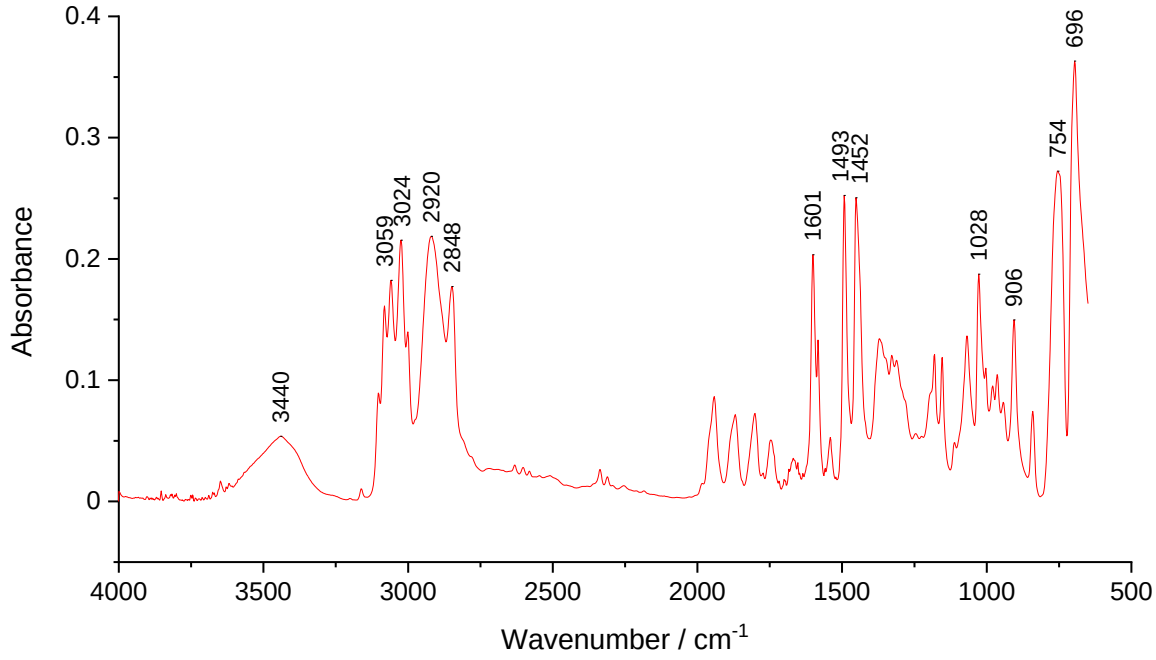

Fig. 1: FTIR spectra of RM BAM-P202. Mean value of six measurements embedded in KBr pills.

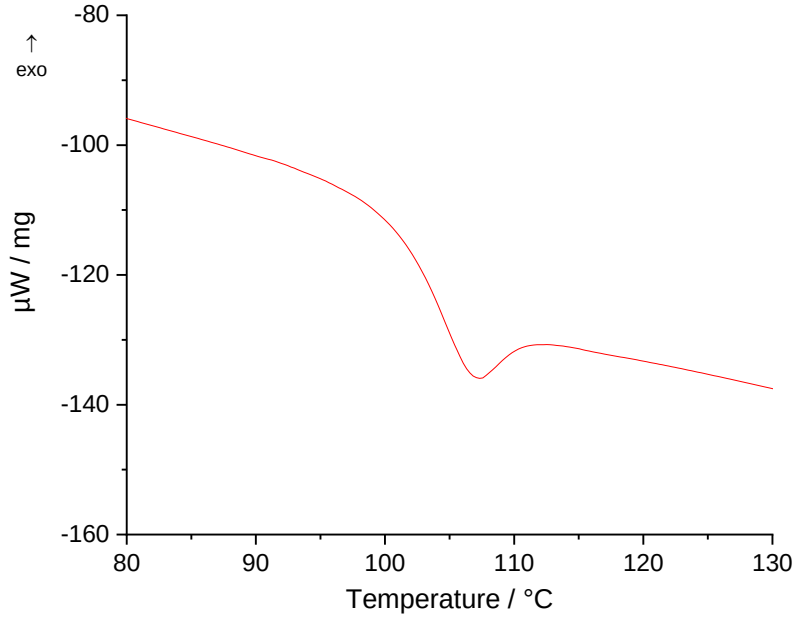

Fig. 2: Section of the DSC measurement showing the glass transition peak of RM BAM-P202. Mean curve of three measurements.

