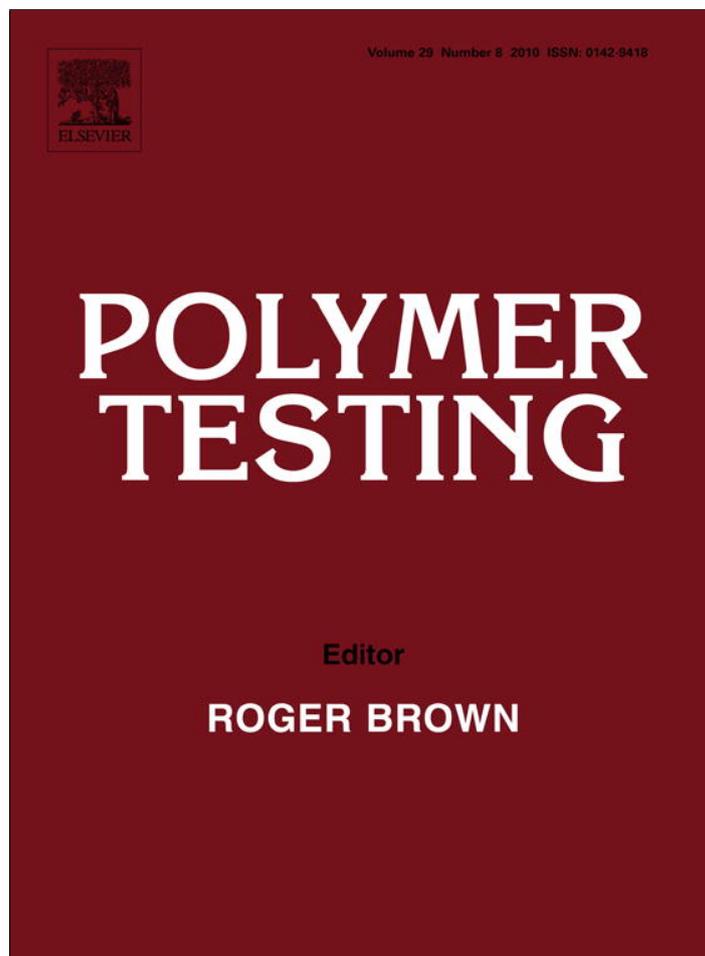


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

## Polymer Testing

journal homepage: [www.elsevier.com/locate/polytest](http://www.elsevier.com/locate/polytest)POLYMER  
TESTING

ROGER BROWN

## Test Method

## Determination of molecular weights by size exclusion chromatography (SEC) – Results of round robin tests

A. Ritter<sup>a,\*</sup>, M. Schmid<sup>b</sup>, S. Affolter<sup>c</sup><sup>a</sup> Swiss Federal Institute for Materials Testing and Research (Empa), CH-9014 St. Gallen, Switzerland<sup>b</sup> Inspire, irpd, Institute for rapid product development (Inspire), CH-9014 St. Gallen, Switzerland<sup>c</sup> University of Applied Science Buchs (NTB), CH-9470 Buchs SG, Switzerland

## ARTICLE INFO

## Article history:

Received 4 June 2010

Accepted 4 August 2010

## Keywords:

Size exclusion chromatography (SEC)

Round robin test

Interlaboratory test comparison

Validation

Repeatability

Reproducibility

## ABSTRACT

Results obtained from two round robin test programmes are summarized and presented. Size exclusion chromatography (SEC) in both organic and aqueous phases was used to determine the average molar masses of six different polymers. The data were collected by Empa and evaluated using a robust statistical method concerning repeatability and reproducibility standard deviations. Refractive index (RI) and light scattering (LS) were utilized for signal detection. The results of the measurements indicate that the determination of molecular weights by LS is associated with a high degree of uncertainty compared to data measured by RI. For the SEC measurements in the aqueous phase, repeatability is good, unlike reproducibility. Comparisons of molar mass data determined by aqueous SEC in different laboratories are regarded as at least doubtful.

© 2010 Elsevier Ltd. All rights reserved.

## 1. Introduction

A key problem for almost any analytical method is its validation [1]. One way of performing a validation is to carry out, or participate in, round robin tests or inter-laboratory comparisons [2]. Data generated from different laboratories on the same sample provide a large amount of valuable information regarding the standard deviations of repeatability and reproducibility for the particular analytical method. In order to eliminate bias and to obtain high independence, round robin tests are often organized by neutral federal institutions such as Empa (Swiss Federal Institute for Materials Testing and Research), BAM (German Federal Institute for Materials Research) or DACH (German Accreditation Body Chemistry). Standards organizations such as ISO and A STM also organize round robin tests but have not done so on this topic of chromatography.

The determination of weight average molar mass ( $M_w$ ) and number average molar mass ( $M_n$ ) of synthetic and

natural polymers by size exclusion chromatography (SEC) is well established and widely used in polymer science [3]. Information about the molecular weight of a polymer and its distribution is of great importance because this affects material processability and almost all material properties [4–8].

