Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2026)11

Unclassified

English - Or. English

19 October 2026

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 7 July 2026

Report on Interlaboratory Comparison: Validation of an OECD Test Guideline (TG 322) on Determination of the Solubility and Dissolution Rate of Nanomaterials for Environmental Fate Assessment

OECD Series on Testing and Assessment

E-mail: ehscont@oecd.org

JT03595623

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

This work was approved and declassified by the Chemicals and Biotechnology Committee on [22/06/2026].

Please cite this publication as:

OECD (2026), *Report on Interlaboratory Comparison: Validation of an OECD Test Guideline on Determination of the Solubility and Dissolution Rate of Nanomaterials for Environmental Fate Assessment*, OECD Series on Testing & Assessment No.433, OECD Environment, Health and Safety, Paris, https://one.oecd.org/document/ENV/CBC/MONO(2026)11/en/pdf

Contact us

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

E-mail: ehscont@oecd.org

© OECD 2026

Attribution 4.0 International (CC BY 4.0)

This work is made available under the Creative Commons Attribution 4.0 International licence. By using this work, you accept to be bound by the terms of this licence (https://creativecommons.org/licenses/by/4.0/).

Attribution – you must cite the work.

Translations – you must cite the original work, identify changes to the original and add the following text: *In the event of any discrepancy between the original work and the translation, only the text of original work should be considered valid.*

Adaptations – you must cite the original work and add the following text: *This is an adaptation of an original work by the OECD. The opinions expressed and arguments employed in this adaptation should not be reported as representing the official views of the OECD or of its Member countries.*

Third-party material – the licence does not apply to third-party material in the work. If using such material, you are responsible for obtaining permission from the third party and for any claims of infringement.

You must not use the OECD logo, visual identity or cover image without express permission or suggest the OECD endorses your use of the work.

Any dispute arising under this licence shall be settled by arbitration in accordance with the Permanent Court of Arbitration (PCA) Arbitration Rules 2012. The seat of arbitration shall be Paris (France). The number of arbitrators shall be one.

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 38 countries in North and South America, Europe and the Asia and Pacific region, as well as the European Union, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several Partner countries and from interested international organisations attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in twelve different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides**; **Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; Safety of Manufactured Nanomaterials;** and **Adverse Outcome Pathways.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (https://www.oecd.org/en/topics/chemical-safety-and-biosafety.html).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Report on Interlaboratory Comparison: Validation of an OECD Test Guideline on Determination of the Solubility and Dissolution Rate of Nanomaterials for Environmental Fate Assessment

Table of contents

About the OECD 3

Report on Interlaboratory Comparison: Validation of an OECD Test Guideline on Determination of the Solubility and Dissolution Rate of Nanomaterials for Environmental Fate Assessment 4

Foreword 9

Acknowledgements 10

1 Organisational details 11
1.1. Participating laboratories 11
1.2. Test materials 11
1.3. Study design 12

2 Results 13
2.1. Results Part A Quick Batch Test on Solubility 13
2.2. Part B Extended dissolution test 25
2.3. Results of the Continuous flow experiments 45

Appendix 57

Part A: Solubility Quick Test 58
Materials: 58
Chemicals: 58
General Procedure 59
Workflow 59
Preparation of the test medium for the testing of a powder NM 60
Preparation of the test dispersions and centrifugal ultrafiltration units 61
Conditioning of the centrifugal ultrafiltration tubes 61
Remarks on centrifugal ultrafiltration: 61
Preparation of the dispersion and first sampling at t_{0h} – from NM Dispersions – 100 ppm batch 62
Determination of C_{tot} in the test dispersions at t_{0h} 63
Preparation of the dispersion and first sampling at t_{0h} – from NM Powders – 100 ppm batch 63
Determination of C_{tot} in the test dispersions 64
Remarks: 64

Part B: Solubility and Dissolution Rate - Extended Test 66
Materials: 66
Chemicals: 66
General Procedure 67
Workflow 67
Preparation of the test media 68
Preparation of the test dispersions 69
Conditioning of the centrifugal ultrafiltration tubes 70
Preparation of the dispersion and first sampling at t_{0h} - NM Dispersions – 10 ppm batch 70
Determination of C_{tot} in the test dispersions 71
Preparation of the dispersion and first sampling at t_{0h} - NM Powders – 10 ppm batch 71
Determination of C_{tot} in the test dispersions 72
Remarks: 73

Part C1: Dissolution Rate – Continuous-Flow Test – High Time Resolution 74
Materials: 74
Chemicals: 74
General Procedure 75
Performing the test 76

Part C2: Dissolution Rate – Continuous-Flow Test – integration method 78
Materials: 78
Chemicals: 78
General Procedure 78
Performing the test 79

FIGURES

Figure 1: Z-Score plots for Part A ZnO based on all data and all data without results of lab 02 15
Figure 2: Relative standard deviations for Lab 03 at time points 0 and 24h 16
Figure 3: Relative standard deviations for Lab 04 at time points 0 and 24h 16
Figure 4: Relative standard deviations for Lab 05 at time points 0 and 24h 17
Figure 5: Relative standard deviations for Lab 06 at time points 0 and 24h 17
Figure 6: Relative standard deviations for Lab 07 at time points 0 and 24h 18
Figure 7: Relative standard deviations for Lab 08 at time points 0 and 24h 18
Figure 8: Relative standard deviations for Lab 09 at time point 24h 19
Figure 9: Relative standard deviations for Lab 10 at time points 0 and 24h 19
Figure 10: Z-Score plots for Part A $BaSO_4$ based on all data 20
Figure 11: Relative standard deviations for Lab 02 at time points 0 and 24h 21
Figure 12: Relative standard deviations for Lab 03 at time points 0 and 24h 21
Figure 13: Relative standard deviations for Lab 04 at time points 0 and 24h 22
Figure 14: Relative standard deviations for Lab 05 at time points 0 and 24h 22
Figure 15: Relative standard deviations for Lab 06 at time points 0 and 24h 23
Figure 16: Relative standard deviations for Lab 07 at time points 0 and 24h 23
Figure 17: Relative standard deviations for Lab 10 at time points 0 and 24h 24
Figure 18: Time resolved plots for ZnO concentrations for participating laboratory 08. Plots are shown for single batches and average values for each pH. 27
Figure 19: Time-resolved plots of ZnO concentrations for laboratory 05. Plots are shown for single batches and average values for each pH. 28
Figure 20: Time-resolved plots for ZnO: Laboratory 06 29

Figure 21: Relative standard deviations for Lab 05 Part B ZnO 30
Figure 22: Relative Standard deviations for Lab 06 Part B ZnO 31
Figure 23: Relative standard deviations for Lab 08 Part B ZnO 32
Figure 24: Time-resolved plots of Ba-concentrations for laboratory 05 (plots shown for single batches and average values for each pH) 34
Figure 25: Time-resolved plots of Ba-concentrations for laboratory 06 (plots shown for single batches and average values for each pH) 35
Figure 26: Relative standard deviations for Lab 05 Part B $BaSO_4$ 37
Figure 27: Relative standard deviations for Lab 06 Part B $BaSO_4$ 38
Figure 28: Plots kt vs t (240 min and whole dataset) for Lab 05 39
Figure 29: Plots kt vs t (240 min and whole dataset) for Lab 06 40
Figure 30: Logarithmic fits of concentration data Lab 05 42
Figure 31: Logarithmic fit functions of concentration data Lab 06 43
Figure 32: Time-resolved concentrations for Sm, Sr and Co for the single batches: Laboratory 05 44
Figure 33: Time-resolved concentrations for Sm, Sr, and Co for the single batches at three pH: Laboratory 06 45
Figure 34: Results for part C1 with ZnO for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales 47
Figure 35: Results for part C1 with ZnO for pH 7, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales 48
Figure 36: Results for part C1 with ZnO for pH 8, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales 49
Figure 37: Results for part C1 with $BaSO_4$ for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. 50
Figure 38: Results for part C1 with $BaSO_4$ for pH 7, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. 51
Figure 39: Results for part C1 with $BaSO_4$ for pH 8, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. 52
Figure 40: Results for part C1 with mixed oxide for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Only one of three reported data sets shown for lab 01. 53
Figure 41: Results for part C1 with mixed oxide for pH 7, for labs 01, 05, 06 and 07. Only one of three reported data sets shown for lab 01. Please note the different scaling for lab 01. 54
Figure 42: Results for part C1 with mixed oxide for pH 8, for labs 01, 05, 06 and 07. Only one of three reported data sets shown for lab 01. Please note the different scaling for lab 01. 55

TABLES

Table 1: Participating laboratories of the ILC, their abbreviation and country code in alphabetical order 11
Table 2: Time schedule of the ILC 12
Table 3: Overview on participation and received data for the different test materials. 13
Table 4: Reported results for ZnO. Mean Zn-concentrations and standard deviation after 24h 14
Table 5: Results for $BaSO_4$ expressed as mean concentrations of Ba and standard deviations in ultrafiltrates. 20
Table 6: Results for the mixed oxide expressed as mean concentrations of Sm, Sr and Co and standard deviations in ultrafiltrates. 25
Table 7: Mean Zn-concentrations and SDs for 24 and 96h at pH 5 25
Table 8: Mean Zn-concentrations and SDs for 24 and 96h at pH 7 25
Table 9: Mean Zn-concentrations and SDs for 24 and 96h at pH 8 26
Table 10: Mean Ba-concentrations and SDs for 24 and 96h at pH 5 33
Table 11: Mean Ba-concentrations and SDs for 24 and 96h at pH 7 33
Table 12: Mean Ba-concentrations and SDs for 24 and 96h at pH 8 33
Table 13: First order rate constants for Part B with $BaSO_4$ 38
Table 14: Dissolution rates derived from concentration data and logarithmic fit 41
Table 15: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 5 43
Table 16: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 7 43
Table 17: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 8 43
Table 18: Results Part C2 concentrations for all three tested materials at pH 5 56
Table 19: Results Part C2 concentrations for all three tested materials at pH 7 56

Table 20: Results Part C2 concentrations for all three tested materials at pH 8 56

Foreword

This validation report presents the outcomes of an interlaboratory comparison conducted under OECD Project WNT 3.10 to support the development of an OECD Test Guideline on the determination of the solubility and dissolution rate of nanomaterials for environmental fate assessment. The project was included in the work plan of the Working Party of the National Coordinators of the Test Guidelines Programme (WNT) in 2019 and was led by Austria and Germany. The draft Test Guideline and the draft validation report were reviewed by the Joint WNT- WPMN Expert Group on physicochemical properties and subsequently underwent two rounds of WNT commenting in October 2025 and January 2026. Both the OECD Test Guideline on the determination of the solubility and dissolution rate of nanomaterials for environmental fate assessment and this accompanying validation report were approved at the 38th WNT meeting in April 2026.

The OECD Working Party on Manufactured Nanomaterials (WPMN) has actively worked towards understanding possible safety issues for manufactured nanomaterials and has contributed significantly to resolve these by supporting the OECD Test Guidelines Programme in the development of Test Guidelines, Guidance Documents, Test Reports and by providing other publications with the aim of a safe use of manufactured nanomaterials. The OECD website (OECD Series on the Safety of Manufactured Nanomaterials and other Advanced Materials | OECD) provides more background information.

The interlaboratory comparison was organised by the Fraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME) (Germany) and the University of Vienna (Austria) as part of the project FKZ 3719 66 401 0 financed by the German Federal Ministry for the Environment, Climate Action, Nature Conservation and Nuclear Safety[1]. The report summarises the study design, SOP implementation, data quality considerations, and statistical evaluation of inter- and intra-laboratory variability.
Reflecting the aims of the test guideline, the Standard Operating Procedure (SOP) for the performance of the ILC was divided into three different parts: a screening test for determination of the solubility (Part A), an extended test providing time-resolved data for derivation of a dissolution rate (Part B), and a dynamic flow-through setup (Part C) for the same purpose. For evaluation of these methodologies, three test materials were included to span a range of behaviours: a highly soluble metal oxide, a medium soluble metal oxide, and a mixed oxide to include a more complex case with fractional dissolution behavior. Furthermore, an excel-spreadsheet for processing of the obtained data was developed and evaluated by applying it to the provided datasets. In total 10 international participants were taking part in the ILC. Depending on the tested nanomaterials, data were provided from five to nine laboratories for Part A, from two to three laboratories for Part B, and from four laboratories for Part C. This distribution provides a robust basis for assessing the fast batch test and initial cross-laboratory evidence for the dissolution-rate methods. Based on evaluation of the reported data, Part C was not further considered for the final test guideline due to too high variability of the reported data and too high dependency on personal experience of the laboratory with these kinds of flow-through methods. Even though not all participants reported data for all test materials for Part A and the limited data provided for Part B, conclusions on the reproducibility of both tests could be made and implemented in the final test guideline draft, e.g. to define thresholds for the validity of the test.

[1] UBA TEXTE 45/2025: F. von der Kammer, B. Song, M. Vital, B. Meisterjahn „Standardization of Methods on the Fate and Behavior of Nanomaterials in Environmental Media – Dissolution and Dissolution Rate“ https://www.umweltbundesamt.de/sites/default/files/medien/11850/publikationen/45_2025_texte.pdf

Acknowledgements

This report was prepared by Dr. Boris Meisterjahn, Fraunhofer Institute for Molecular Biology and Applied Ecology (Germany) and Dr. Frank von der Kammer, University of Vienna (Austria).

1 Organisational details

1.1. Participating laboratories

In total 10 laboratories had participated in the interlaboratory comparison (ILC) on nanomaterial solubility and dissolution rate. Table 1 lists the participants.

Table 1: Participating laboratories of the ILC, their abbreviation and country code in alphabetical order

Laboratories	Abbreviation	Country code
BASF	BASF	DE
Center for the Development of Nanoscience and Nanotechnology	CEDENNA	CL
Centre for Nanotechnology and Advanced Materials	CENTI	PT
Fraunhofer Institute for Molecular Biology and Applied Ecology IME	IME	DE
International Iberian Nanotechnology Laboratory	INL	PT
LAUS GmbH	LAUS	DE
Laboratoire national de métrologie et d'essais	LNE	FR
Sciensano	Sciensano	BE
UK Health Security Agency	UKHSA	UK
University of Vienna	UNIVIE	AT

1.2. Test materials

For the ILC three inorganic nanomaterials were selected as test materials:

- Zinc oxide (NM-110)
- $BaSO_4$ (NM-220)
- A mixed oxide $Sm_{0.5}Sr_{0.5}Co_2O_x$

Zinc oxide was selected being a material of highly pH-depending saolubility in the relevant pH-range (5-8) and thus can serve also an additional check on the stability of buffer systems used in the standard operating procedure (SOP). Barium sulphate was chosen as being a material of pH-independent medium solubility and thus serves for highlighting sources of error beyond the stability and precision of pH-adjustment. The mixed oxide was added to the test material set to have a more complex case with fractional dissolution where the three contained metals dissolve

at different concentrations with possibly different dependencies on pH-adjustment of the test media.
All materials were provided as powders to the participating laboratories.

1.3. Study design

The interlaboratory comparison was designed to test three different approaches regarding the solubility and dissolution rate:

A) A quick batch test at test item concentration of 100 mg/l. This test was performed for 24h at three different pH values. The evaluated endpoint was the dissolved content after 24h.
B) An extended test at lower concentration level (10 mg/l) running for a longer time period with higher time resolution regarding the sampling schedule. The aim of this test was to evaluate whether meaningful dissolution rates could be derived from such a simplified test
C) Continuous flow experiments. These were divided in two sub-variants:
    a. C1 being a continuous flow experiment with samples taken every 5 min using a fraction collector (total time for the experiment: 5 h)
    b. C2 being an integrating continuous flow experiment with only one vessel receiving the complete outflow over a time period of 3 h

All experimental procedures were described in detail in a SOP that was provided to the participants before start of the experimental work. Besides a hands-on-training in person at the Fraunhofer Institute for Molecular Biology and Applied Ecology two virtual questions/answers sessions were organized for the participants as opportunities to clarify open issues with the described procedures. Upon these events the SOP was modified where uncertainties were identified. In addition, experimental observations made during the hands-on training at Fraunhofer IME were considered in the final version of the SOP (see A.1).