Table 1: Glass transition temperature of BAM-P202 from DSC measurements. Mean value of three measurements.

T_g in °C	**Standard deviation *s*** in °C	**Rel. standard deviation *s*** in %
104.68	0.14	0.14

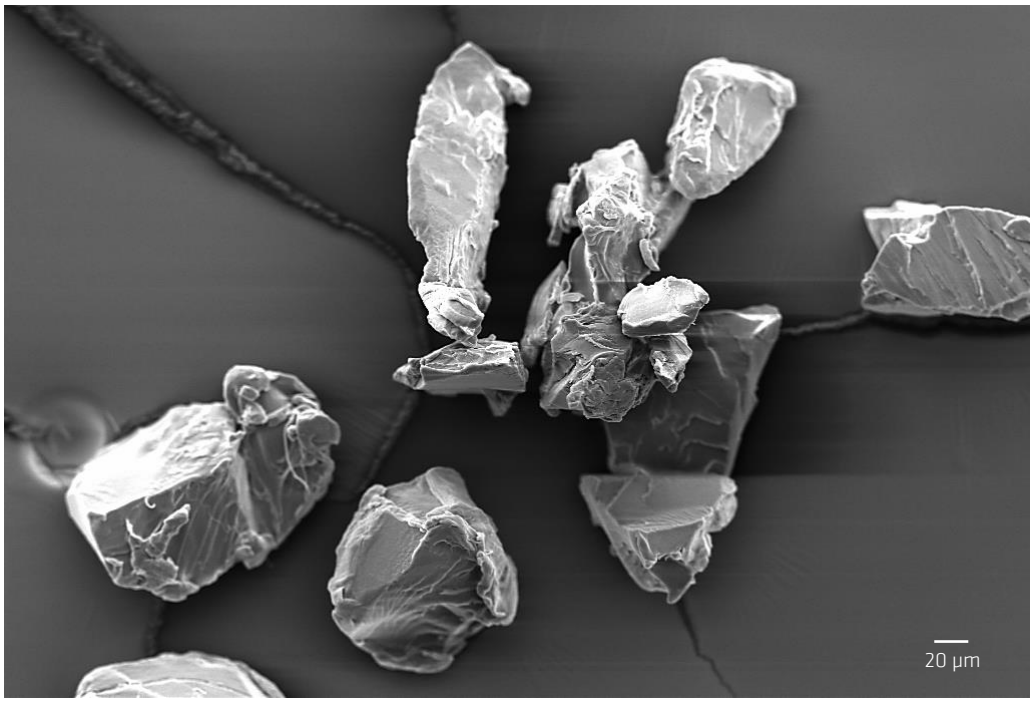

Fig. 3: SEM image showing near to reality decomposed shapes of RM BAM-P202 particles.

2.2. Particle size distribution

The RM BAM-P202 was characterised by determining the particle size distribution by laser diffraction (HELOS/BR + RODOS/L + ASPIROS, Sympatec, Germany, ISO 13320:2009 certified). Measurements were carried out under dry dispersion with 100 mg material, at a pressure of 5.5 bar and with a velocity of ASPIROS sleigh of 100 mm/s. Ten randomly selected bottles were measured three times each. Evaluation was executed applying Fraunhofer approximation.

The results are shown as cumulative and density distribution curves in Figure 4 and are summarized as mean values and corresponding standard deviations in Table 2.

Table 2: Mean values of PSD and respective standard deviations of homogeneity test of RM BAM-P202.

Particle size distribution	**Particle size** in µm	**Standard deviation *s*** in µm	**Relative standard deviation *s*** in %
D10	91.15	4.61	5.06
D50	206.24	13.43	6.51
D90	310.98	13.96	4.49