SEC measurements can be divided, in a similar way to other chromatographic methods, into two parts: separation and detection. Separation is carried out by means of a porous separation matrix with different sized cavities. Completely solvated polymer chains diffuse according to their hydrodynamic volume into the cavities of the separation matrix and are sorted in terms of their size. For more detailed information about the SEC method, see references [9–13]. Various detection techniques are common. Concentration detectors are utilized in most cases (differential refractometer or UV detector). The evaluation of unknown polymers takes place in comparison with a calibration curve established with narrow distributed polymer standards (“relative method”). Light scattering (LS), as another option for detection, permits the determination of absolute  $M_w$  values (“absolute method”) if the refractive

\* Corresponding author. Tel.: +41 71 2747782; fax: +41 71 2747788.

E-mail address: [axel.ritter@empa.ch](mailto:axel.ritter@empa.ch) (A. Ritter).

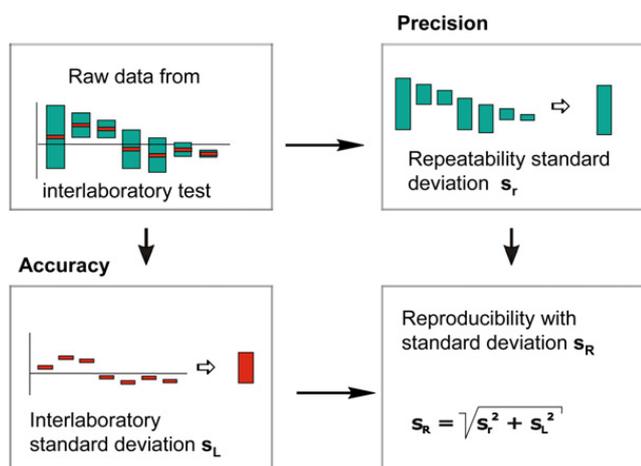


Fig. 1. Relationship between statistical parameters.

index ( $dn/dc$ ), the Rayleigh ratio ( $R_\theta$ ) and the concentration of the polymer in solution are known exactly [14].

Some SEC round robin tests in organic phase for the determination of uncertainty of measurements have already been described in the literature [15,16]. Some classical polymers such as polystyrene (PS) and polymethylmetacrylate (PMMA), epoxies [17] and polyolefins at high temperature [18] have also been examined in SEC round robins. The results described were somewhat heterogeneous and inconsistent, being influenced by widely differing boundary conditions and non-precisely defined measurement parameters.

Recently, the German Federal Office for Material Testing (BAM) in Berlin has tried to subject SEC reference materials to appropriate interlaboratory tests [19,20]. Detection of the molar masses must take place using an absolute detection method (e.g. light scattering) and not a method relative to calibration standards. Such round robin tests require, in addition to the specific selection of suitable samples, a stringent definition of all measurement parameters and almost identical equipment for all participants. SEC reference polymers analysed in this way have meanwhile become partially commercially available and are likely to

play a prominent role in future improvement of the SEC method. No corresponding reference substances of the same polymer class were used for the equipment calibration for the EMPA round robin tests, as this has been unusual in daily practice in almost all laboratories to date.

In the case of organic solvent, the present work compares 'refractive index' RI and 'light scattering' LS data obtained from four polymers investigated in two round robin test programmes where the participants use both detection options for every sample. This should give an indication as to which of the two detection methods is more precise with respect to repeatability and reproducibility.

In addition, two polymer samples were investigated in the aqueous phase and evaluated solely by RI detection. The presented data are, to our knowledge, the first round robin test data for aqueous SEC. The normative requirements for determination of average molecular weights are described in detail in ISO, DIN and ASTM standards [21–24].

## 2. Materials and methods

### 2.1. Evaluation and statistics

The principles and intentions of the Empa round robin tests are described elsewhere. The meaning of terms such as repeatability and reproducibility and their related standard deviations is discussed and many statistic expressions are explained [25]. Fig. 1 summarizes the most important statistical terms used in the following.

Robust statistics were used for the evaluation of the round robin test data. Robust statistics are characterized by the fact that no outlier tests are applied. All the data of the round robin test participants are considered for the evaluation. During an iterative process, the data records are weighted depending on how far away they are from the median value ( $x_{\text{median}}$ ). The wider the gap between the data and the calculated median value, the weaker the influence of this data on the expression of the round robin test becomes. Thus, the "good data", close to  $x_{\text{median}}$  has more of an impact on the result. The median value itself, called Hämpel-Schätzer [26] is also re-calculated after every iterative