Table 2: Time schedule of the ILC

Activity	Date
Virtual questions/answers sessions	July 12 and August 09, 2023
Hands-on training at Fraunhofer IME	September 12, 2023
Start of the ILC (shipment of test materials)	End of November 2023
Final SOP available for participants	January 09, 2024
Completion	October 2024
Discussion of ILC-results	November 22, 2024

2 Results

Table 3 gives an overall summary on the number of labs that were providing data for the three parts described in the SOP for the different test items.

Table 3: Overview on participation and received data for the different test materials.

Test material	Number of labs			
	Part A	Part B	Part C1	Part C2
ZnO	9	3	4	4
BaSO4	7	2	4	4
Mixed oxide	5	2	4	4

While for Part A a sufficient number of laboratories had submitted data, for Part B only two labs had performed the experiments for all three test materials. However, as the experimental procedures for both Part A and B are highly similar the conclusions from the evaluation of Part A can be partially transferred to Part B. For Part C 4 labs reported data which in principle allows to draw some conclusions on the robustness of the methodology.

2.1. Results Part A Quick Batch Test on Solubility

2.1.1. ZnO

Overall results

As shown in Table 3, in total 9 laboratories provided data for the test described in Part A of the SOP for ZnO.

Table 4 gives an overview on the number of labs providing data for each of the materials as well as the respective mean concentration value for the determined metal reported by the respective lab after 24h. All subsequent data are presented as the quantified dissolved metal concentration, not converted to total nanomaterial mass. The dissolved metal concentrations as the directly determined quantity are considered to be sufficient for evaluation of the validity of the methodology outlined in the SOP applied within this ILC. The potential error introduced in results given with regard to the amount of nanomaterial was not the primary scope of the ILC. However, it should be noted that in the final TG both values (metal concentrations and extrapolation to material) will be required as the solubility or dissolution rate of a given material will be the endpoint of the test.

Table 4: Reported results for ZnO. Mean Zn-concentrations and standard deviation after 24h

Laboratory	Mean concentration [mg/l]	Overall mean concentration and standard deviation	
		Including lab 02	Excluding lab 02
02	2.56 ± 0.39	58.87 ± 25.06	65.91 ± 14.42
03	57.78 ± 2.87		
04	34.84 ± 1.24		
05	78.82 ± 1.07		
06	68.79 ± 1.22		
07	74.74 ± 0.60		
08	73.16 ± 6.00		
09	76.33 ± 3.79		
10	62.82 ± 3.95		

Z-Scores

For the calculation of the z-scores the mean of the reported mean values and standard deviations of all labs were calculated according to equation 1.

$$z = \frac{x_i - \bar{x}}{\sigma} \qquad \text{eq 1}$$

x_i: value of lab i. x_i: mean of all labs, σ: standard deviation of all reported values

The z-score indicates how the result of a participant deviates from the assigned value (in this case the overall mean) in units of the standard deviation (as for example the reproducibility SD in ISO 5725-2[2]). Z-scores of $|z| \leq 2.0$ are considered acceptable[3], which is fulfilled for all labs with an exception of lab 4 in the case of the scores calculated without the data of lab 02. In case all data are used for the z-score calculations, only for lab 02 a relevant deviation could be detected and all other labs were in the range of $|z| \leq 2.0$.

The resulting z-scores with and without taking lab 02 into account are shown in Figure 1. As can be seen already in Table 4, only one lab reported significantly deviating values for the Zn-concentration in the test vessels after 24h of shaking. This observation cannot be explained by a different weighed amount of test item. However, this lab was also the only lab that reported no precise values for the pH in the test tubes at the sampling points. As the dissolution of ZnO is highly dependent on the pH with a huge impact of even small changes of this parameter, this might be a possible explanation for the results.

However, even without exclusion of lab 02 from the Z-score calculation all other labs are within a range of 1 standard deviation of the overall mean, which indicates a good reproducibility of the applied protocol.

[2] ISO/FDIS 5725-2 Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

[3] ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison

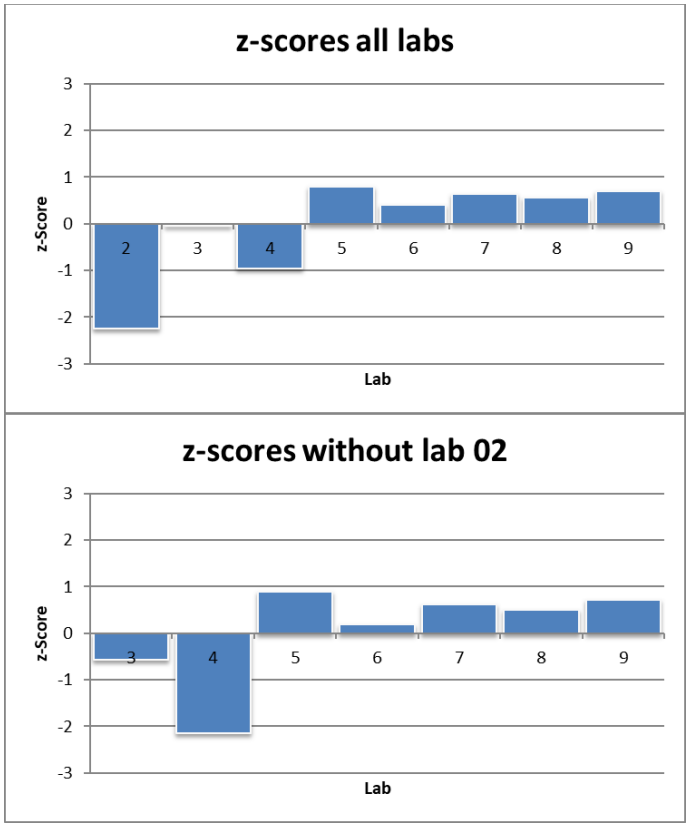


Figure 1: Z-Score plots for Part A ZnO based on all data and all data without results of lab 02

2.1.1.1. Variability within the reported datasets

For identification of possible or major error sources the reported datasets were evaluated for the within and in-between-batch variations. While the within-batch variations account mainly for the reproducibility of the filtrations, the in-between batches are an indicator for the reproducibility of the protocol for preparation of the test dispersions and the influence of general parameters such as the pH, which might differ between different individually prepared batches.

The following diagrams show the within and in-between batch-variations in form of the relative standard variations (RSDs) for the results collected during the ILC for Part A with ZnO. For lab 02 no diagram was prepared since the reported data were clearly outliers. Figure 8 shows only RSDs for the 24h time points as no data for 0h were reported from this lab. Please note that for preparation of these diagrams no outliers were removed from the dataset.

With only a few exceptions the within batch variations were found to be < 5% and the in-between as well as overall variations <10%.