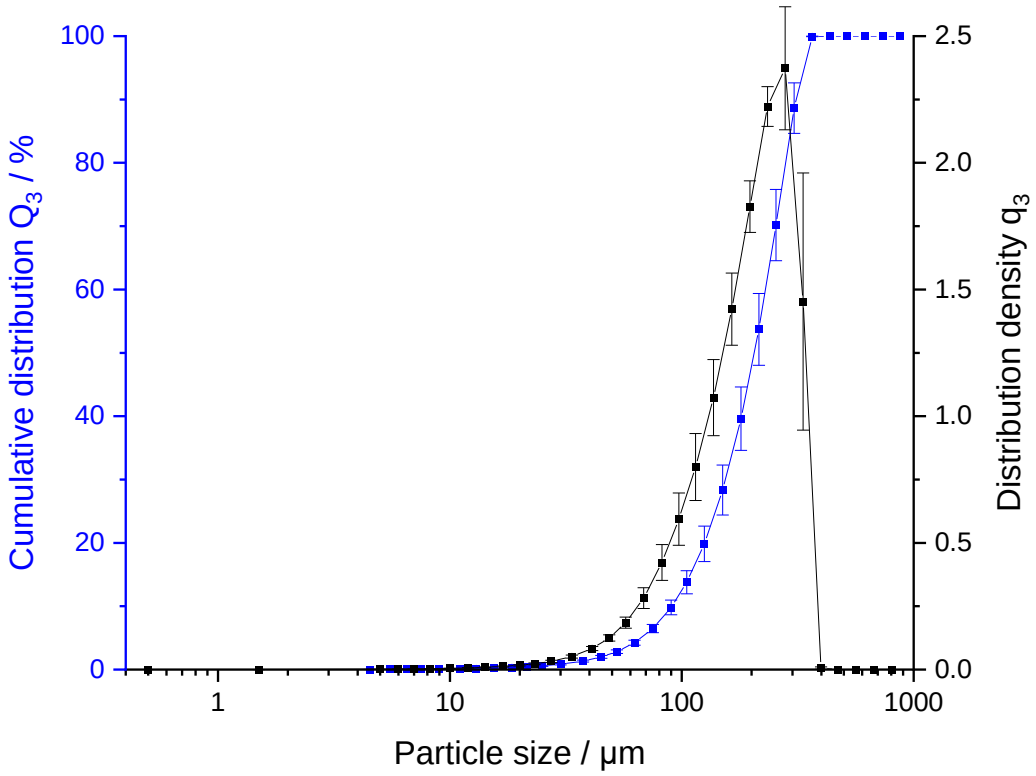

Fig. 4: Particle size distribution with standard deviation of RM BAM-P202. The blue curve represents the cumulative distribution $Q_3(x)$ and shows the volume fraction of particles smaller or larger than x in relation to the total volume. The black curve represents the distribution density $q_3(x)$ (1^{st} derivate of Q_3) and displays the probability of finding a particle with a particle size x in the population. Total number of measurements 30.

3. Homogeneity

For testing the homogeneity, 10 individual units of BAM-P202 were randomly selected for particle size distribution measurements by laser diffraction with dry powder dispersion (HELOS/BR + RODOS/L + ASPIROS, Sympatec, Deutschland, ISO 13320:2009 certified). Three replicate measurements per bottle were carried out under repeatability conditions with approximately 100 mg each to detect the standard deviation between and within the bottles (see Appendix Table 1).

The homogeneity contribution to the total measurement uncertainty was calculated using a 1-way-ANOVA (Table 3 and Appendix Table 3).

Table 3: Analysis of variances calculated for PSD analysis of RM BAM-P202.

PSD	Source of variation	Mean sum of squares (MS)	Test value (F)	Critical F-value 95%
D10	Between Groups	33.505	2.126	2.393
	Within Groups	15.759		
D50	Between Groups	312.046	2.579	2.393
	Within Groups	120.972		
D90	Between Groups	338.202	2.595	2.393
	Within Groups	130.353		

ANOVA results show that for larger particle fractions (D50, D90) there is a slight source of variance. However, this is not relevant for the recommended use of RM BAM-P202 and can be ascribed to the favored near to reality character of the material.

The estimation of inhomogeneity contribution u_{hom} to be included into the total uncertainty budget was calculated from the maximum uncertainty using Equation (1) to Equation (3) according to ISO Guide 35 [3], with $M_{between}$ mean of squared deviations between bottles, M_{within} mean of squared deviations within one bottle, n number of replicate measurements per bottle and N number of bottles selected for homogeneity study. Test results to determine the maximum (in)homogeneity contribution to the total uncertainty are summarized in Table 4.

Standard deviation of repeatability within bottles $s_w = s_r = \sqrt{M_{within}}$ (1)

Standard uncertainty due to between-bottle variation $s_{bb} = \sqrt{\frac{M_{between} - M_{within}}{n}}$ (2)

Standard uncertainty due to within bottle variation $u_{bb} = \sqrt{\frac{M_{within}}{n}} \sqrt[4]{\frac{2}{N(n-1)}}$ (3)

Table 4: Results to determine the maximum inhomogeneity contribution u_{hom} of BAM-P202.