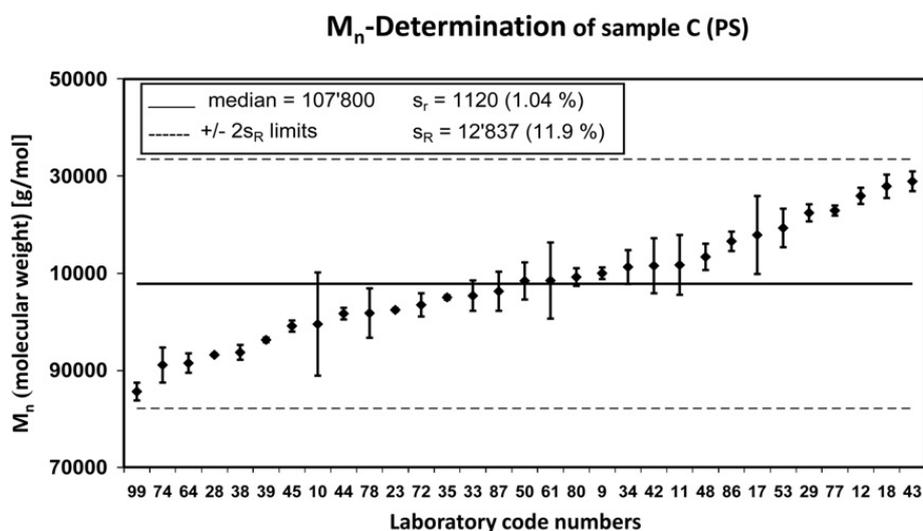


Fig. 2. Plot of interlaboratory test for determination of number average molecular weights ( $M_n$ ) of sample C (PS 143 E, BASF) in THF.

**Table 1**  
Description of materials.

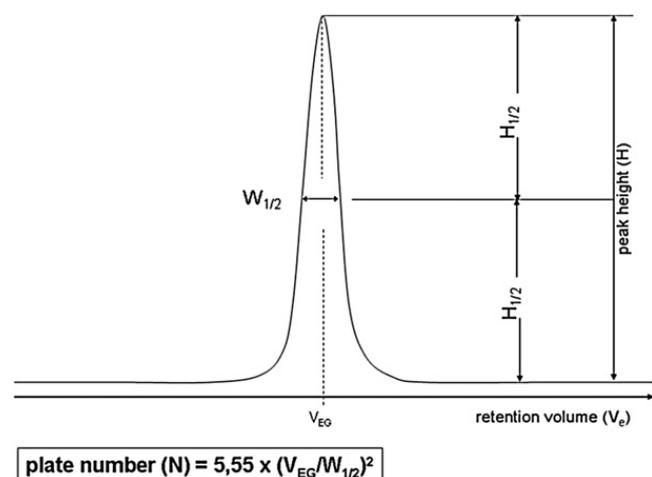
Sample	Material	Producer Type	$M_w$ Region	Description
A	Polystyrene (PS)	BASF 165 H bright	300 kD $\pm$ 50 kD	Bright granules
B	Polymethylmethacrylate (PMMA)	Rhöm Acryrex CM207 nature	100 kD $\pm$ 30 kD	Bright granules
C	Polystyrene (PS)	BASF 143 E bright	250 kD $\pm$ 50 kD	Bright granules
D	Poly(R-3-hydroxy-octanoate) (PHO)	Empa research product	250 kD $\pm$ 50 kD	Bright granules

step. The exact mathematics, which is fundamental to all statistical calculations of our round robin tests, is described elsewhere [27].

The repeatability standard deviation  $s_r$  is defined as the standard deviation of the test results obtained under repeatability conditions and calculated by robust statistics. In contrast, the reproducibility standard deviation  $s_R$  is defined as the standard deviation of the test results obtained under reproducibility conditions and calculated by robust statistics.

The resulting combined uncertainty of measurement ( $u_c = 3 s_r$ ) for a single laboratory, a most important statistical term estimated from the statistical interlaboratory values, is thus more affected by matrix effects and less by sampling and measuring methods [25]. The uncertainty of the result of a measurement generally consists of several components which may be grouped into those evaluated by statistical methods and those evaluated by other means. The guidelines for evaluating and expressing the uncertainty of measurement results are described in detail in a NIST technical note [28].

There are two important molecular weight parameters used in polymer characterization. The number average molecular weight is one way of determining the molecular weight of a polymer. Polymer molecules arrive in different sizes (chain lengths, for linear polymers), so the average molecular weight will depend on the method of averaging. The number average molecular weight is the ordinary arithmetic average of the molecular weights of the individual macromolecules. It is determined by measuring the molecular weight of  $n$  polymer molecules, summing the weights and dividing by  $n$ . The second option for characterization of polymers is the weight average molecular weight described by a more mathematical expression [29].



**Fig. 3.** Chromatogram of BHT for determination of the theoretical number of plates ( $N$ ) according to chromatography theory.

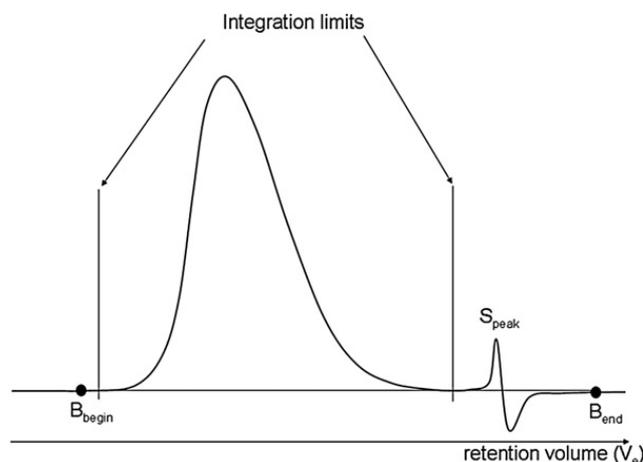
Fig. 2 shows a typical distribution plot for one experiment of SEC analysis with all representative data of the round robin test. The code number of the participating laboratory with the corresponding result is plotted together with the median and different kinds of standard deviations.