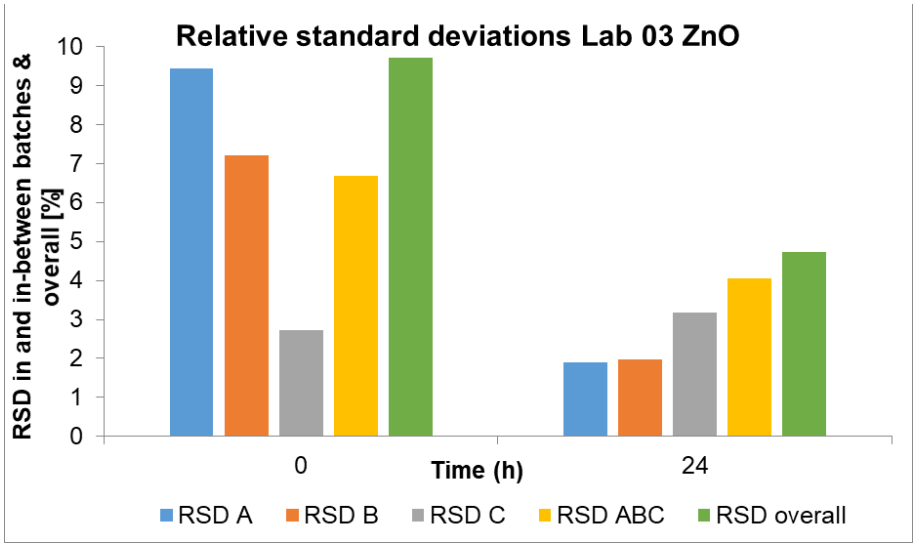


Figure 2: Relative standard deviations for Lab 03 at time points 0 and 24h

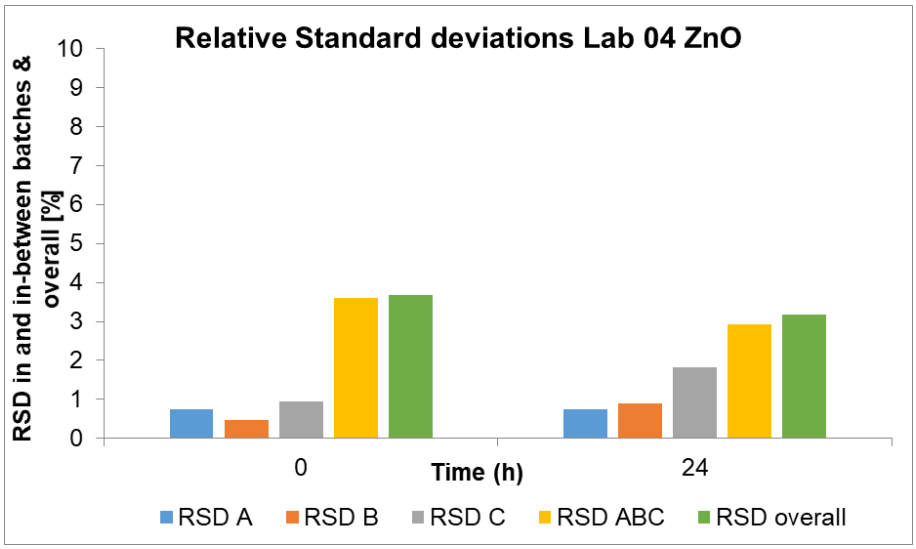


Figure 3: Relative standard deviations for Lab 04 at time points 0 and 24h

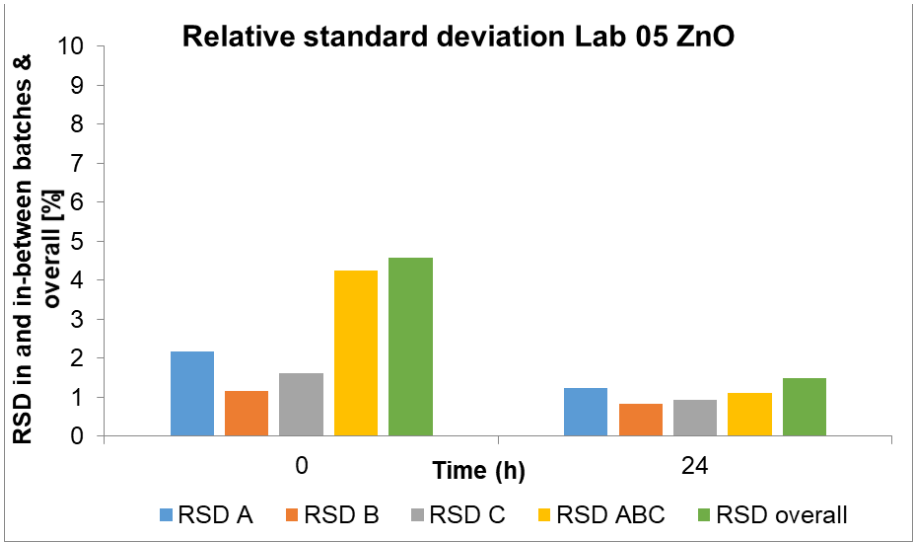


Figure 4: Relative standard deviations for Lab 05 at time points 0 and 24h

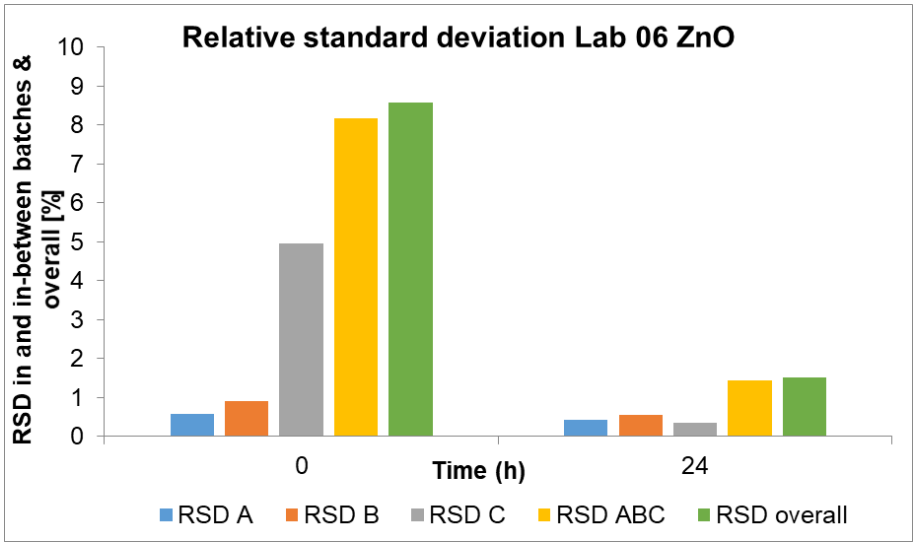


Figure 5: Relative standard deviations for Lab 06 at time points 0 and 24h

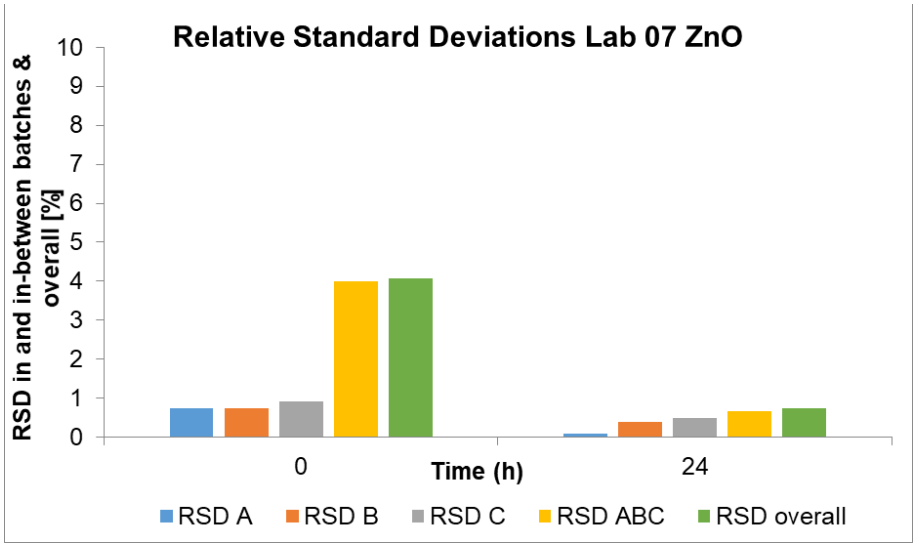


Figure 6: Relative standard deviations for Lab 07 at time points 0 and 24h

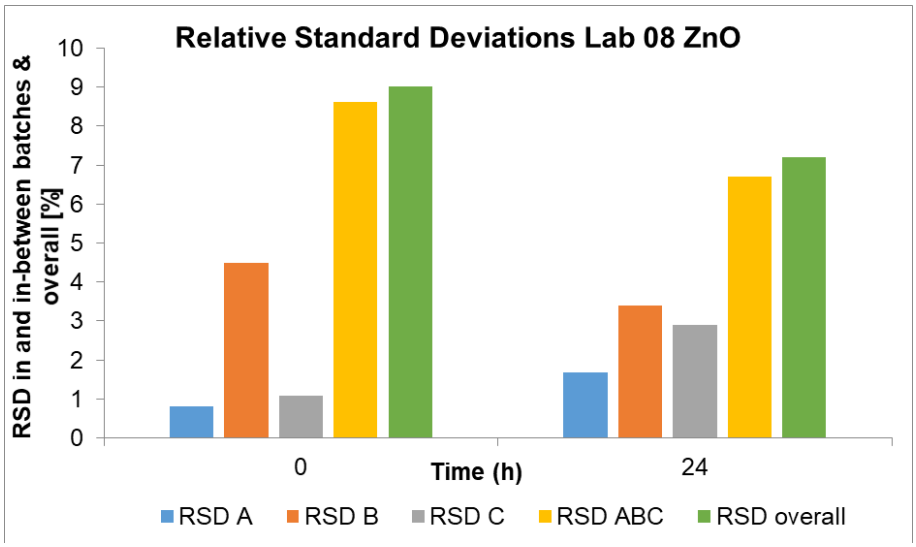


Figure 7: Relative standard deviations for Lab 08 at time points 0 and 24h

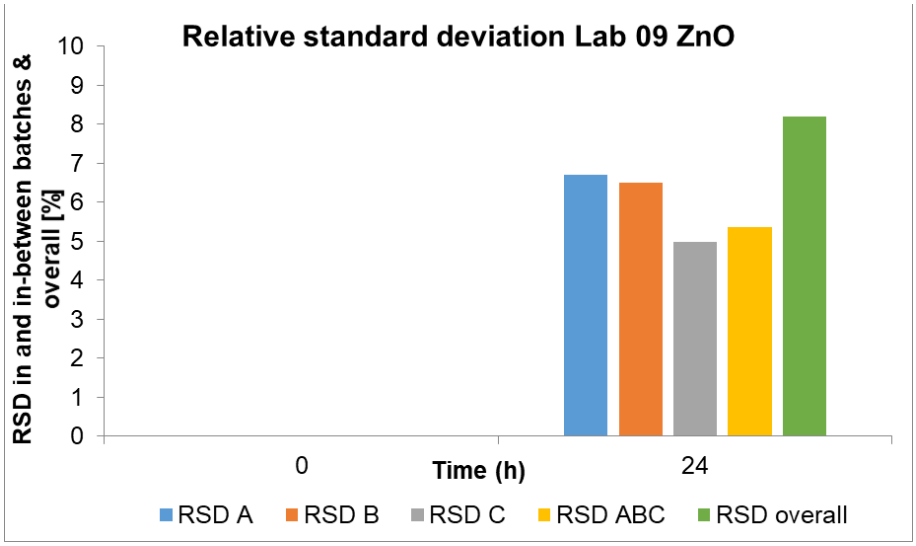


Figure 8: Relative standard deviations for Lab 09 at time point 24h

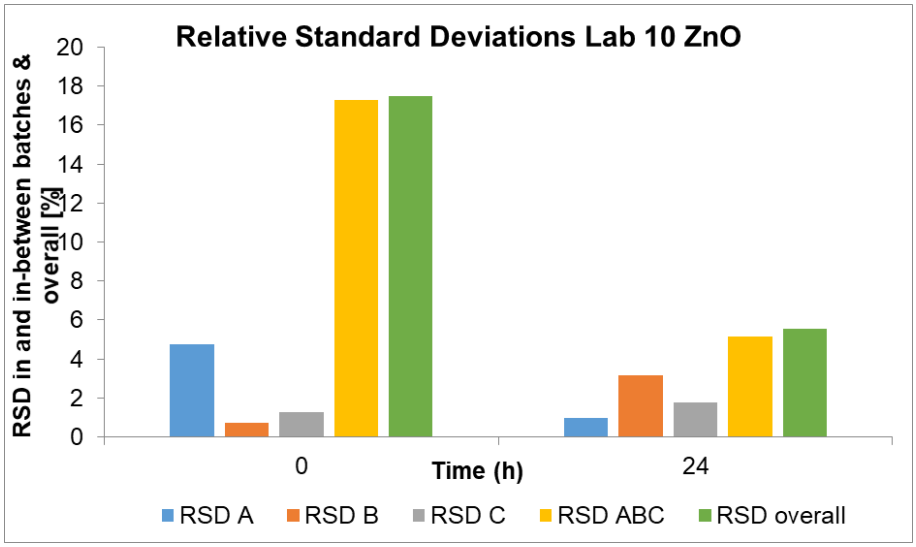


Figure 9: Relative standard deviations for Lab 10 at time points 0 and 24h

2.1.2. $BaSO_4$

Overall results

As shown in Table 3, in total 7 laboratories provided data for the test described in Part A of the SOP for $BaSO_4$.

Table 5 gives an overview on the number of labs providing data for $BaSO_4$ as well as the respective mean concentration value for Ba reported by the respective lab after 24h. All data shown in the following are based on the quantified Ba concentration and were not converted to the total material (see also 10345021).

Table 5: Results for $BaSO_4$ expressed as mean concentrations of Ba and standard deviations in ultrafiltrates.

Laboratory	Mean conc and standard deviation / [mg/l]	Overall mean and standard deviation / [mg/l]
02	3.56 ± 0.49	4.34 ± 0.79
03	4.06 ± 0.08	
04	3.51 ± 0.01	
05	5.78 ± 0.04	
06	4.16 ± 0.02	
07	4.82 ± 0.06	
10	4.52 ± 0.29	

Z-Scores

For the reported $BaSO_4$ data the z-scores were calculated as stated in 10345021 and are plotted in Figure 10. All labs are within a range of two standard deviations of the overall mean, which still indicates a good reproducibility of the applied protocol.



Figure 10: Z-Score plots for Part A $BaSO_4$ based on all data

Variability within the reported datasets

The within and in-between batch relative standard deviations were also calculated for the data on $BaSO_4$. For the time point 24h the within and in-between variations were found to be excellent (<5%) for all labs with only Lab 02 being an exception, while higher values are observed for the 0h sampling, which is probably due to lower measured values at the initial phase of dissolution. Please note that for these plots no outliers were removed from the dataset.

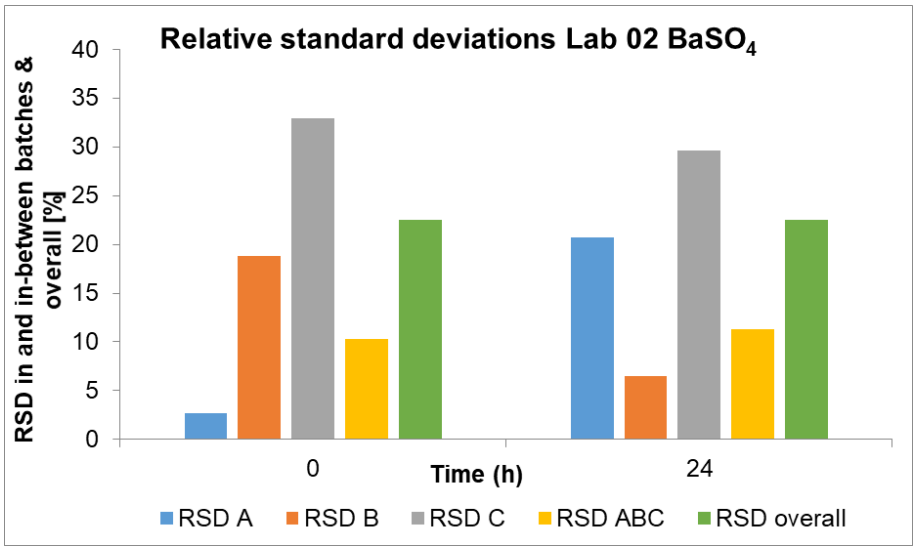


Figure 11: Relative standard deviations for Lab 02 at time points 0 and 24h

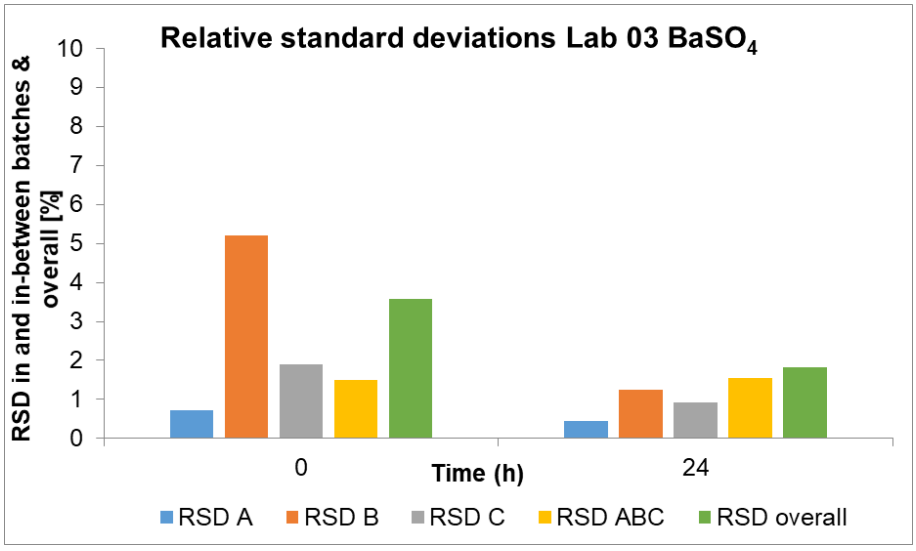


Figure 12: Relative standard deviations for Lab 03 at time points 0 and 24h

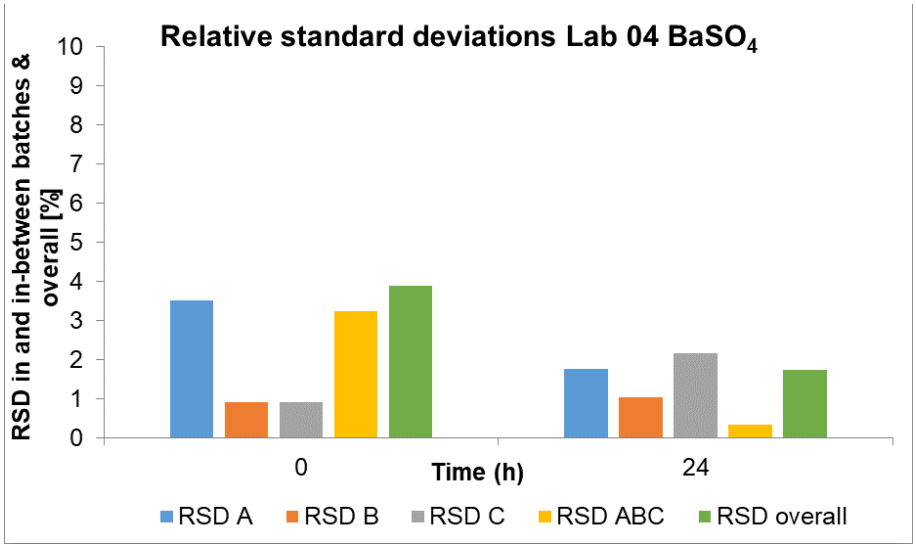


Figure 13: Relative standard deviations for Lab 04 at time points 0 and 24h

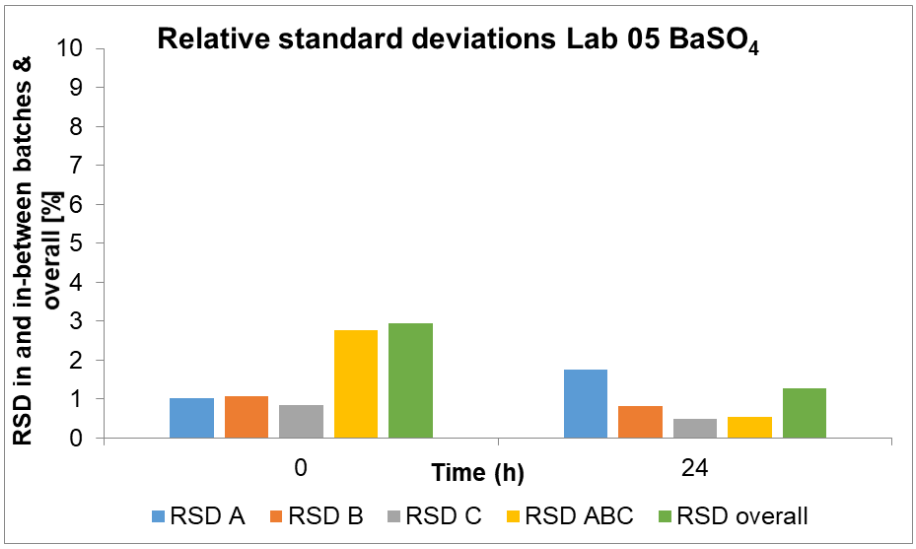


Figure 14: Relative standard deviations for Lab 05 at time points 0 and 24h

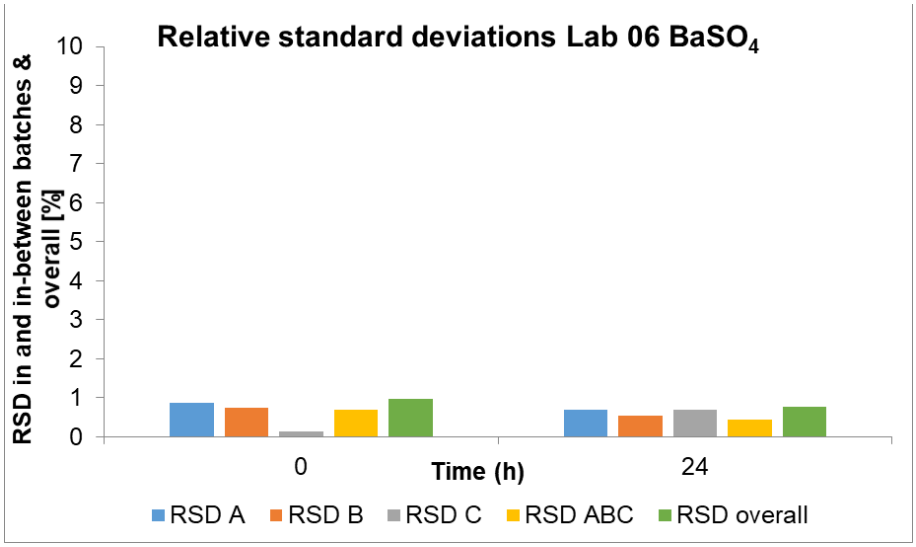


Figure 15: Relative standard deviations for Lab 06 at time points 0 and 24h

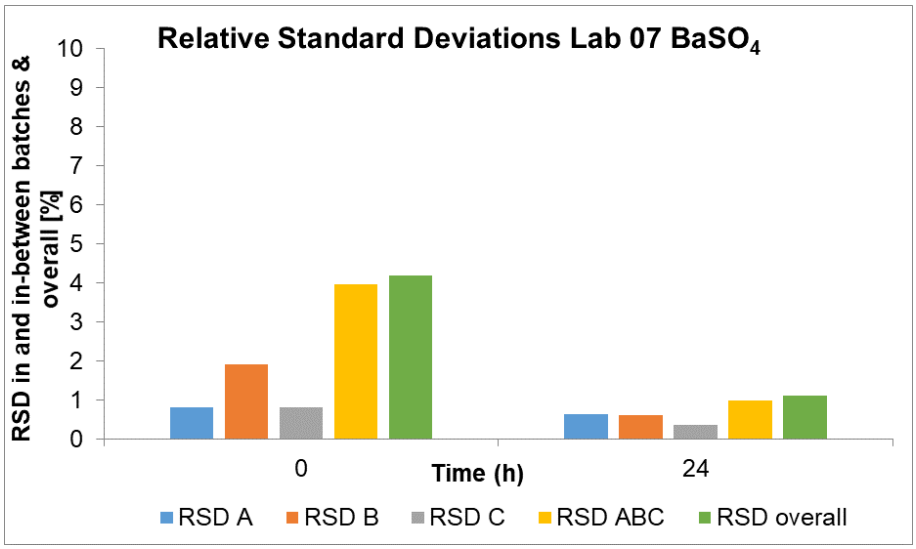


Figure 16: Relative standard deviations for Lab 07 at time points 0 and 24h

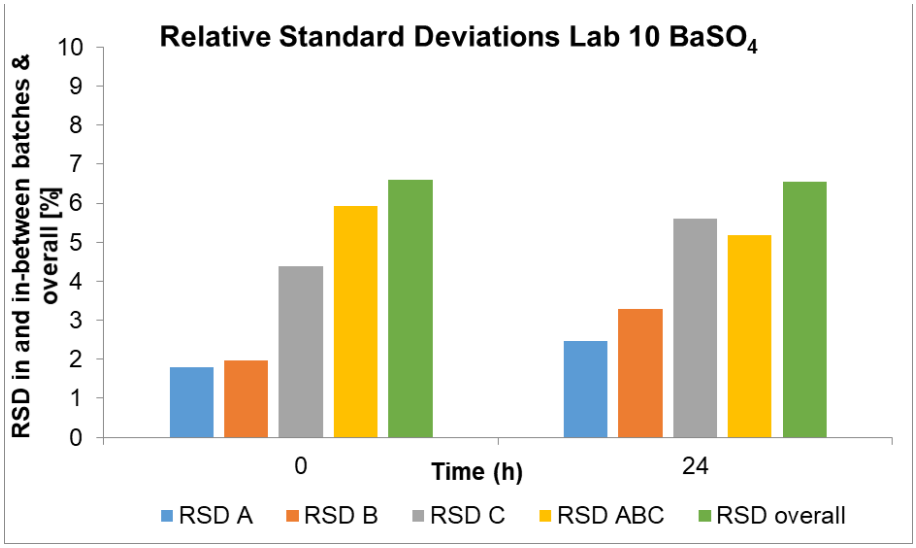


Figure 17: Relative standard deviations for Lab 10 at time points 0 and 24h

2.1.3 Mixed oxide

Overall results

As shown in Table 3, in total 5 laboratories provided data for the test described in Part A of the SOP for the mixed oxide. One lab provided only data for Co. Table 6 summarizes the mean concentrations and standard deviations for the reporting labs.