PSD	**D10**	**D50**	**D90**
$\bar{x}_{hom}$ **in µm**	91.15	206.24	310.98
$M_{between}$	33.50	312.05	338.20
M_{within}	15.76	120.97	130.35
s_r **in µm**	3.97	11.00	11.42
s_r **rel. in %**	4.36	5.33	3.67
s_{bb} **in µm**	2.43	7.98	8.32
s_{bb} **rel. in %**	2.67	3.87	2.68
u_{bb} **in µm**	1.29	3.57	3.71
u_{bb} **rel. in %**	1.41	1.73	1.19
$u_{hom}(s_r)$	3.97	11.00	11.42
$u_{hom}(s_r)$ **rel.**	4.36	5.33	3.67

4. Stability

Based on expert knowledge and literature in the field of polymers, it is very unlikely that BAM-P202 composition and particle size will change, provided that the samples are stored and handled properly. Nevertheless, a stability check of the bottled material was performed. Therefore, immediately after bottling selected units were stored at a temperature of 5 °C (the indicated temperature values imply a tolerance of ±3 °C). After a storage period of 0, 1, 3, 6 and 12 months, respectively, three randomly selected bottles, each with 1 g PS per bottle, were tested in terms of particle size distribution. Three measurements (100 mg each) per bottle were carried out (see Appendix Table 2). The results are summarized in Table 5 and presented in Figure 5.

Table 5: Average values of stability test of RM BAM-P202.

Storage period	Particle size distribution	Particle size in µm	Standard deviation s in µm	Relative standard deviation s in %
0 d	**D10**	91.69	3.38	3.69
	D50	204.90	7.25	3.54
	D90	308.85	7.96	2.58
1 m	**D10**	90.62	6.45	7.11
	D50	206.11	17.02	8.26
	D90	311.68	15.58	5.00
3 m	**D10**	86.47	4.80	5.55
	D50	194.79	10.86	5.57
	D90	301.10	9.71	3.22
6 m	**D10**	89.49	3.63	4.06
	D50	199.92	9.82	4.91
	D90	304.02	11.41	3.75
12 m	**D10**	100.44	5.78	5.75
	D50	211.27	11.81	5.59
	D90	312.16	14.22	4.56

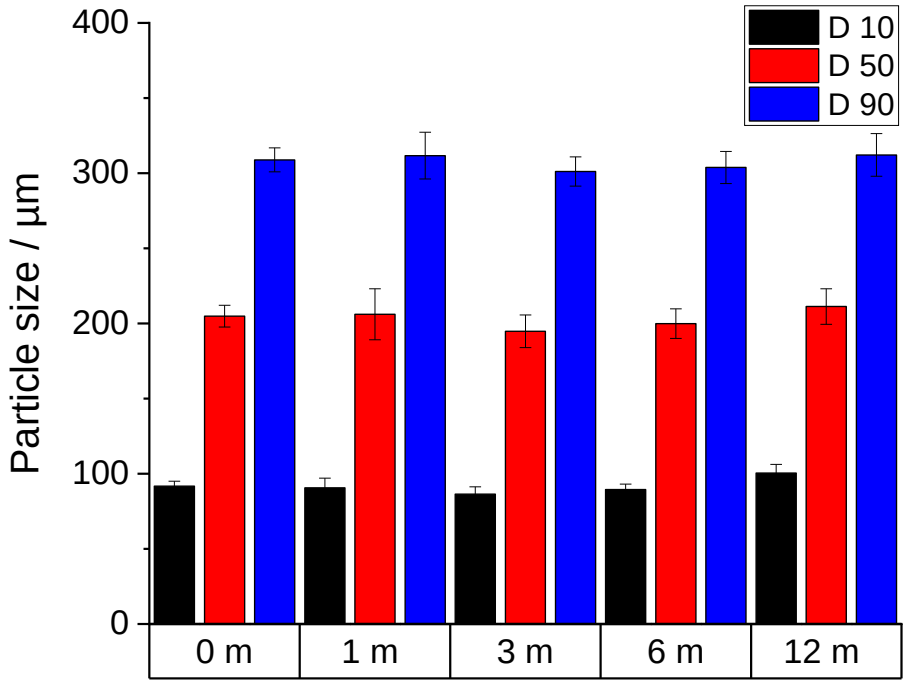

Fig. 5: Comparison of mean values (n = 9) of measured samples in particle size distribution after storage time of 0, 1, 3, 6 and 12 months.