## 2.2. SEC in organic phase (THF)

The set of raw data of these round robin tests evaluated by Empa are presented in the following. Four different polymers in organic phase were used in the round robin test (see Table 1 for description of the materials).

Three of them were common commercial polymers (sample A and C (polystyrene, PS) and sample B (polymethylmethacrylate, PMMA)). Sample D was poly(R-3-hydroxyoctanoate, PHO). PHO is an isotactic aliphatic polyester produced in a biotechnological process by fermentation of octanoic acid with *Pseudomonas putida* [30,31].

As it is known that SEC measurements are influenced by many different parameters, mandatory settings for concentration, flow rate, temperature and injection volume were prescribed for all participants. Furthermore, the influence of different calibration standards used for the refractive index evaluation was excluded by supplying them: Each laboratory received an identical set of PS and PMMA standards which had to be used to generate the calibration curve for the related polymer. PHO was evaluated using the PS calibration. An internal standard (2,6-di-tert-butyl-hydroxytoluene (BHT)) had to be used for control of flow rate and the determination of the theoretical plate number of the columns. The selected column combination should have at least a plate number of  $>20,000$  and should cover a range of



**Fig. 4.** Guidelines for integration limits.

**Table 2**

Technical equipment of participating laboratories.

Equipment-Type	Hitachi, Waters, Viscotek, Gynothek, Knauer, Kontron, Agilent, Shimadzu
Columns	Analytical columns 300 mm L × 7.8 or 8 mm ID, provider PSS, Polymer Labs, Macherey Nagel, Waters
Detectors	RI-detector of Waters, Knauer, Bischoff, Shodex, LS-detector of Minid. Wyatt, Viskotec, PSS

**Table 3**

Samples for SEC in Aqueous Phase.

Sample	Material	$M_w$ -range [kD]	Description
E	Pullulan (Pul)	200,000 ± 50,000	White powder
F	Poly(ethylenoxide) (PEO)	200,000 ± 50,000	White powder

**Table 4**

Technical equipment of participating laboratories for SEC in aqueous phase.

Equipment-Type	Waters, Viscotek, Agilent, Shimadzu, Dionex, Wyatt, Erc, Jasco, Perkin Elmer
Columns	Analytical columns 300 mm L × 7.8 or 8 mm ID, provider PSS, Waters, Varian
Detectors	RI-detector of Waters, Merck, Erc, Shodex

separation from 0.2 kD to 1,500 kD. For additional characterization of the performance of the columns, the plate number and specific resolution ( $R_{sp}$ ) had also to be determined as described in Fig. 3 and in reference [32].

In addition, all participants were recommended to use a 3rd order function to fit their calibration curve, and a recommendation for the setting of base line and integration limits for the calculation of the molar masses ( $M_w$ ,  $M_n$ ) was also given. The sample concentration should be around 3.0 mg/L after more than 24 h solution time. All inspection data of equipment performance were checked by Empa in order to ensure that the SEC systems used are at a certain level of capability. Furthermore, the test guideline described the methods to fulfill the various recommendations, such as. baseline settings and integration limits for signal evaluation (see Fig. 4).

To indicate the different measuring systems, Table 2 shows a list of the equipment elements, such as GPC-system, columns and detectors used in the round robin test.

### 2.3. SEC in aqueous phase

As in the case of trials in organic phase, the guidelines, measuring parameters and requirements for equipment performance were pre-defined for the participants for the aqueous measurements. A polysaccharide (Pullulan) and a poly(ethylene oxide) (PEO) were used as test material (Table 3).

Pullulan (CAS 9057-02-7, provider Fluka) is a linear edible polysaccharide polymer consisting of maltotriose units, also known as  $\alpha$ -1,4-; $\alpha$ -1,6-glucan. Three glucose units in maltotriose are connected by an  $\alpha$ -1,4 glycosidic bond, whereas consecutive maltotriose units are connected to each other by an  $\alpha$ -1,6 glycosidic bond. Pullulan is produced biotechnologically from starch by the fungus *Aureobasidium pullulans*. It is also known as a food additive with E number E1204.

All participants also received an identical set of suitable polymer standards of pullulan and PEO provided for these measurements by the PSS company (Mainz, Germany) in order to calibrate the SEC for refractive index measurements, and to exclude the (negative) influence of different calibration standards on the final results. An internal standard (ethyleneglycol, EC) was recommended for flow control and determination of the theoretical number of plates. The measurement parameters such as eluent, concentrations, temperature and injection volume were exactly specified. The selected column combination was to have at least a theoretical plate number > 10,000 and was to exhibit a separation range from 0.2 kD to 1500 kD. The specific resolution of the column combination ( $R_{sp}$ ) was to be determined with selected signals.