Table 6: Results for the mixed oxide expressed as mean concentrations of Sm, Sr and Co and standard deviations in ultrafiltrates.

Laboratory	Mean conc Sm ± SD / [mg/l]	Overall mean Sm ± SD	Mean conc Sr ± SD / [mg/l]	Overall mean Sr ± SD	Mean conc Co ± SD / [mg/l]	Overall mean Co ± SD
03	0.70 ± 0.05		11.19 ± 1.25		0.85 ± 0.06	
04					2.23 ± 0.05	
05	0.08 ± 0.03	0.28 ± 0.28	21.39 ± 0.10	14.25 ± 4.82	1.65 ± 0.06	1.21 ± 0.70
06	0.20 ± 0.02		11.50 ± 0.31		0.61 ± 0.04	
07	0.16 ± 0.01		12.92 ± 0.41		0.72 ± 0.03	

The mixed oxide dissolves non-stoichiometrically with high variation between the different labs for Sm and Co. For Sr, where all labs reported the highest concentrations, the values are more homogeneous for 4 out of 5 participants.

2.2. Part B Extended dissolution test

For Part B only 2 laboratories generated data for all three materials and with one more lab in the case of ZnO. Therefore, only the reported results for the 24h and 96h sampling times are reported as well as the plots of the concentration increase over time for the different media.

2.2.1. ZnO

Table 7 – Table 9 summarize the 24h and 96h mean concentrations for Zn with their standard deviations at the different pH values tested.

Table 7: Mean Zn-concentrations and SDs for 24 and 96h at pH 5

Lab	Mean conc 24h / [mg/l]	Overall mean 24h ± SD / [mg/l]	Mean conc 96 h / [mg/l]	Overall mean 96h ± SD / [mg/l]
05	8.19 ± 0.65		8.28 ± 0.66	
06	7.37 ± 0.07	8.49 ± 1.31	7.36 ± 0.07	8.54 ± 1.33
08	9.92 ± 2.44		9.98 ± 2.52	

Table 8: Mean Zn-concentrations and SDs for 24 and 96h at pH 7

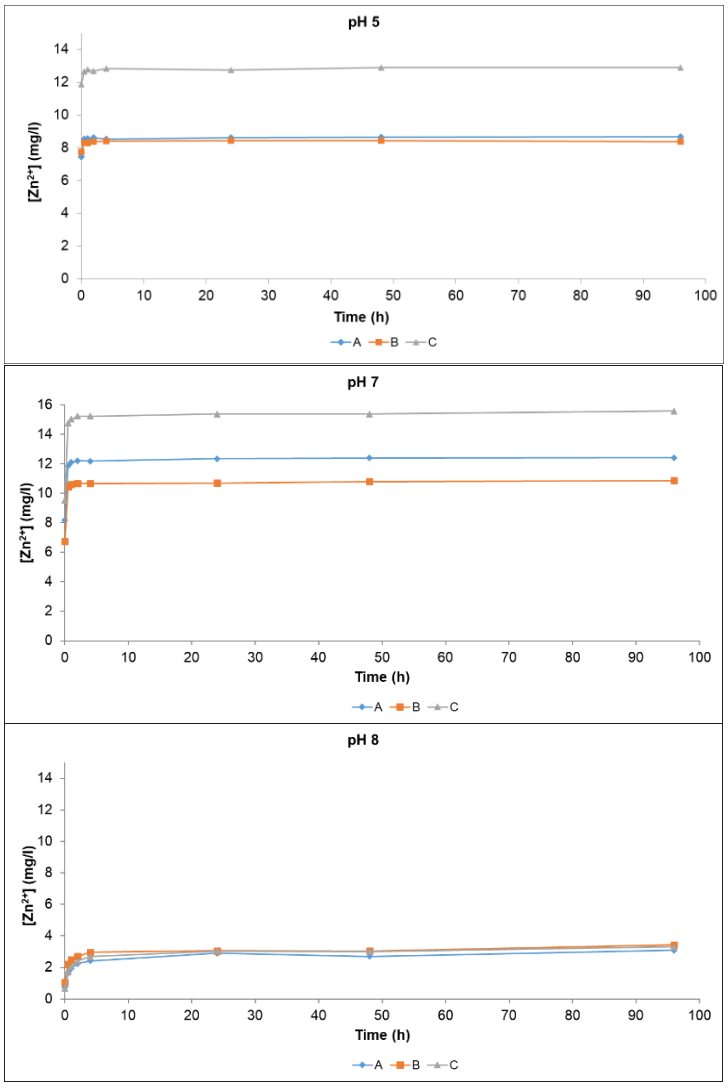
Lab	Mean conc 24h / [mg/l]	Overall mean 24h ± SD / [mg/l]	Mean conc 96 h / [mg/l]	Overall mean 96h ± SD / [mg/l]

05	8.81 ± 0.36		8.86 ± 0.34	
06	7.36 ± 0.07	9.66 ± 2.82	7.56 ± 0.03	9.79 ± 2.81
08	12.81 ± 2.37		12.94 ± 2.39	

Table 9: Mean Zn-concentrations and SDs for 24 and 96h at pH 8

Lab	Mean conc 24h / [mg/l]	Overall mean 24h ± SD / [mg/l]	Mean conc 96 h / [mg/l]	Overall mean 96h ± SD / [mg/l]
05	2.36 ± 0.04		2.54 ± 0.03	
06	2.17 ± 0.00	2.51 ± 0.44	2.38 ± 0.02	2.73 ± 0.47
08	3.00 ± 0.08		3.27 ± 0.18	

The higher values for the concentrations and also standard deviations found for lab 08 at pH 5 and 7 cannot be explained by e.g. higher variations of the weighed amount of test material in the individual batches of the test, which are not correlated to the found concentration levels in the single batches. For instance, the weighed amounts of test material for batches A, B and C at pH 5 were 11.0, 9.0 and 10.5 mg, respectively, and the highest concentration level different to the other batches was found in batch C (see Figure 18, where the time-resolved data for lab 08 are shown). This high variation between the single batches was not observed for the data reported by labs 05 and 06.



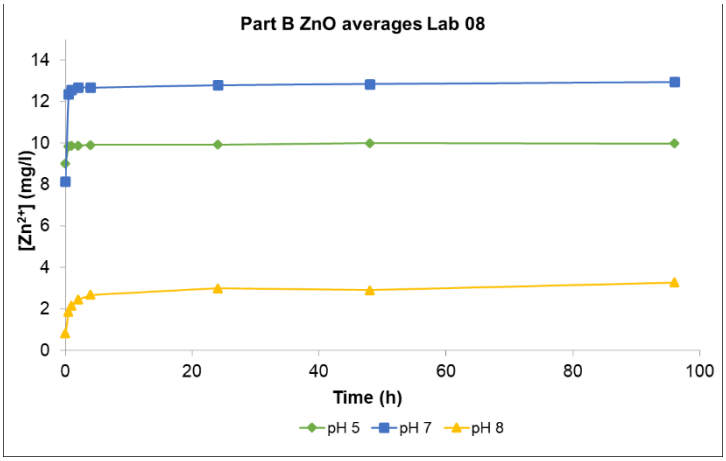
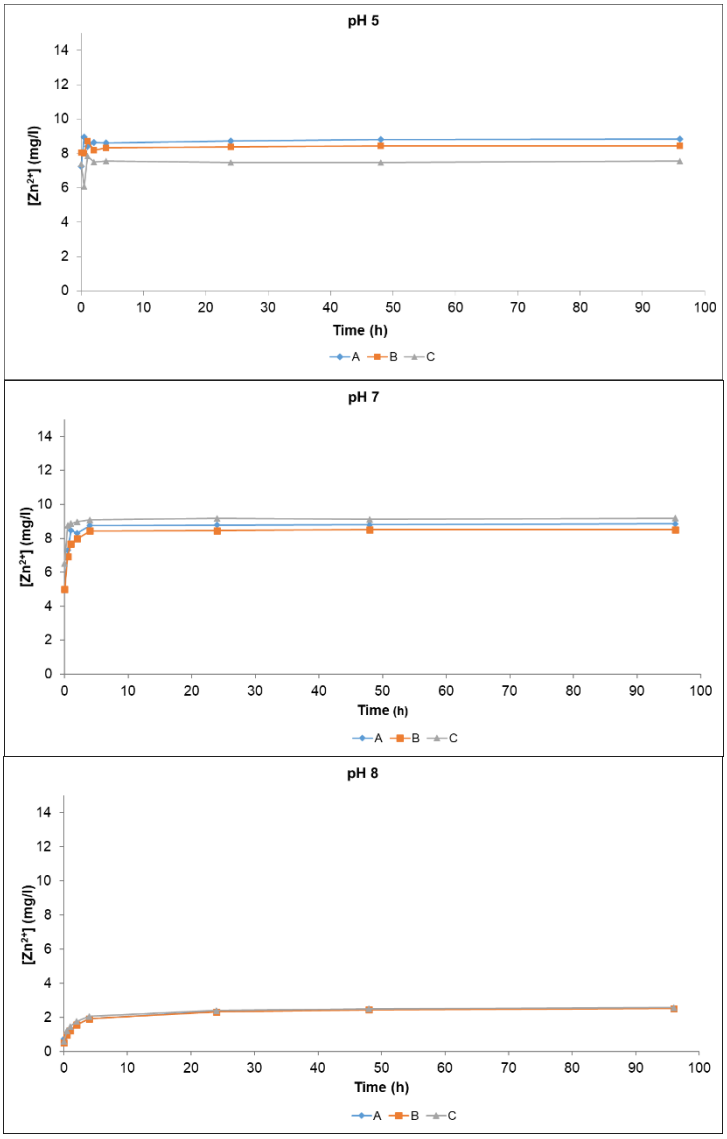


Figure 18: Time resolved plots for ZnO concentrations for participating laboratory 08. Plots are shown for single batches and average values for each pH.



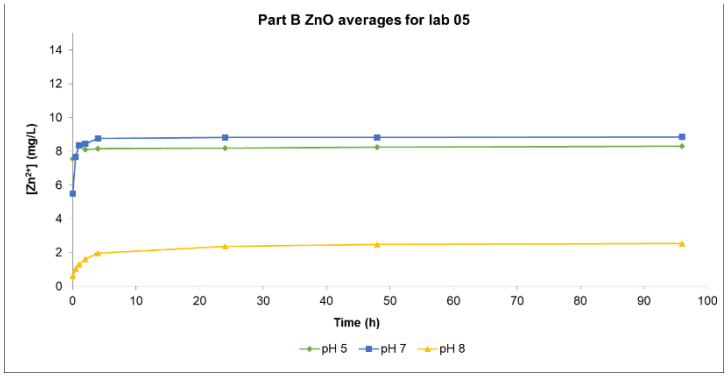
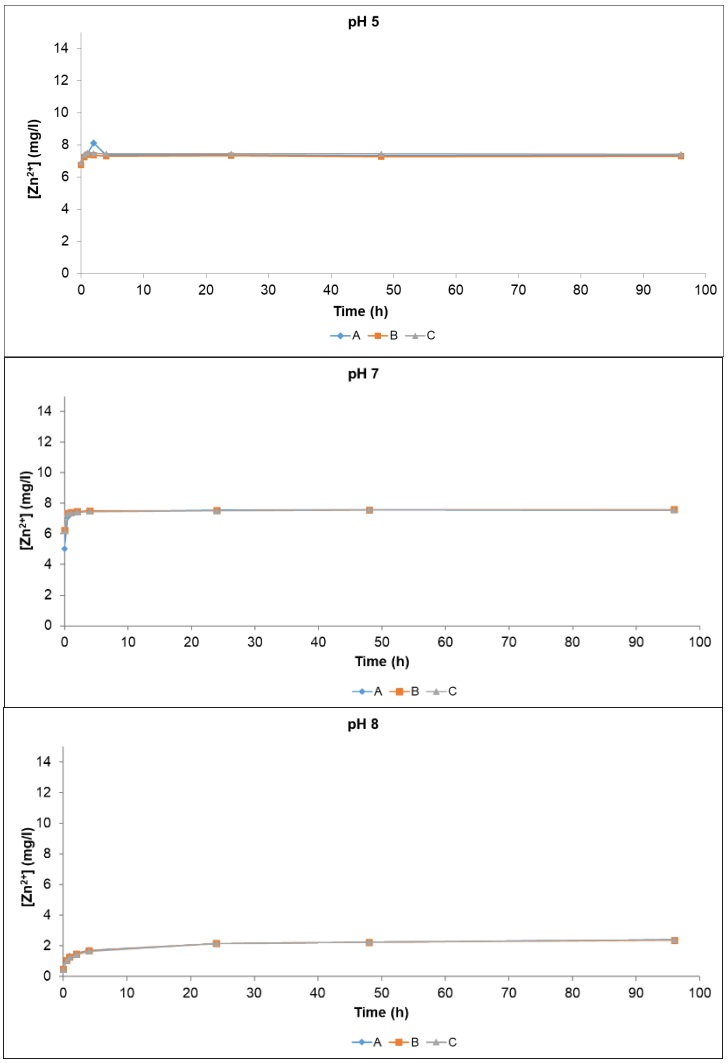


Figure 19: Time-resolved plots of ZnO concentrations for laboratory 05. Plots are shown for single batches and average values for each pH.



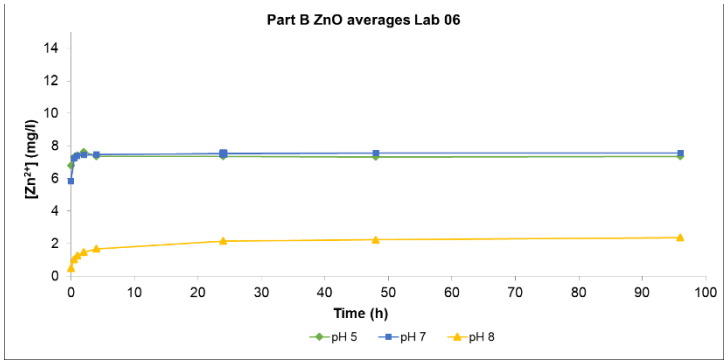


Figure 20: Time-resolved plots for ZnO: Laboratory 06

Therefore, one possible reason for the observed high variation for Lab 08 may be variations occurring already in the preparation phase for the single batches, possibly resulting in less homogenous particle dispersions.

To conclude, even though the data set was limited, the data still demonstrate the good reproducibility of the protocol.

The concentrations reached a plateau already during the first 10h of the test performance.

2.2.1.1. Variability within the reported datasets

Analogously to the evaluation of variations within and in-between batches the relative standard variations were calculated. The resulting diagrams for the three datasets obtained for Part B with ZnO are shown in Figure 21 - Figure 23. Please note that also for these plots no outlier removal was performed. Again, the in-between batch variations are far more relevant than the within-batch variations.
The high variations between the batches in the dataset reported by lab 08 are also clearly visible in the RSD-plot for this lab.



Figure 21: Relative standard deviations for Lab 05 Part B ZnO



Figure 22: Relative Standard deviations for Lab 06 Part B ZnO



Figure 23: Relative standard deviations for Lab 08 Part B ZnO

2.2.2. $BaSO_4$

Table 10 - Table 12 summarize the 24h and 96h mean concentrations for Ba with their standard deviations for the two labs that reported results for this material.