The stability was assessed according to ISO Guide 35 [3], using linear regression (Figure 6) followed by testing for statistically significant slopes different from zero. The t-test statistics for slopes were calculated using Equation (4) and compared with the critical values at the 95 % level of confidence.

t-statistic for slope b significance $$t_b = \frac{|b|}{s(b)} \quad (4)$$

Results of linear regression are presented in Figure 6 and values of *t*-statistics in Table 6. For all particle size distributions, the *t*-statistics are below the critical value and therefore slopes are not significantly different from zero, and thus, no significant instability is detected.

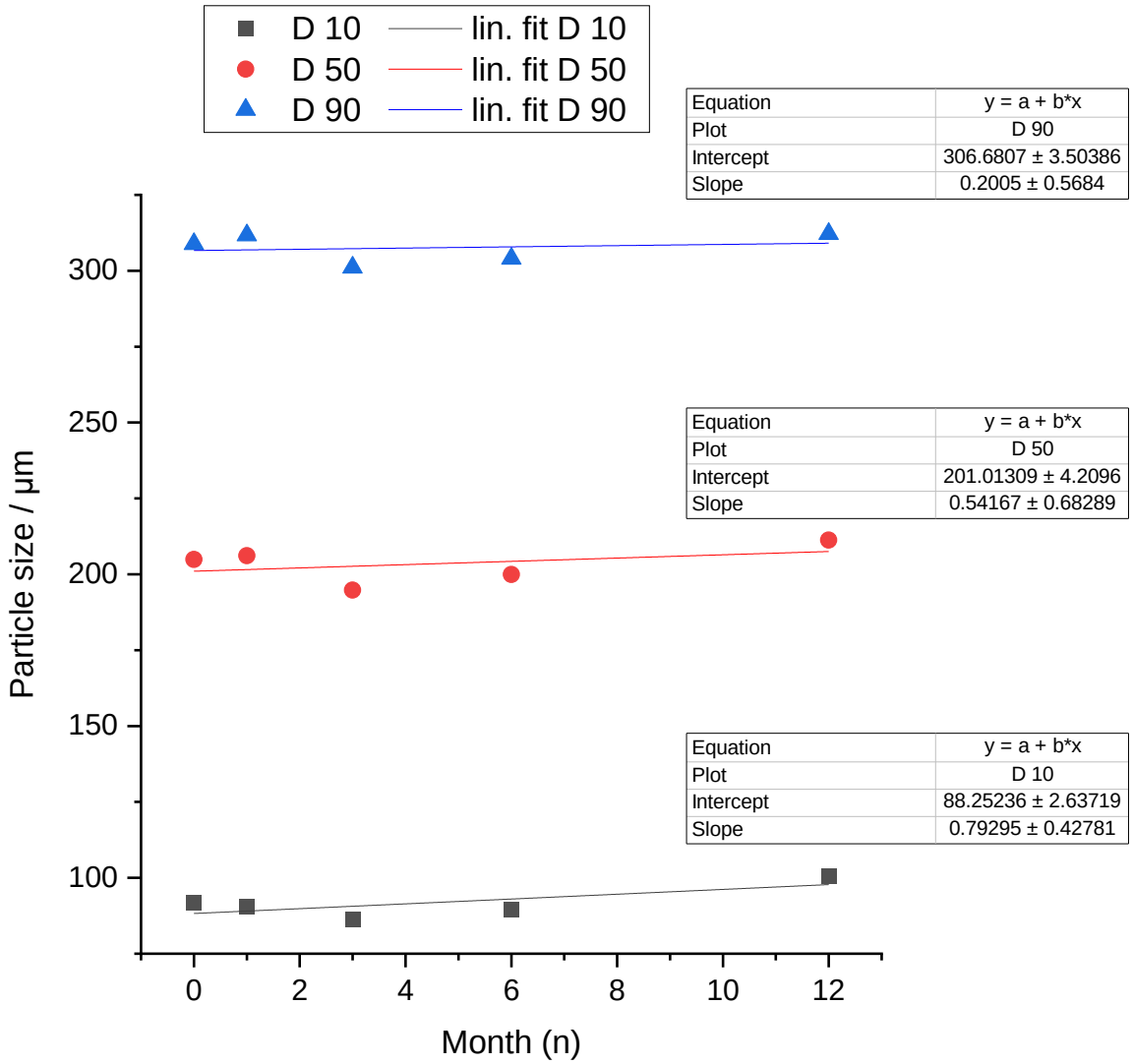

Fig. 6: Stability monitoring of PSD by laser diffraction and linear regression analysis for statistical evaluation of stability of BAM-P202.