A 3rd order function was to be used to fit the calibration curves, and a recommendation was also given for the setting of baseline and integration limits. All participants had to use an RI detector and perform 3-fold samples with

**Table 5**Number average molecular weight ( $M_n$ ) results with conventional calibration.

	Unit	number average molecular weight ( $M_n$ )			
		Sample A PS 165 H	Sample B PMMA	Sample C PS 143 E	Sample D PHO
Median Value (Hämpel)	kD	134.9	47.0	107.8	133.1
Repeatability standard deviation ( $s_r$ )	kD	1.15	0.36	1.12	1.64
$s_r$ relative	[%]	0.85	0.80	1.04	1.24
Reproducibility standard deviation ( $s_R$ )	kD	17.5	4.8	12.8	15.9
$s_R$ relative	[%]	13.0	10.2	11.9	12.0
quality of a determination ( $s_R/s_r$ )		15.2	10.0	11.5	9.7
Number of participants		21	20	32	32

**Table 6**  
Weight average molecular weight ( $M_w$ ) results with conventional calibration.

	Unit	Molecular weight average weight ( $M_w$ )			
		Sample A PS 165 H	Sample B PMMA	Sample C PS 143 E	Sample D PHO
Median Value (Hämpel)	kD	322.8	85.7	245.8	224.0
Repeatability standard deviation ( $s_r$ )	kD	1.08	0.5	0.74	1.26
$s_r$ relative	[%]	0.33	0.6	0.30	0.56
Reproducibility standard deviation ( $s_R$ )	kD	17.0	4.72	15.5	17.8
$s_R$ relative	[%]	5.3	5.5	6.3	7.9
quality of a determination ( $s_R/s_r$ )		15.7	9.4	20.9	14.1
Number of participants		21	20	32	32

double injection. The mean value of the double injection had to be reported. The technical equipment of the participating laboratories is shown in the Table 4; and it is clear that many different brands were used.

### 3. Results and discussion

#### 3.1. SEC in organic phase (THF)

Due to the fact that the interlaboratory test participants are members of diverse laboratories in industry and research, the SEC equipment used, the column combinations and the detectors differ widely, as shown in Table 2. However, all combinations fulfill the default requirements regarding theoretical plate number ( $>20,000$ ) and specific resolution ( $R_{sp} > 2$ ). The molar mass data of all laboratories were compared with their theoretical plate number and their specific resolution (data not presented here). There does not appear to be a significant dependence (trend) of median values and repeatability or reproducibility on the equipment parameters. A dependency of measured data on equipment performance could not be found in the given range.

##### 3.1.1. Test results using conventional calibration with reference polymers

Tables 5–7 combine the statistical results for  $M_n$  (Table 5) and  $M_w$  (Table 6) of the four samples and compare them with data from standards (Table 7) [23,24].

In the case of conventional calibration, all four samples show repeatability standard deviations ( $s_r$ , relative) between 0.3% and 1.2% for  $M_n$  and  $M_w$ . They are lower than comparable data of standards (about 1%–3%). On the other

hand, the relative reproducibility standard deviations  $s_R$  with values of approximately 6%–13% produced similar results to the standards mentioned.

The relative  $s_r$  and  $s_R$  values of the number average molecular weights ( $M_n$ ) were about two times higher than the corresponding values of the weight average molecular weights ( $M_w$ ). The reasons for this are not clear and still under discussion.

The quality of determination of this round robin test (relationship:  $s_R/s_r$ ) amounted to 10 and 20 (see Table 5 and 6) and was insufficient, due to the high values of the reproducibility standard deviations. The ratio  $s_R/s_r$  is a measure of the quality of a determination and should be in a range of approximately 2–3 for good interlaboratory tests such as analysis of potable water [33].

In this evaluation, the combined uncertainty was calculated between 15 and 30% for the weight average ( $M_w$ ), and between 17 and 41% for the number average ( $M_n$ ). These can be regarded as rather high values.

##### 3.1.2. Results with the light scattering detector (LS)

15 participants took part in the absolute calculation with the light scattering detector. All kinds of available commercial systems were used, Right-Angle Light Scattering (RALS), Low-angle Laser Light Scattering Detector (LALS) and the Multi-angle Laser Light Scattering Detector (MALLS). Table 8 shows the results by light scattering and compares them with conventional calibration.

The calculations of  $M_w$  using light scattering detectors show similar results to conventional calibration for all samples regarding  $s_r$  ( $s_r$ , relative: 0.3%–0.6%). However, the relative reproducibility standard deviations ( $s_R$ , relative) of polystyrene and PHO samples were twice as high as the

**Table 7**  
Comparison of the standard deviations ( $s_r$ ,  $s_R$ ) of the Empa round robin test with the data of corresponding standards.

Norm/Round robin	Material	Molecular weight average weight ( $M_w$ )			Number average molecular weight ( $M_n$ )		
		$s_r$ , rel. %	$s_R$ , rel. %	$u_c^a$	$s_r$ , rel. %	$s_R$ , rel. %	$u_c^a$
DIN 55672-1	Not defined	2	approx. 10	30%	3	approx. 11	33%
ISO 16014-3	PS (Mw approx. 37 kD)	1.3	8.1	24.3%	3.1	8.0	24%
ISO 16014-3	PS (Mw approx. 226 kD)	2.1	6.1	18.3%	3.1	5.6	16.8%
ISO 16014-3	PS (Mw approx. 157 kD)	2.4	4.9	14.7%	2.8	6.9	20.7%
sample A	PS (Mw approx. 323 kD)	0.33	5.3	15.9%	0.9	13.0	39.0%
sample C	PS (Mw approx. 245 kD)	0.3	6.3	18.9%	1.0	11.9	35.7%
ISO 16014-3	PMMA (Mw approx. 834 kD)	1.4	8.4	25.2%	2.4	5.7	17.1%
sample B	PMMA (Mw approx. 86 kD)	0.6	5.5	16.5%	0.8	10.2	30.6%
sample D	PHO (Mw approx. 224 kD)	0.6	7.9	23.7%	1.2	12.0	36%
Range		0.5–2%	5–10%	15–30%	5–10%	5–15%	17–41%