Table 10: Mean Ba-concentrations and SDs for 24 and 96h at pH 5

Lab	Mean conc 24h / [mg/l]	Mean conc 96 h / [mg/l]
05	2.89 ± 0.10	3.09 ± 0.03
06	2.61 ± 0.04	2.81 ± 0.01

Table 11: Mean Ba-concentrations and SDs for 24 and 96h at pH 7

Lab	Mean conc 24h / [mg/l]	Mean conc 96 h / [mg/l]
05	2.79 ± 0.11	3.08 ± 0.05
06	2.22 ± 0.03	2.36 ± 0.06

Table 12: Mean Ba-concentrations and SDs for 24 and 96h at pH 8

Lab	Mean conc 24h / [mg/l]	Mean conc 96 h / [mg/l]
05	2.71 ± 0.06	2.93 ± 0.03
06	2.05 ± 0.04	2.18 ± 0.05

In Figure 24 and Figure 25 the time-resolved Ba-concentrations for laboratories 05 and 06 are shown for both the single batches and the average values for each pH. The data from laboratory 06 show a pH-dependency of the dissolution that is also visible for the results of lab 05, even though it is less pronounced for that dataset.
Due to the limited dataset with only two participants reporting data, conclusions on the reproducibility of the applied method are not possible to state. Both labs report results that are in a similar range with one lab reporting higher concentrations for all conditions and the other lab reporting a visible difference between the batches tested at different pH-values.
While the dissolution was much faster for ZnO in the beginning of the test, the time-resolved data for $BaSO_4$ should allow for a derivation of dissolution rates.
The concentrations in the test batches reached a plateau already at ~30h of testing time. Taking into consideration also the data for the fast-dissolving ZnO, a reduction of the test duration to e.g. 72 h seems to be feasible without losing too much information.

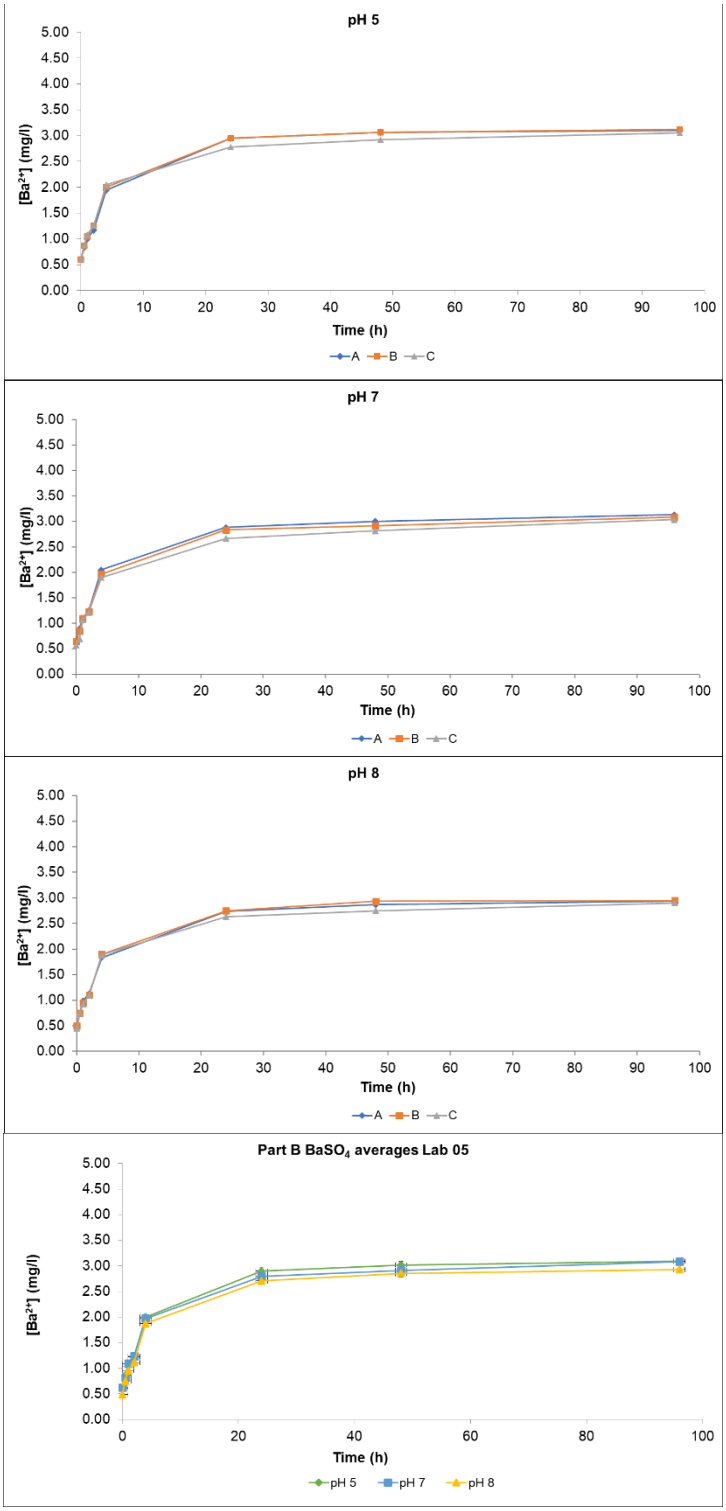


Figure 24: Time-resolved plots of Ba-concentrations for laboratory 05 (plots shown for single batches and average values for each pH)

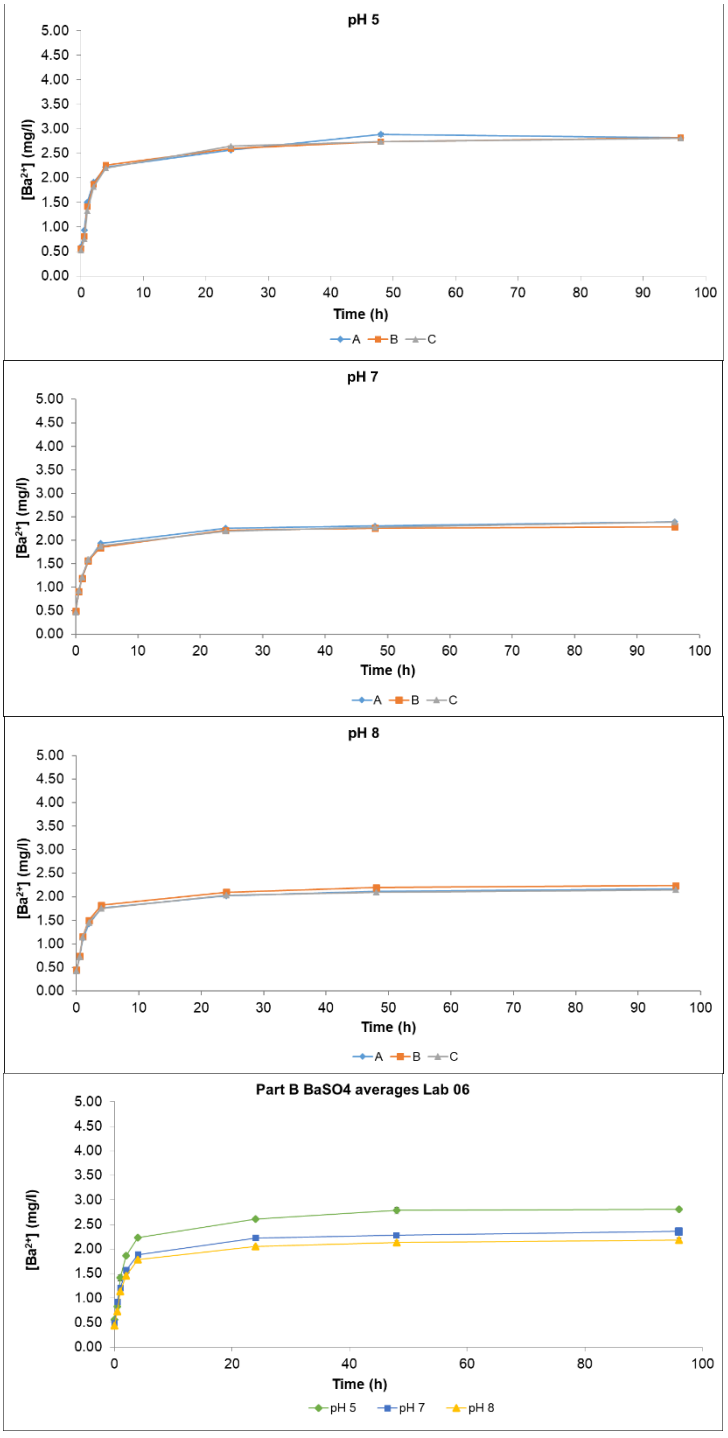
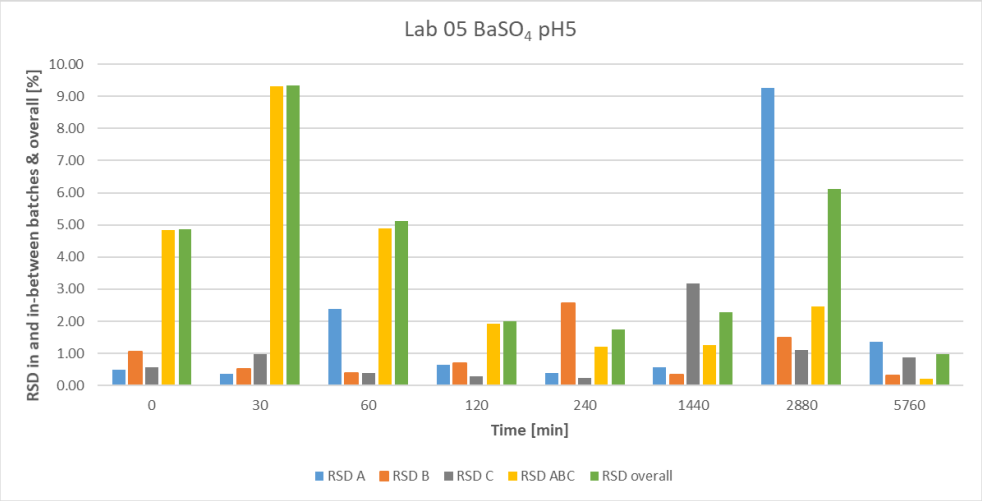
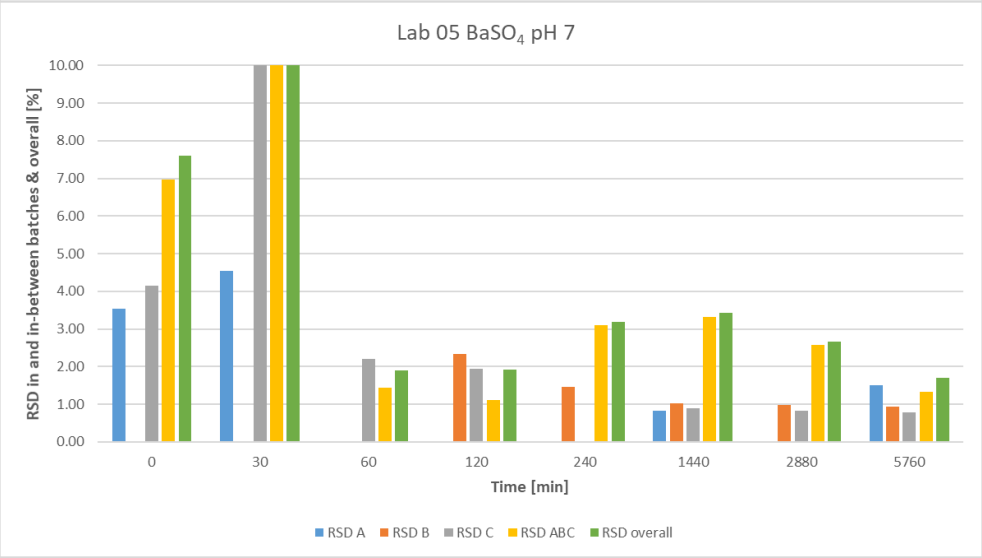


Figure 25: Time-resolved plots of Ba-concentrations for laboratory 06 (plots shown for single batches and average values for each pH)

2.2.2.1. Variability of within the reported datasets

As for Part A also the relative standard deviations within and in-between batches were calculated for Part B. Figure 26 and Figure 27 show the analogous diagrams for both labs that reported data. Please note that also for these diagrams the removal of outliers was not performed. However, even from the uncorrected data the standard deviations were mainly found < 10% (both within and in-between batch variations). This is in general agreement with the data from Part A and shows the excellent reproducibility of the applied protocol.





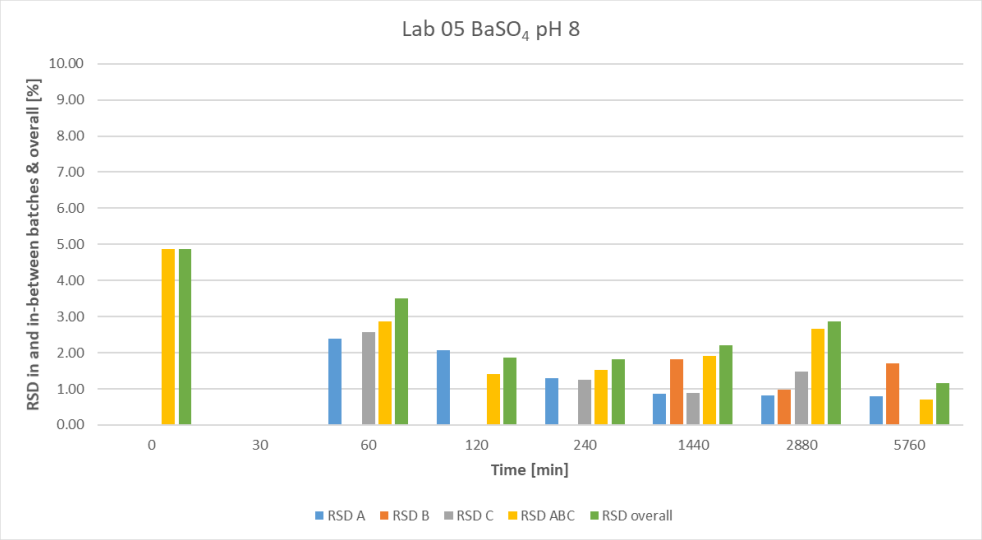
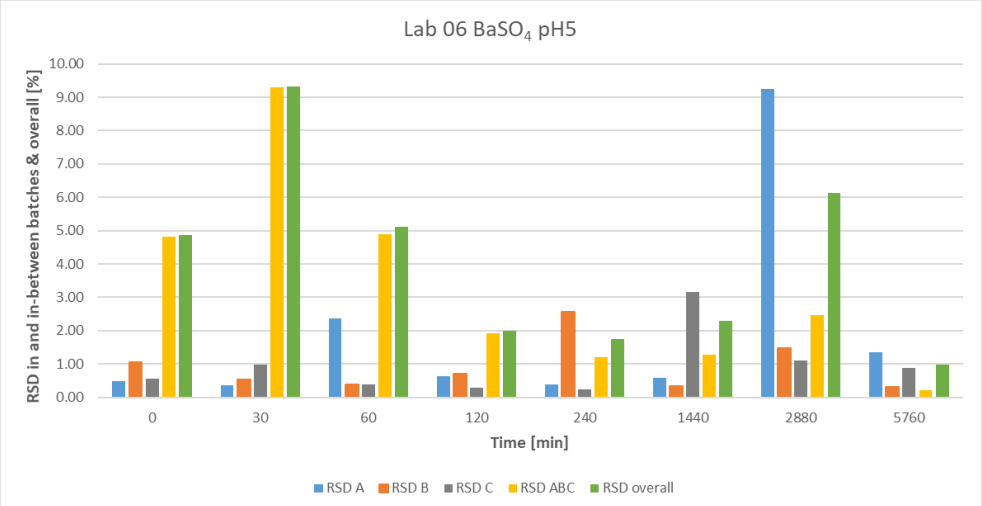
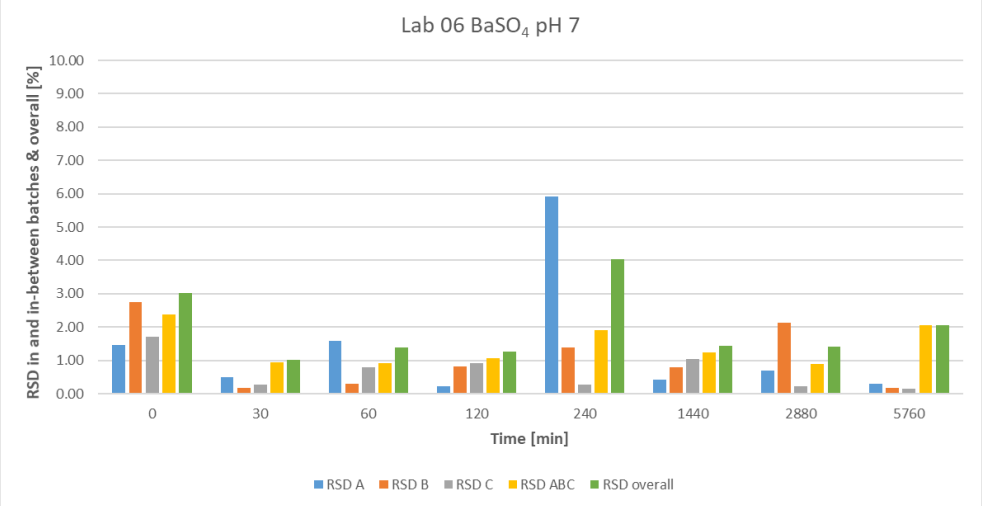