Table 6: Testing for significant instability. Values of slope from linear regression of stability data, calculated *t*-statistics and critical value at the 95 % level of confidence.

Particle size distribution	Slope b	*s* (b)	*t*-statistic (b)	Critical value at 95 %
D10	0.79	0.43	1.85	2.15
D50	0.54	0.68	0.79	2.71
D90	0.20	0.57	0.35	2.01

Altogether, results of the statistical evaluation of the stability data indicate that there is no instability and that RM BAM-P202 is stable regarding particle size distribution over a 12 months period at least (see Figure 5 and 6, and Table 5, 6 and 7). However, the contribution of uncertainty of the long term

stability u_{lts} was calculated according to Equation (5), as in ISO Guide 35 [3], with *s(b)* as standard error for estimated slope, t_m time interval between value assignment and initial stability monitoring point, and t_{cert} period of validity issued during that time. Results of uncertainty of the long term stability are summarized in Table 7.

Contribution of standard uncertainty of long term stability $u_{lts} = s(b)(t_m + t_{cert})$ (5)

Table 7: Results of stability data evaluation of BAM-P202 according to ISO Guide 35 [3].

PSD	$\bar{x}$ stab in µm	$s_{x,stab}$ in µm	Intercept	Slope	s (slope)	u_{lts}(x) in µm	Rel. u_{lts}(x) in %
D 10	91.74	5.24	88.25	0.79	0.43	5.13	5.60
D 50	203.40	6.28	201.01	0.54	0.68	8.19	4.02
D 90	307.56	4.85	306.68	0.20	0.57	6.82	2.22

5. Statistical evaluation

The combined uncertainty $u_c(x)$ was calculated according to Equation (6), using the numerical values summarized in Table 8. This equation is a combination of the standard uncertainty due to characterization, the contribution of the variation between the bottles and the long-term stability contribution.

combined uncertainty $u_c^2(x) = u_{\text{char}}^2 + u_{\text{hom}}^2 + u_{\text{lts}}^2$ with $u_{\text{char}}^2 = \frac{s_x^2}{N}$ (6)

Table 8: Values of the uncertainty components for the PSD of RM BAM-P202.

PSD	$\bar{x}$ in µm	u_{char} in µm	u_{hom} in µm	u_{lts} in µm	u_c in µm	U in µm
D 10	91.15	1.46	3.97	5.13	6.65	13.30
D 50	206.24	4.25	11.00	8.19	14.36	28.72
D 90	310.98	4.41	11.42	6.82	14.01	28.03

The final attested values of the particle size distribution with a reasonable number of digits (rounded according to DIN 1333 [5]) and the respective expanded uncertainties U (U=k·u_c with k=2) are summarized in Table 9.

Table 9: Final attested values for PSD of RM BAM-P202.

PSD	Particle size **D** in µm	Twofold standard deviation **2 · s** in µm	Expanded uncertainty U in µm
D10	91	9	13
D50	206	27	29
D90	311	28	28

6. Information on the proper use of the reference material

6.1 Recommended use

The present material is a reference material close to reality for the validation of sampling, sample preparation and detection of microplastics. At the same time, it can be used for the evaluation of effects in the field of ecotoxicology, human toxicology, pollutant transport and agglomeration behaviour related to microplastics.

6.2 Transport, storage and handling

RM BAM-P202 can be shipped at ambient temperature. It should be stored under dark and dry conditions at a temperature of 5 ± 3 °C in its original tightly closed bottle. BAM cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened bottles. In case of further transport, the packaging should be free of plastic to avoid contamination.

6.3 Shelf life

The stability study after storage for 12 months of selected units at temperatures of 5 ± 3 °C did not reveal any statistically significant deterioration of the particle size distribution. However, starting with dispatch of the material from BAM the validity of the data sheet expires after 24 months. Post-certification measurements will be conducted in appropriate periods to keep this information up to date.

6.4 Safety information

The usual laboratory safety precautions must be applied. No hazardous effects are to be expected when the material is used under conditions commonly adopted for the analysis of environmental samples.

6.5 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

(b) assume any liability with respect to, or for damages resulting from, the use of any information, material, apparatus, method or process disclosed in this document.

7. Information on purchase of the reference material

Reference material BAM-P202 is supplied by:

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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

https://www.bam.de
www.webshop.bam.de

Each bottle of RM BAM-P202 will be distributed together with a data sheet containing the mean values, standard deviations and uncertainties of all data sets and information on the analytical methods used.