<sup>a</sup>  $u_c$ : = combined uncertainty of measurement ( $3 \times s_R$ ). The combined uncertainty of measurement ( $u_c$ ) in a laboratory is greatly affected by standard deviation of reproducibility ( $s_R$ ). Within a 99.7% confidence interval the combined uncertainty of measurement ( $i$ ) is the threefold reproducibility ( $s_R$ ).

**Table 8**

Comparison of weight average molecular weight ( $M_w$ ) results obtained by conventional calibration respectively without calibration using light scattering detectors.

	Unit	Molecular weight average weight ( $M_w$ )							
		Sample A PS 165 H		Sample B PMMA		Sample C PS 143 E		Sample D PHO	
		CC <sup>a</sup>	LS <sup>a</sup>	CC <sup>a</sup>	LS <sup>a</sup>	CC <sup>a</sup>	LS <sup>a</sup>	CC <sup>a</sup>	LS <sup>a</sup>
Median Value (Hämpel)	kD	322.8	345.8	85.7	87.8	245.8	282.2	224.0	210.7
Repeatability standard deviation ( $s_r$ )	kD	1.08	0.91	0.50	0.48	0.74	1.83	1.26	0.92
$s_r$ relative	[%]	0.33	0.26	0.60	0.54	0.30	0.65	0.56	0.44
Reproducibility standard deviation ( $s_R$ )	kD	17.0	36.3	4.72	4.73	15.5	39.9	17.8	30.5
$s_R$ relative	[%]	5.3	10.5	5.5	5.4	6.3	14.1	7.9	14.4
quality of a determination ( $s_R/s_r$ )		15.7	39.9	9.4	10	20.9	21.8	14.1	33.1
combined uncertainty of measurement ( $u_c$ )		15.9%	31.5%	16.5%	16.2%	18.9%	42.3%	23.7%	43.2%
Number of participants			8	20	8	32	7	32	7

<sup>a</sup> CC = conventional calibration; LS = light scattering.

conventional calibration. Only the PMMA sample provides nearly the same statistical values  $s_r$  and  $s_R$  for light scattering values, but light scattering is normally not suitable for low molecular weights. The results are, therefore, not trustworthy. The combined uncertainty of measurement ( $u_c$ ) was between 16 and 43% for light scattering and between 16 and 24% for conventional calculation. The values are very high and show that the determination of molecular weights is not very precise.

Furthermore, detailed study of the data shows that there are significant deviations of molecular weight between the different detection systems. SEC systems with RALS/LALS detection provide higher results for molecular weight average ( $M_w$ ) than systems with MALS detectors. However, it should be mentioned that only eight data sets could be used for calculation of the results and the exact  $dn/dc$  values (differential index of refraction) of the samples were not available. The trend was significant, but proof was impossible due to an insufficient number of data sets.

The  $M_w$  calculation with light scattering provides a quality of determination (relation:  $s_R/s_r$ ) of 10–30 and was unacceptable for a usual round robin test measurement.

### 3.2. SEC in aqueous phase

With the exception of the interlaboratory test mentioned in DIN 55672-3[34], there is no literature concerning statistical data relevant to SEC in aqueous solution for the comparison of standard deviations of repeatability and reproducibility. The standard interlaboratory test mentioned only describes data concerning relatively low molecular weight samples of synthetic polymers (aqueous PU, acrylate dispersions and copolymers).

This interlaboratory test with pullulan (polysaccharide, sample E) and poly(ethylenoxide) (PEO, sample F) is the first experiment to compare higher molecular weight and water soluble polymers with relevant statistical data. The test was performed with 11 participants.

The equipment used by participants concerning SEC system, column combination and detectors was again very varied as in the round robin test using organic solvent THF (see Table 4). However, all the systems fulfilled the defaults

regarding the theoretical number of plates (>10,000) and specific resolution ( $R_{sp} > 1.5$ ).

No significant dependence of the interlaboratory test data on theoretical number of plates and specific resolution could be found. The evaluation of the number average molecular weight ( $M_n$ ) and the molecular weight average ( $M_w$ ) likewise showed no tendency regarding particular SEC systems or columns combinations. However, it should be mentioned that the number of participating laboratories was not sufficiently high to draw definite conclusions.

Table 9 shows the statistical results of  $M_n$  and  $M_w$  with deviations for both aqueous GPC-samples, and Table 10 give a comparison of these values with data published in standard DIN 55672-3 [35]. One participant calculated values of  $M_w$  using the light scattering detector (absolute method).