Figure 26: Relative standard deviations for Lab 05 Part B $BaSO_4$





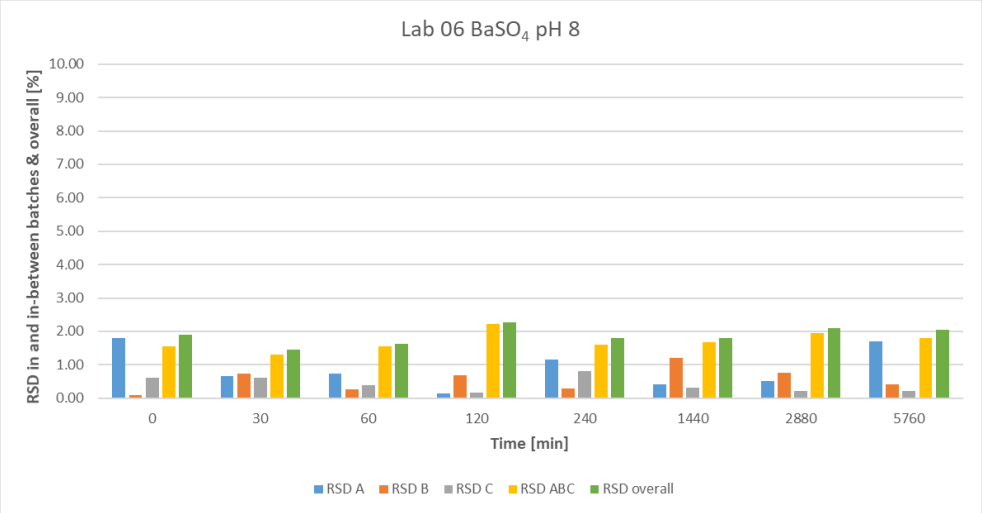


Figure 27: Relative standard deviations for Lab 06 Part B $BaSO_4$

2.2.2.2. Evaluation of $BaSO_4$-data using the evaluation spreadsheet

An excel spreadsheet was developed for kinetic evaluations of the time -resolved extended test. The spreadsheet calculates the within batch means and standard deviations, as well as the means and standard deviations between the batches, thus enabling the identification of outliers in the overall dataset. Besides this general evaluation of the obtained dataset, the sheet calculates the following parameters are derived from the dataset:

- The first order rate constant for the initial 240 min of the test (k_{240}) and R^2 for the fit
- The first order rate constant for the whole dataset (k_{all}) and R^2 for the fit
- The dissolution rates at 15 and 120 min in both µg/l*min and ng/sec*m^2 from concentration data
- The slope of the logarithmic fit of the data
- The dissolution rates for 15 and 120 min in µg/l*sec and ng/sec*m^2 derived from the logarithmic fit of the data

The following figures show the respective fits for the data of both lab 05 and 06 for comparison. Table 13 and Table 14 summarize the derived kinetic parameters for the two labs that had reported data.

Table 13: First order rate constants for Part B with $BaSO_4$

Parameter	Lab 05			Lab 06		
	pH 5	pH 7	pH 8	pH 5	pH 7	pH 8
k_{all} [min^{-1}]	0.00144	0.00115	0.00138	0.00170	0.00137	0.00148
k_{240} [min^{-1}]	0.00420	0.00416	0.00406	0.00680	0.00698	0.00720

For both labs the derived rate constants show no influence of pH as expected. As visible already in Figure 24 and Figure 25 the dissolution of $BaSO_4$ was apparently faster in the experiments performed by Lab 05.

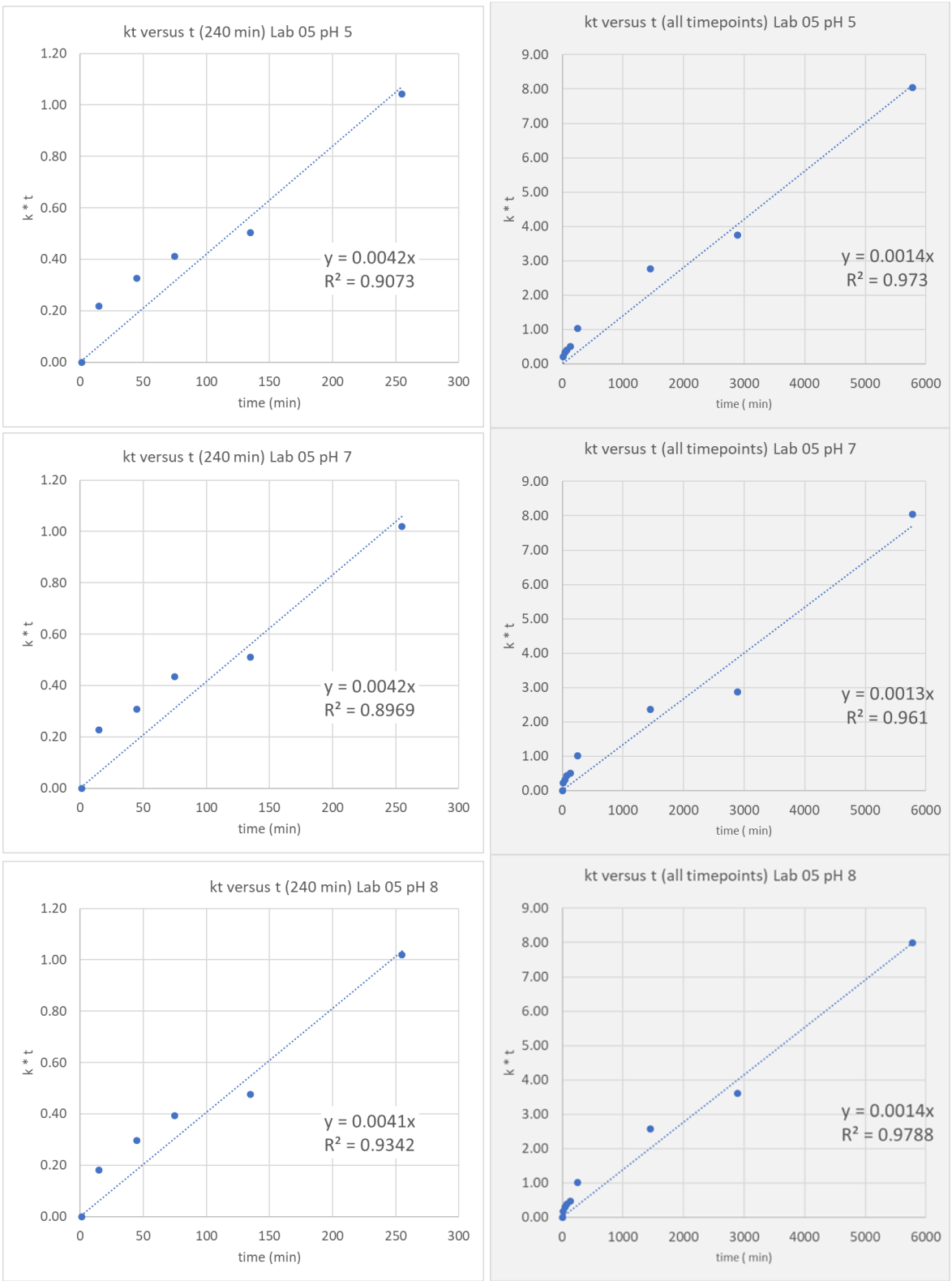


Figure 28: Plots kt vs t (240 min and whole dataset) for Lab 05

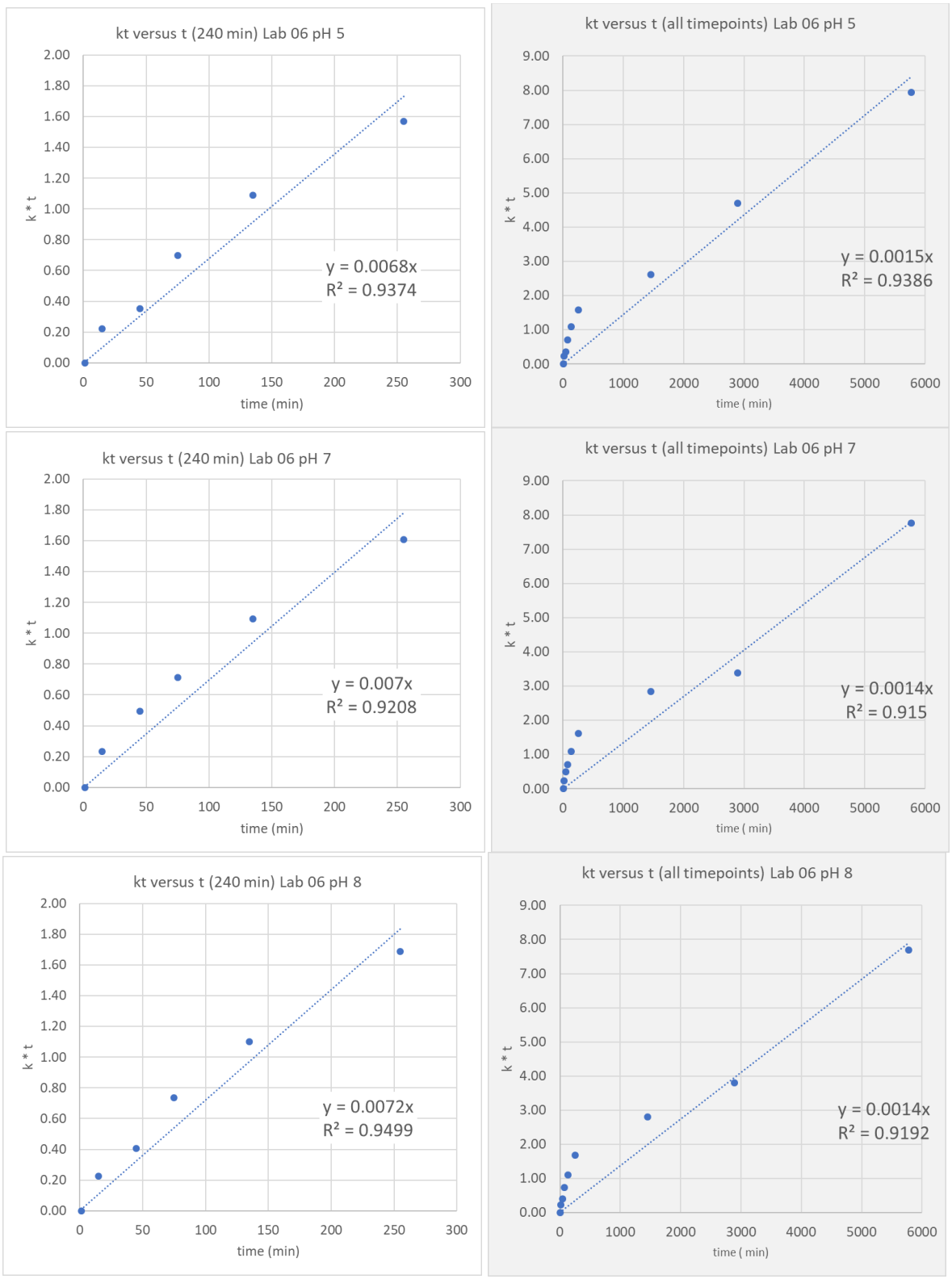
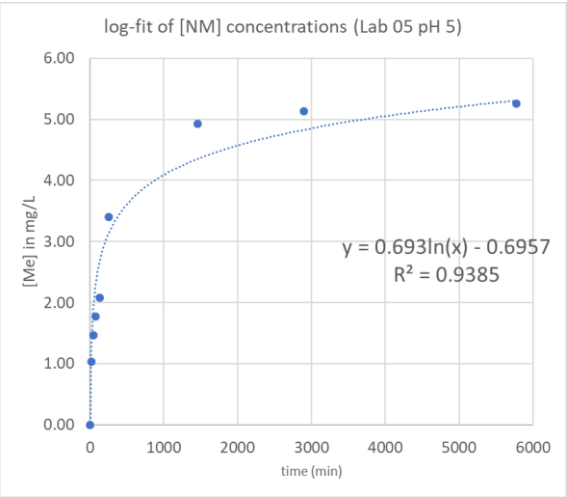
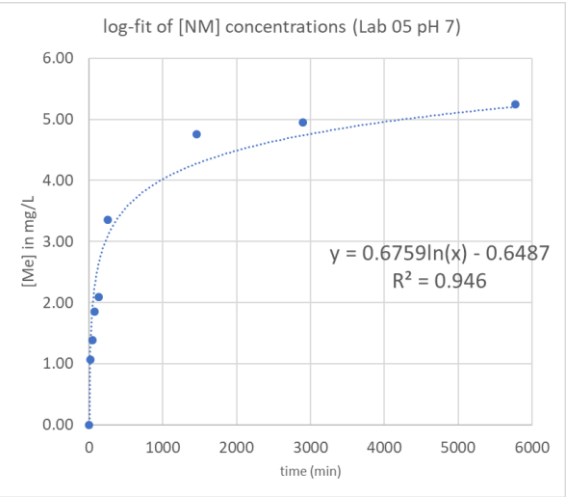


Figure 29: Plots kt vs t (240 min and whole dataset) for Lab 06

Table 14: Dissolution rates derived from concentration data and logarithmic fit

Parameter	Lab 05			Lab 06		
	pH 5	pH 7	pH 8	pH 5	pH 7	pH 8
Rates concentration data						
Dissolution rate [µg/l*sec) 15 min	1.132	0.988	0.891	1.226	1.271	0.979
Dissolution rate [µg/l*sec) 120 min	0.214	0.174	0.152	0.084	0.068	0.076
Dissolution rate [ng/sec*m^2) 15 min	2.709	2.365	2.132	2.947	3.055	2.352
Dissolution rate [ng/sec*m^2) 120 min	0.809	0.607	0.519	0.280	0.231	0.345
Rates from logarithmic fit						
Dissolution rate [µg/l*sec) 15 min	0.694	0.569	0.535	0.770	0.751	0.736
Dissolution rate [µg/l*sec) 120 min	0.077	0.063	0.059	0.086	0.083	0.082
Dissolution rate [ng/sec*m^2) 15 min	1.661	1.363	1.280	1.851	1.805	1.769
Dissolution rate [ng/sec*m^2) 120 min	0.291	0.221	0.203	0.285	0.282	0.264