8. References

[1] Managementhandbuch der BAM MH-3.5.
Bundesanstalt für Materialforschung und –prüfung (BAM), Berlin 2020.

[2] ISO 17034, General requirements for the competence of reference material producers.
International Organization for Standardization, Geneva 2016.

[3] ISO Guide 35, Reference materials - Guidance for characterization and assessment of homogeneity and stability.
International Organization for Standardization, Geneva 2017.

[4] ISO Guide 31, Reference materials - Contents of certificates, labels and accompanying documentation.
International Organization for Standardization, Geneva 2015.

[5] DIN 1333, Zahlenangaben.
DIN, Berlin 1992.

Appendix

Table 1: Results of homogeneity test of 10 randomly selected samples with three replicate measurements (Rep. Meas.) from a single bottle.

Bottle ID	Rep. Meas.	D10 in µm	D10 average per bottle [µm] with standard deviation s	D50 in µm	D50 average per bottle [µm] with standard deviation s	D90 in µm	D90 average per bottle [µm] with standard deviation s
009	1 2 3	79.56 90.65 88.31	86.17 ± 5.85 (6.79 %)	172.46 201.19 196.70	190.11 ± 15.45 (8.13 %)	281.86 302.12 301.65	295.21 ± 11.56 (3.92 %)
013	1 2 3	91.68 86.81 90.27	89.58 ± 2.51 (2.80 %)	207.47 194.84 200.54	200.95 ± 6.33 (3.15 %)	310.93 301.79 302.92	305.21 ± 4.98 (1.63 %)
101	1 2 3	86.86 95.44 89.13	90.48 ± 4.45 (4.92 %)	196.38 219.35 197.06	204.27 ± 13.07 (6.40 %)	305.35 326.34 296.57	309.42 ± 15.30 (4.94 %)
135	1 2 3	92.66 95.03 99.13	95.61 ± 3.28 (3.43 %)	208.85 212.11 230.53	217.16 ± 11.69 (5.38 %)	315.64 314.07 335.29	321.67 ± 11.82 (3.68 %)
173	1 2 3	87.09 90.94 94.20	90.74 ± 3.56 (3.92 %)	190.80 201.77 207.17	199.91 ± 8.34 (4.17 %)	293.73 302.21 305.77	300.57 ± 6.19 (2.06 %)
183	1 2 3	96.77 90.45 91.48	92.90 ± 3.39 (3.65 %)	224.88 205.02 210.01	213.30 ± 10.33 (4.84 %)	329.48 305.66 320.28	318.47 ± 12.01 (3.77 %)
211	1 2 3	88.81 84.04 86.77	86.54 ± 2.39 (2.76 %)	202.17 189.50 192.59	194.75 ± 6.60 (3.39 %)	317.57 296.46 295.08	303.04 ± 12.61 (4.16 %)
218	1 2 3	92.85 97.26 85.84	91.98 ± 5.75 (6.26 %)	202.39 222.80 189.26	204.81 ± 16.90 (8.25 %)	300.91 328.29 293.04	307.42 ± 18.50 (6.02 %)
253	1 2 3	93.53 100.85 94.89	96.42 ± 3.89 (4.04 %)	215.12 227.16 210.82	217.70 ± 8.47 (3.89 %)	322.75 329.42 312.71	321.62 ± 8.41 (2.62 %)
281	1 2 3	87.92 91.66 93.64	91.08 ± 2.90 (3.19 %)	211.48 223.29 223.20	219.32 ± 6.79 (3.10 %)	322.62 330.48 328.43	327.18 ± 4.08 (1.25 %)

Table 2: Results of the stability test after storage times of 0, 1, 3, 6 and 12 months with three replicate measurements (Rep. Meas.) from a single bottle.