Both samples E and F have very low repeatability standard deviations of  $M_w$  and  $M_n$  determinations ( $s_r$ , relative) of about 1%. They showed a better performance by a factor of two compared with the results in the standard DIN 55672-3 (approx. 2%).

On the other hand, the reproducibility standard deviations ( $s_R$ , relative) showed values up to 30% (exception approx. 9% for  $M_w$  of the PEO-sample), which are twice as high as described in the above mentioned standard.

The substantial discrepancy between  $s_r$  and  $s_R$  suggests that the different SEC systems of the individual participants were not compatible, although identical calibration standards had to be used. The quality of the separation columns used was probably too different and caused the very high values for  $s_R$ , in relative terms.

**Table 9**

Molecular weight average results ( $M_w$ ,  $M_n$ ) of aqueous SEC.

	Unit	Sample A		Sample B	
		$M_w$	$M_n$	$M_w$	$M_n$
Median value (Hämpel)	kD	324.9	99.3	197.9	135.0
$s_r$	kD	3.25	1.32	1.06	1.51
$s_r$ relative	%	1.0	1.33	0.54	1.12
$s_R$	kD	93.1	31.9	18.1	42.9
$s_R$ relative	%	28.7	32.1	9.2	31.8
$s_R/s_r$		28.6	24.3	17.1	28.4
$u_c$	%	86.1	96.3	27.6	95.4
Participants		11			

**Table 10**Comparison of  $s_T$  and  $s_R$  with data of corresponding standards.

Standard/Interlaboratory test	Material	Molecular weight average weight ( $M_w$ )		Number average molecular weight ( $M_n$ )	
		$s_T$ , rel. (%)	$s_R$ , rel. (%)	$s_T$ , rel. (%)	$s_R$ , rel. (%)
DIN 55672-3	PU, Acrylate	2	16	2	16
Sample E	Pullulan	1.0	28.7	1.3	32.1
Sample F	PEO	0.5	9.2	1.1	31.8
Range		0.5–2%	10–30%	1–2%	15–35%

The quality of the round robin test data (relation  $s_R/s_T$ ) in DIN 55672-3 was about 8 (for  $M_w$  and  $M_n$  data) and, therefore, much lower than the quality values of sample E and F (17–29%), which has to be judged as unacceptable. The combined uncertainty of measurement  $u_c$  of standard data is also only 48% in comparison with the wide range 27–96% in sample E and F. Only the  $u_c$  of  $M_w$  in sample B was quite low (27%). It is difficult to obtain an explanation because no detailed information about the measuring conditions in the mentioned standard is available.

#### 4. Conclusions

The present report of SEC round robin test in organic and aqueous phases indicates what repeatability and reproducibility standard deviations must be taken into account if measurements of molecular weight average weight ( $M_w$ ) and number average molecular weight ( $M_n$ ) are performed. The parameters for sample pretreatment and chromatography were prescribed for all participants. All calibration standards were supplied to the participants to perform a comparable calibration for the measurements.

In the case of SEC in the organic phase (THF), four different samples (2 × PS, PMMA and PHO) were analysed for  $M_w$  and  $M_n$ . 43 participants provided sufficient data in the case of conventional calibration to generate statistically trustworthy median values and standard deviations. The resulting standard deviations of  $M_w$  values were in the same range as published in the SEC standards. The deviations of  $M_n$  values were twice as high as the standards. The  $M_w$  evaluations with light scattering detector showed nearly twice as high reproducibility standard deviations ( $s_R$ , relative) as the conventional calibration for all samples. Some trends concerning the detectors used could be shown. The detector system with RALS/LALS detection provided higher values than the MALLS detectors. However, it should also be mentioned that only seven or eight data sets were available for light scattering analysis. The precise  $dn/dc$  values of the samples were not available in this experiment.

In a second experimental setup, one pullulan sample with 11 data sets and one poly(ethylene oxide) (PEO) sample with 10 data sets in aqueous phase were analysed and evaluated. One participant calculated the  $M_w$  values with a light scattering detector absolutely. It was shown here again that the repeatability standard deviation within a laboratory was low (approx. 1%). However, the comparison between different laboratories showed a reproducibility standard deviation up to 30%. The wide difference between

$s_T$  and  $s_R$  is assumed to be caused by the different SEC systems despite using identical calibration standards.

#### Acknowledgements

The authors wish to thank all the participants in the round robin test campaigns who produced the basic data for this presentation and all colleagues of Empa who made it possible for the round robin tests to be carried out. Special thanks go to the PSS company (Mainz, Germany) for supplying all the polymer standards for calibrations to the participants free of charge.