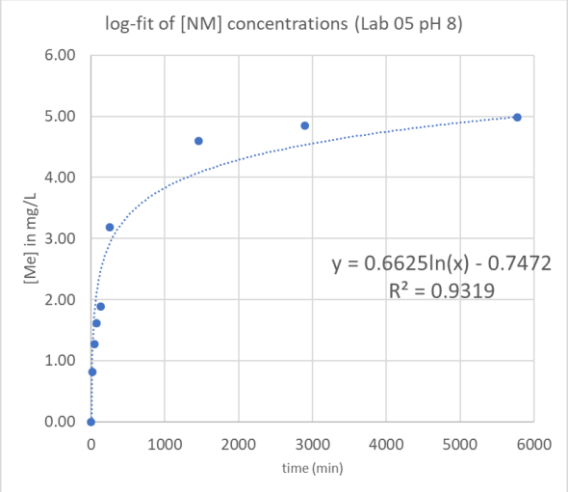
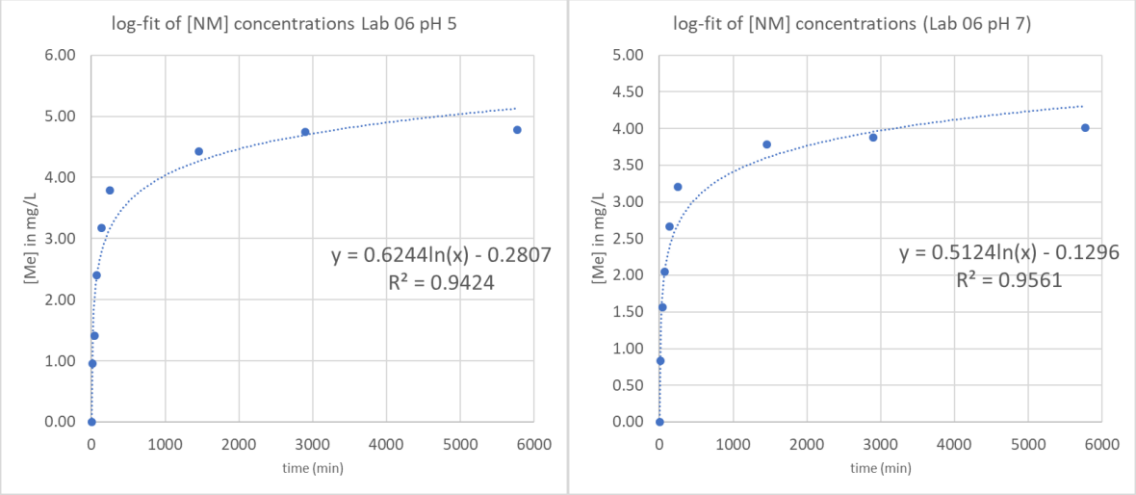


Figure 30: Logarithmic fits of concentration data Lab 05



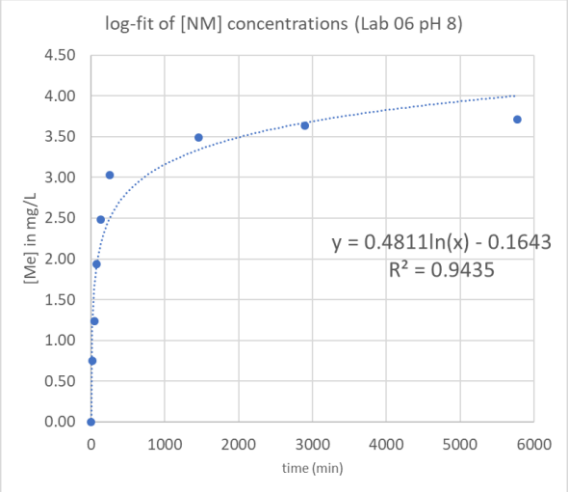


Figure 31: Logarithmic fit functions of concentration data Lab 06

2.2.3. Mixed oxide

Table 10 - Table 12 summarize the 24h and 96h mean concentrations for Sm, Sr and Co with their standard deviations for the two labs that reported results for this material.

Table 15: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 5

Lab	Mean Sm conc 24h / [mg/l]	Mean Sm conc 96h / [mg/l]	Mean Sr conc 24h / [mg/l]	Mean Sr conc 96h / [mg/l]	Mean Co conc 24 h / [mg/l]	Mean Co conc 96 h / [mg/l]
05	1.49 ± 0.10	1.64 ± 0.10	2.93 ± 0.07	2.99 ± 0.04	3.50 ± 0.08	3.76 ± 0.08
06	2.12 ± 0.02	2.24 ± 0.07	1.61 ± 0.03	1.67 ± 0.08	1.55 ± 0.00	1.75 ± 0.09

Table 16: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 7

Lab	Mean Sm conc 24h / [mg/l]	Mean Sm conc 96h / [mg/l]	Mean Sr conc 24h / [mg/l]	Mean Sr conc 96h / [mg/l]	Mean Co conc 24 h / [mg/l]	Mean Co conc 96 h / [mg/l]
05	0.08 ± 0.02	0.26 ± 0.07	2.28 ± 0.17	2.55 ± 0.06	0.38 ± 0.06	0.81 ± 0.32
06	0.06 ± 0.00	0.17 ± 0.01	1.36 ± 0.05	1.50 ± 0.05	0.14 ± 0.00	0.27 ± 0.01

Table 17: Mean Sm, Sr and Co-concentrations and SDs for 24 and 96h at pH 8

Lab	Mean Sm conc 24h / [µg/l]*	Mean Sm conc 96h / [µg/l]*	Mean Sr conc 24h / [mg/l]	Mean Sr conc 96h / [mg/l]	Mean Co conc 24 h / [mg/l]	Mean Co conc 96 h / [mg/l]
05	0.77 ± 0.61	20.68 ± 11.24	2.29 ± 0.17	2.45 ± 0.20	0.15 ± 0.05	0.29 ± 0.16
06	0.15 ± 0.04	0.20 ± 0.05	1.19 ± 0.04	1.30 ± 0.05	0.06 ± 0.01	0.09 ± 0.01

* Please note that for Sm at pH 8 the concentrations are given in µg/l.

Besides the non-stochiometric dissolution behaviour of the mixed oxide, the limited dataset of only two labs does not allow for any conclusions regarding the reproducibility of the applied method based on this test material.

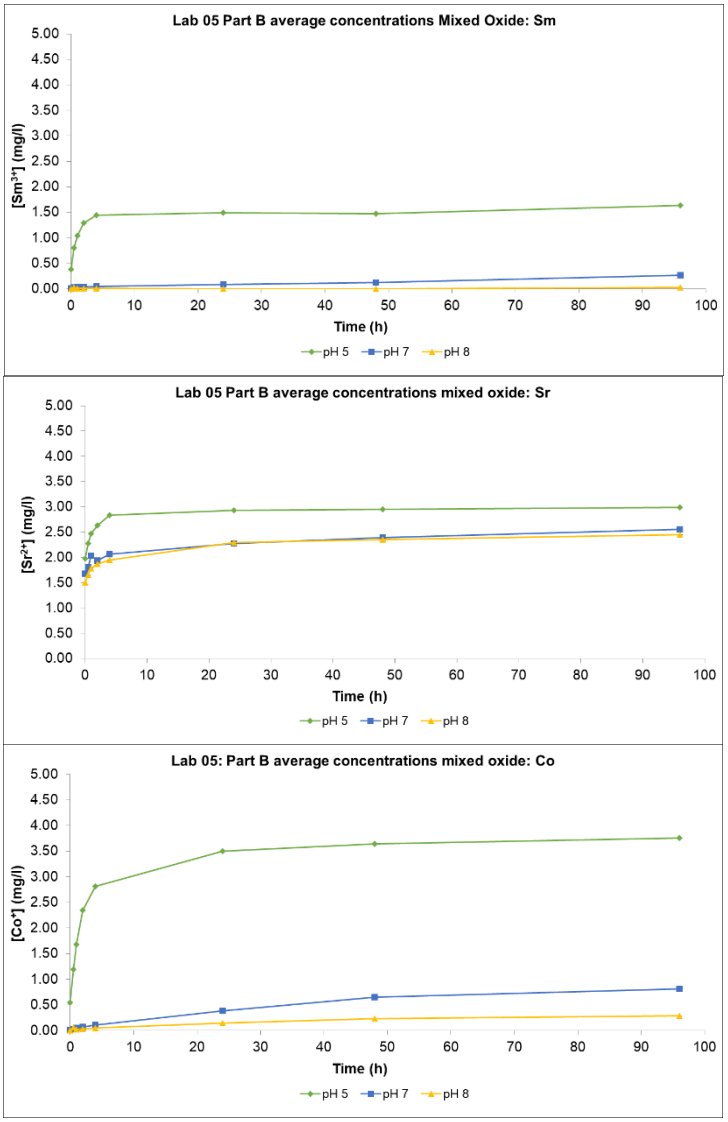


Figure 32: Time-resolved concentrations for Sm, Sr and Co for the single batches: Laboratory 05

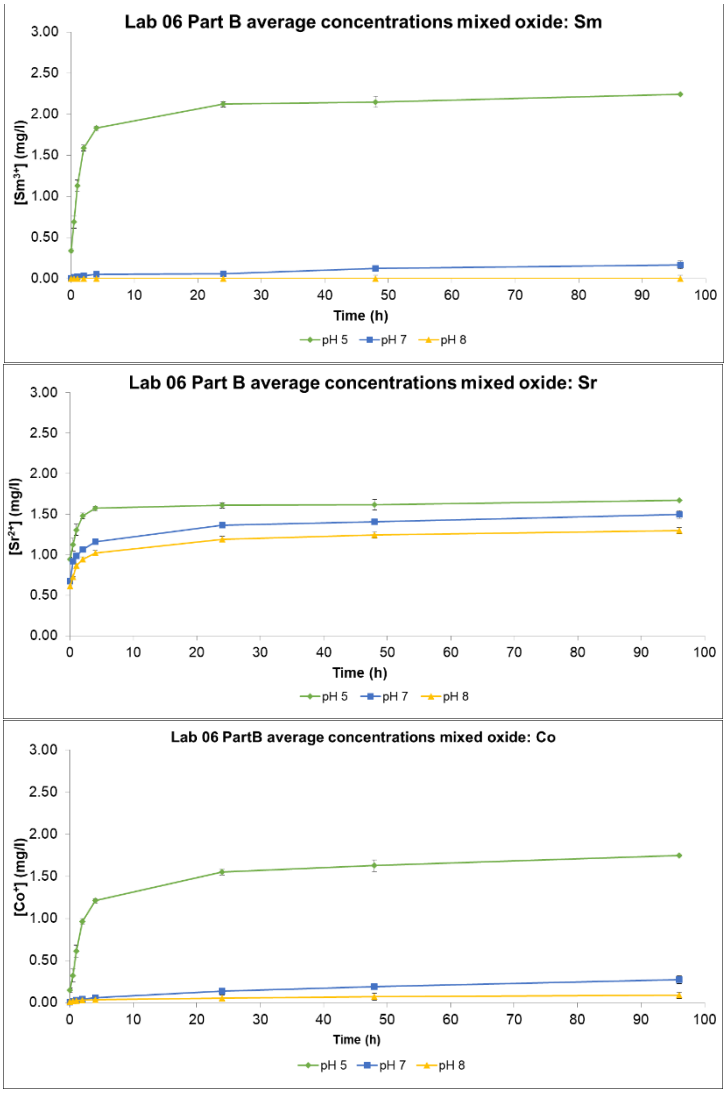


Figure 33: Time-resolved concentrations for Sm, Sr, and Co for the single batches at three pH: Laboratory 06

2.3. Results of the Continuous flow experiments

For Part C1 and C2 only four laboratories reported data for both test variants and all test materials.

2.3.1. Part C1 ZnO

Figure 34 - Figure 36 show the measured concentrations in the outflow over time for pH 5, 7 and 8 for the four labs providing data.

Lab 01 reported triplicate measurements for each pH and the results from these triplicate measurements show excellent inhouse-reproducibility for this lab.

Lab 01 reported significantly higher concentrations especially for pH 5 and 7. For pH 7 and 8 labs 05, 06 and 07 reported similar concentrations for later sampling points. However, the shape of the concentration curve was not always the same with an apparently still increasing curve for lab 05, constant values from start on for lab 06 and initial decrease from higher start to a lower steady-state concentration for lab 07. For pH 5, where the material is highly soluble, the differences between the four labs are more pronounced with different shapes and also concentration levels.
The overall conclusion of these observations for this test is that it needs personal experience and training of the technical staff to achieve reproducible results.

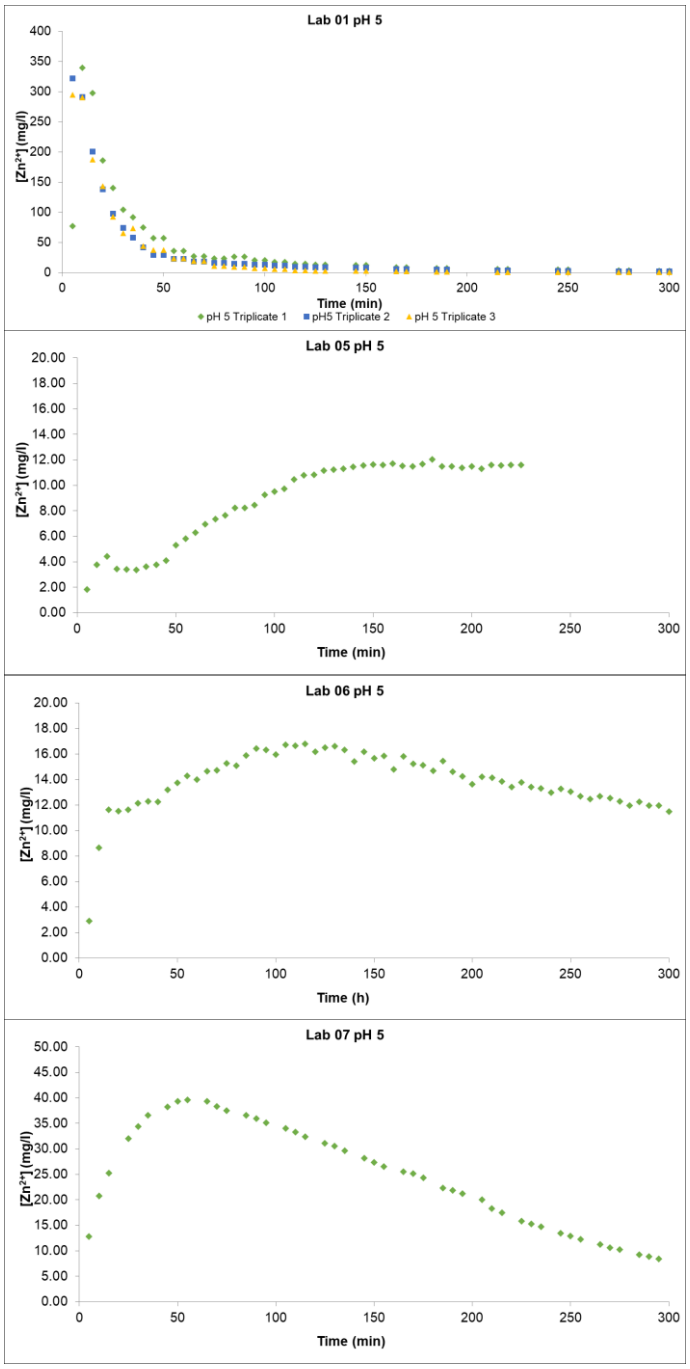
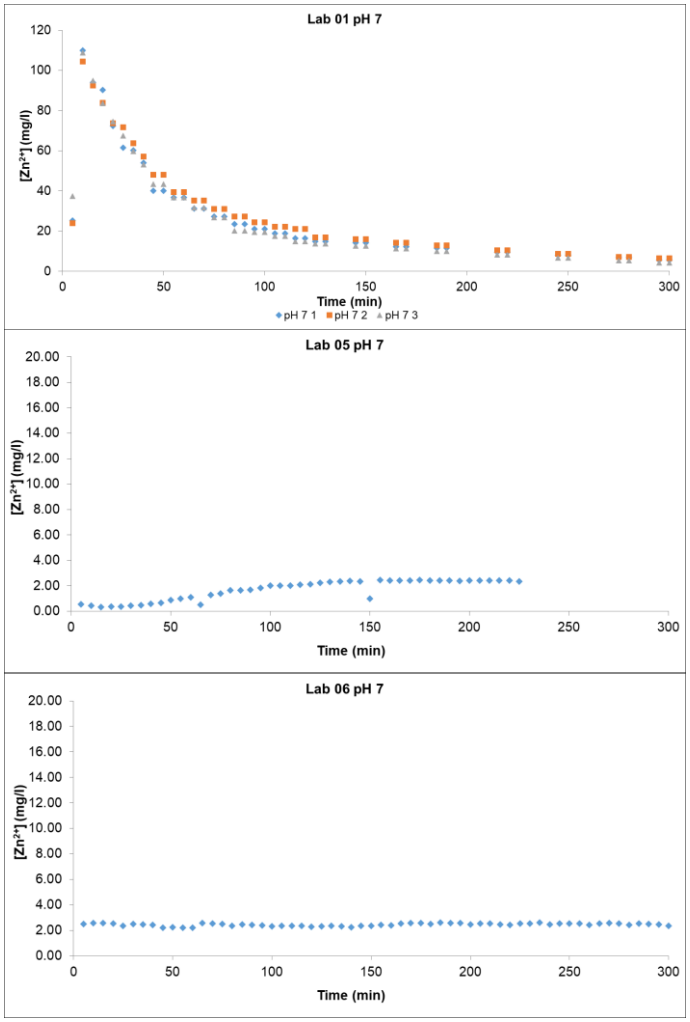