Storage time	Bottle ID	Rep. Meas.	D10 in µm	D10 average per bottle [µm] with standard deviation s	D50 in µm	D50 average per bottle [µm] with standard deviation s	D90 in µm	D90 average per bottle [µm] with standard deviation s
0 d	072	1	87.30	89.34 ± 3.19 (3.57 %)	198.08	200.63 ± 6.87 (3.42 %)	305.44	305.97 ± 6.33 (2.07 %)
		2	87.72		195.41		299.92	
		3	93.02		208.41		312.54	
	090	1	98.04	94.47 ± 3.19 (3.37 %)	218.41	211.71 ± 6.33 (2.99 %)	323.53	317.29 ± 6.34 (2.00 %)
		2	91.89		205.83		310.85	
		3	93.49		210.88		317.48	
	103	1	93.16	91.26 ± 2.28 (2.50 %)	205.19	202.35 ± 4.06 (2.01 %)	304.96	303.28 ± 2.70 (0.89 %)
		2	91.88		204.16		304.72	
		3	88.73		197.70		300.17	
1 m	144	1	86.96	93.18 ± 5.88 (6.31 %)	195.42	214.83 ± 17.15 (7.98 %)	307.91	323.31 ± 13.43 (4.16 %)
		2	98.64		227.96		332.62	
		3	93.93		221.10		329.40	
	200	1	85.37	92.09 ± 7.33 (7.96 %)	193.43	207.78 ± 17.93 (8.63 %)	301.36	311.68 ± 16.55 (5.31 %)
		2	90.99		202.02		302.91	
		3	99.91		227.89		330.78	
	256	1	79.25	86.59 ± 6.36 (7.35 %)	177.01	195.72 ± 16.23 (8.29 %)	287.92	300.06 ± 10.52 (3.51 %)
		2	90.03		204.23		306.44	
		3	90.49		205.92		305.82	
3 m	068	1	86.75	85.91 ± 0.80 (0.94 %)	192.67	190.59 ± 2.01 (1.05 %)	298.61	297.38 ± 1.48 (0.50 %)
		2	85.15		188.65		295.74	
		3	85.82		190.44		297.79	
	111	1	91.42	92.03 ± 2.32 (2.52 %)	203.21	207.65 ± 8.93 (4.30 %)	302.64	310.79 ± 12.52 (4.03 %)
		2	94.59		217.94		325.21	
		3	90.07		201.81		304.51	
	261	1	79.90	81.48 ± 1.38 (1.70 %)	185.93	186.13 ± 0.68 (0.37 %)	297.18	295.14 ± 1.77 (0.60 %)
		2	82.09		185.58		294.18	
		3	82.45		186.90		294.06	
6 m	018	1	85.98	87.50 ± 5.84 (6.67 %)	189.64	192.23 ± 12.43 (6.46 %)	294.00	295.26 ± 9.85 (3.34 %)
		2	93.94		205.74		305.68	
		3	82.57		181.30		286.10	
	070	1	86.76	89.48 ± 2.48 (2.77 %)	195.15	200.51 ± 5.02 (2.50 %)	302.55	303.06 ± 1.15 (0.38 %)
		2	91.64		205.10		304.38	
		3	90.03		201.29		302,24	
	180	1	90.63	91.48 ± 0.82 (0.90 %)	199.62	207.01 ± 6.46 (3.12 %)	300.69	313.15 ± 10.82 (3.45 %)
		2	92.28		209.80		318.62	
		3	91.54		211.60		320.14	

Table 2: continued.

Storage time	Bottle ID	Rep. Meas.	D10 in µm	D10 average per bottle [µm] with standard deviation s	D50 in µm	D50 average per bottle [µm] with standard deviation s	D90 in µm	D90 average per bottle [µm] with standard deviation s
12 m	102	1	93.64	100.45 ± 5.93 (5.90 %)	196.58	211.94 ± 13.30 (6.28 %)	294.82	313.65 ± 16.31 (5.20 %)
		2	103.27		219.76		323.55	
		3	104.45		219.48		322.57	
	182	1	106.37	104.78 ± 1.54 (1.47 %)	217.88	219.21 ± 4.72 (2.15 %)	317.38	320.72 ± 7.16 (2.23 %)
		2	103.30		215.30		315.84	
		3	104.69		224.46		328.95	
	258	1	93.78	96.08 ± 6.25 (6.51 %)	196.13	202.65 ± 12.33 (6.08 %)	290.85	302.12 ± 15.06 (4.99)
		2	91.30		194.96		296.28	
		3	103.15		216.87		319.23	

Table 3: ANOVA results of homogeneity testing of 10 randomly selected units and their replicate measurements within the units.

	Source of variation	Square sum (SS)	Degree of freedom (df)	Mean square sum (MS)	Test value (F)	P-Value	Critical F-value
D10	Between groups	301.544	9	33.505	2.126	0.077	2.393
	Within groups	315.173	20	15.759			
	Total	616.717	29				
D50	Between groups	2808.411	9	312.046	2.579	0.037	2.393
	Within groups	2419.446	20	120.972			
	Total	5227.857	29				
D90	Between groups	3043.815	9	338.202	2.595	0.036	2.393
	Within groups	2607.061	20	130.353			
	Total	5650.876	29				