#### References

- [1] S.L.R. Ellison, M. Roesslein, A. Williams (Eds.), EURACHEM/CITAC Guide, second ed. (2000), p. 7.
- [2] J. Mandel, Chemometrics and Intelligent Laboratory Systems, vol. 11 (1991) 109.
- [3] T. Hofe, G. Reinhold, Grundlagen der GPC, Chem. Labor Biotechnik 50 (1999) 14.
- [4] E.N. Peters, Introduction to Polymer Characterization, Comprehensive Desk Reference of Polymer Characterization and Analysis (2003) p. 3–29.
- [5] A.R. Cooper, Molecular Weight Determination, Characterization and Analysis of Polymers (2008) pp. 65–89.
- [6] H.C. Barth, Measurement of Molecular Weight and Molecular Weight Distribution, Comprehensive Desk Reference of Polymer Characterization and Analysis (2003) pp. 30–45.
- [7] M. Camus, Molecular weight of polymers. Part 3, Schweizerische Laboratoriums-Zeitschrift 59 (10) (2002) 230.
- [8] A.V. Volokhina, Effect of the molecular weight of fibre-forming polymers on the mechanical properties of polymer fibres (review), Fibre Chem. (Translation Khimicheskije Volokna) 34 (1) (2002) 1.
- [9] P. Kitz, Optimization of GPC/SEC Separations by Appropriate Selection of the Stationary Phase and Detection Mode, HPLC Made to Measure (2006) pp. 359–381.
- [10] J.M. Bruna, Determination of molecular weights of polymers. Gel-permeation chromatography (GPC)/size-exclusion chromatography (SEC) method with detection of differential viscosity and light scattering, Ing. Quim 25 (296) (1993) 113.
- [11] S. Shiga, Modern characterization of long-chain branching, Polym. Plast. Technol. Eng. 28 (1) (1989) 17.
- [12] M. Mourey, SEC molecular-weight-sensitive detection, Int. J. Polym. Anal. Charact 9 (1–3) (2004) 97.
- [13] D.M. Meunier, Molecular Weight Determinations of Polymers, Handbook of Instrumental Techniques for Analytical Chemistry (1997) pp. 853–866.
- [14] M. Haney, Low-angle light scattering (LALS) detector. Basis of GPC/SEC with triple detection. Part 2, GIT Labor Fachz 24 (1) (2004) 14.
- [15] R.J. Bruessau, Experiences with interlaboratory GPC experiments, Macromol. Symp. 110 (1996) 15.
- [16] H. Goetz, H. Schulenberg-Schell, Intra- and Interday precision of molecular weight data determined by GPC: precision of  $M_n$  and  $M_w$ , Int. J. Polym. Anal. Char 6 (2001) 565.
- [17] S. Podzimek, Round-Robin test on the molecular characterisation of epoxy resins by liquid chromatography, Int. J. Polym. Anal. Charact 5–6 (2004) 305.
- [18] L. D'Agnillo, et al., Round-robin experiment in high-temperature gel permeation chromatography, J. Poly. Sci. B. Polym. Phys. 10 (2002) 905.

- [19] U. Just, B. Werthmann, Static light scattering of polystyrene reference materials: round-robin test, *Int. J. Polym. Anal. Charact* 5 (1999) 195.
- [20] U. Just, et al., Polymer reference materials: round-robin tests for the determination of molar mass, *Int. J. Polym. Anal. Charact* 10 (2005) 225.
- [21] ISO 16014–1, Plastics, Determination of Average Molecular Mass and Molecular Mass Distribution of Polymers Using Size-exclusion Chromatography – Part 1: General Principles (2003).
- [22] ISO 16014–3, Plastics, Determination of Average Molecular Mass and Molecular Mass Distribution of Polymers Using Size-exclusion Chromatography – Part 3: Low-temperature Method (2003).
- [23] DIN 55672–1, Gelpermeationschromatographie (GPC), Teil 1: Tetrahydrofuran Als Elutionsmittel (1995).
- [24] ASTM D5296, Standard Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography (1997).
- [25] A. Ritter, E. Michel, M. Schmid, S. Affolter, Interlaboratory test on polymers: determination of heavy metals in polymer matrices, *Polym. Test.* 23 (4) (2004) 467.
- [26] F.R. Hampel, et al., *Robust Statistics. The Approach Based on Influence Functions*. Wiley, New York, 1986, ISBN 0-471-73577-9.
- [27] ISO 5725–6, Accuracy (Trueness and Precision) of Measurement Methods and Results – Part 6: Use in Practice of Accuracy Values (1994).
- [28] Guidelines for evaluating and expressing the uncertainty of NIST measurement results, NIST Technical Note 1297; 1994.
- [29] Krzysztof Matyjaszewski, Thomas P. Davis, *Handbook of Radical Polymerization* (2002), ISBN 978-0-471-39274-3.
- [30] R. Sariri, F. Aghaghaziani, Biopolyesters from microorganisms: biochemical basis of microbial synthesis, properties and applications, *J. Appl. Biomater.* 4 (1) (2006) 1.
- [31] J.M. Luengo, B. García, A. Sandoval, G. Naharro, E.R. Olivera, Bioplastics from microorganisms, *Curr. Opin. Microbiol.* 6 (3) (2003) 251.
- [32] S. Yu., K.D. Gao, M.N. Caldwell, J.C. Myers, Giddings, extension of thermal field-flow fractionation to ultrahigh ( $20 \times 10^6$ ) molecular weight polystyrenes, *Macromolecules* 18 (6) (1985) 1272.
- [33] A. Ritter, E. Michel, M. Schmid, S. Affolter, Interlaboratory test on polymers: determination of antioxidants in polyolefins, *Polym. Test.* 24 (4) (2005) 498.
- [34] DIN 55672-3, Gelpermeationschromatographie (GPC) – Teil 3: Wasser Als Elutionsmittel (2004).