Figure 34: Results for part C1 with ZnO for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales



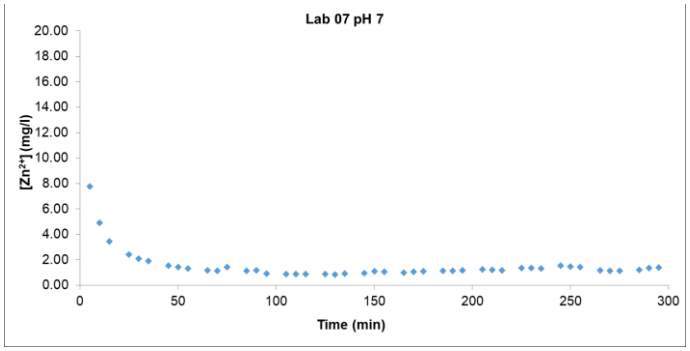
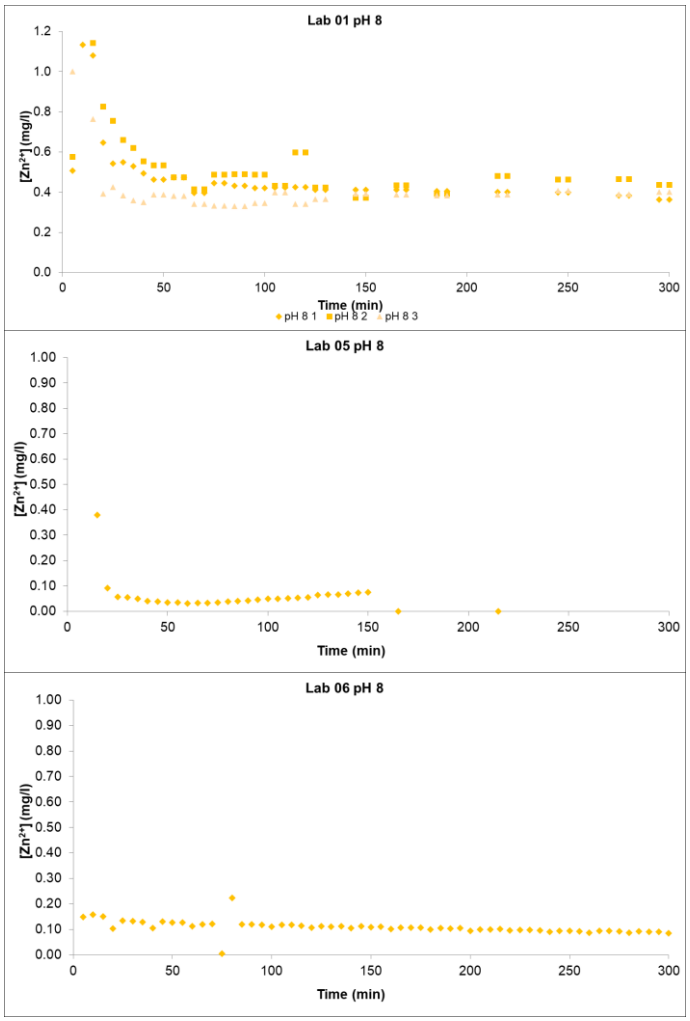


Figure 35: Results for part C1 with ZnO for pH 7, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales



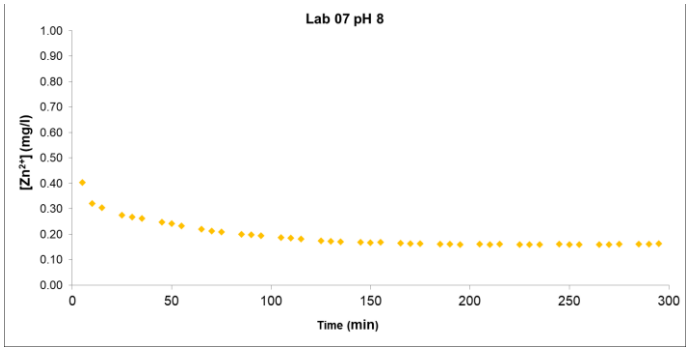
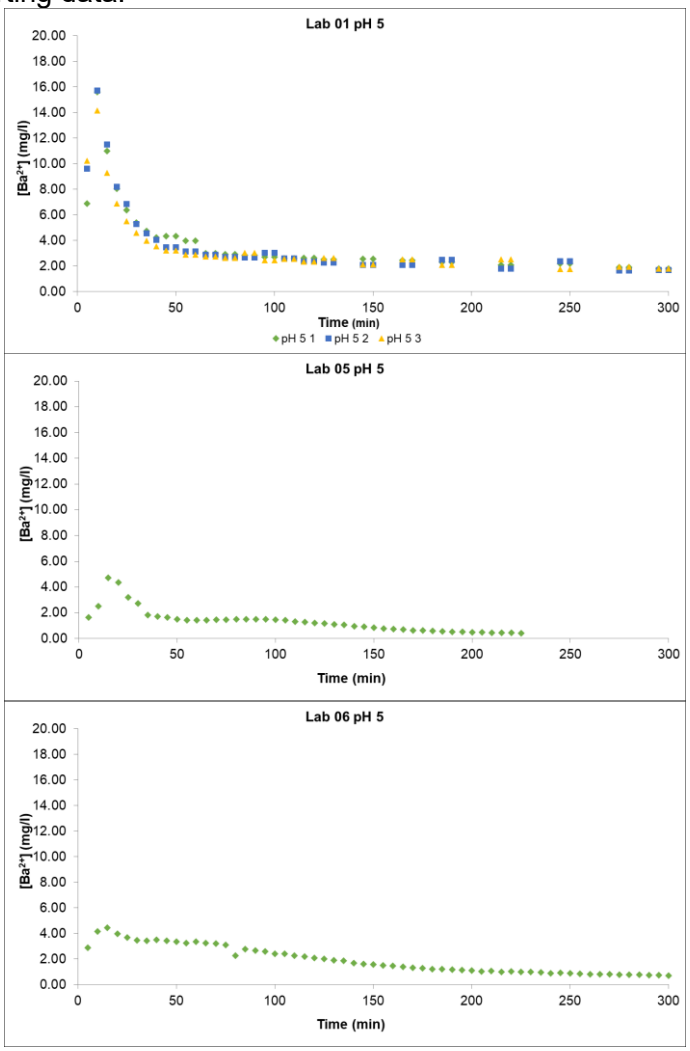


Figure 36: Results for part C1 with ZnO for pH 8, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Please note the different scales

2.3.2. Part C1 $BaSO_4$

Figure 37 to Figure 39 show the concentrations measured in the outflow of the setup over time for the four labs reporting data.



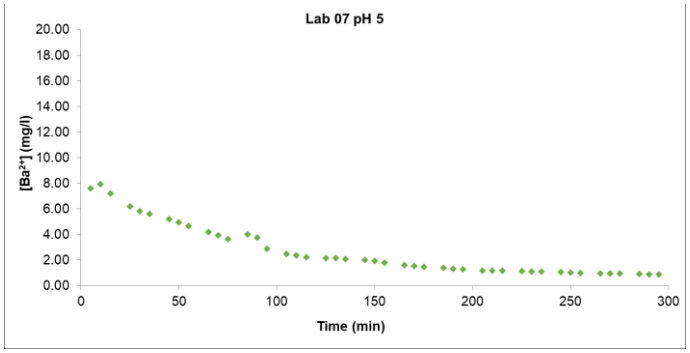
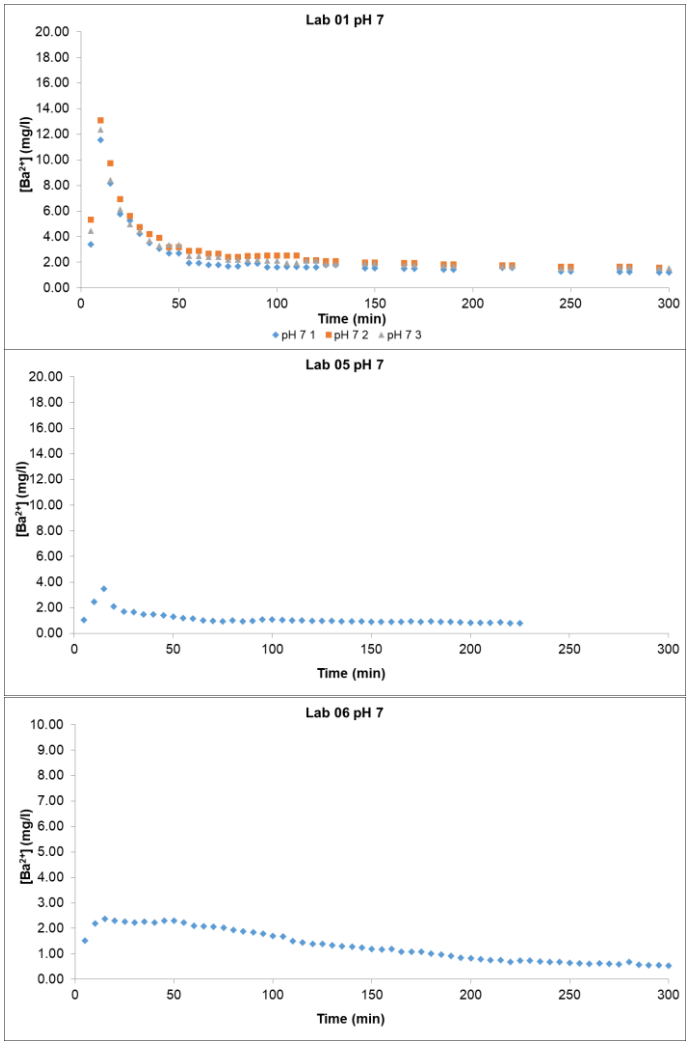


Figure 37: Results for part C1 with $BaSO_4$ for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates.



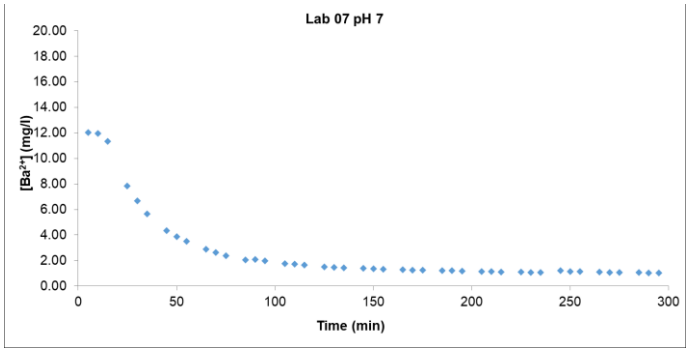
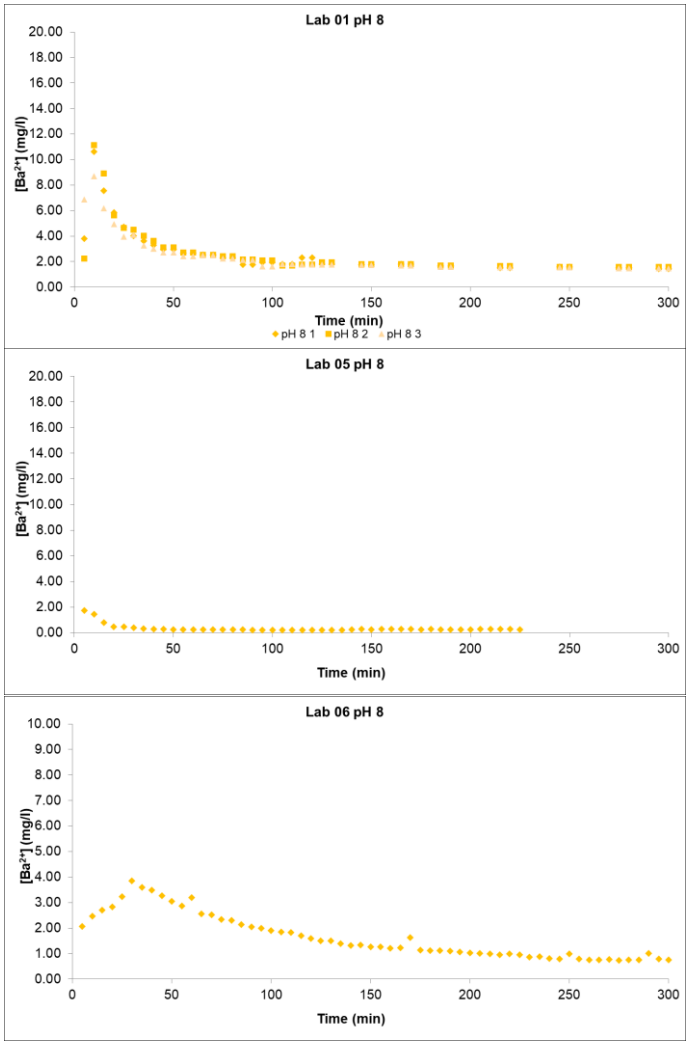


Figure 38: Results for part C1 with $BaSO_4$ for pH 7, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates.



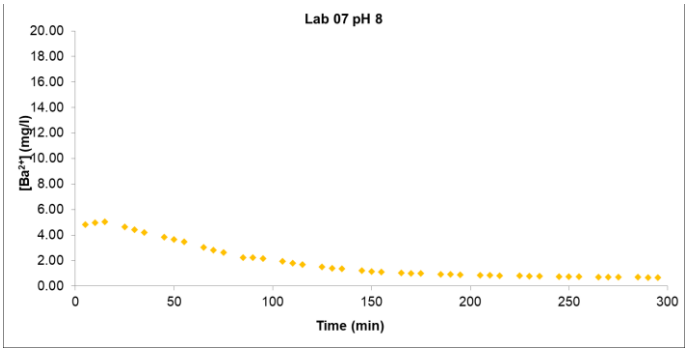


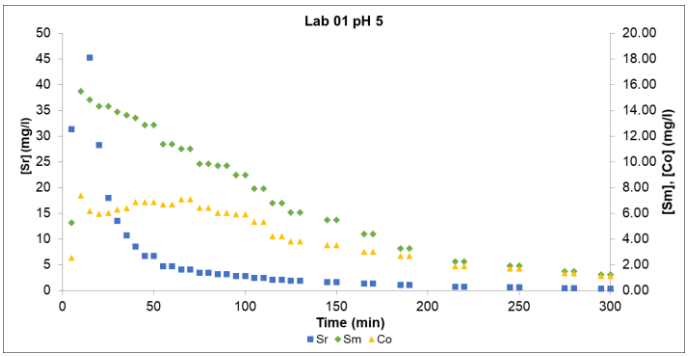
Figure 39: Results for part C1 with $BaSO_4$ for pH 8, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates.

With regard to the concentration levels all labs reported data on a similar concentration level for the steady state part of the curves. However, the shape of the reported curves was again highly variable between the labs. The triplicate measurements provided by lab 01 suggest again an excellent inhouse - reproducibility of the applied protocol for this laboratory.

Overall, the results suggest that there might be a significant influence of the experience of technical staff and individual implementation of these setup in the different labs. The observed different curves might be explainable e.g. by non-homogenous distribution of the material on the filter, air-pockets still present in the filter holder etc.

2.3.3. Part C1 Mixed Oxide

Figure 40 to Figure 42 show the outflow concentrations for the part C1 setup with the mixed oxide.



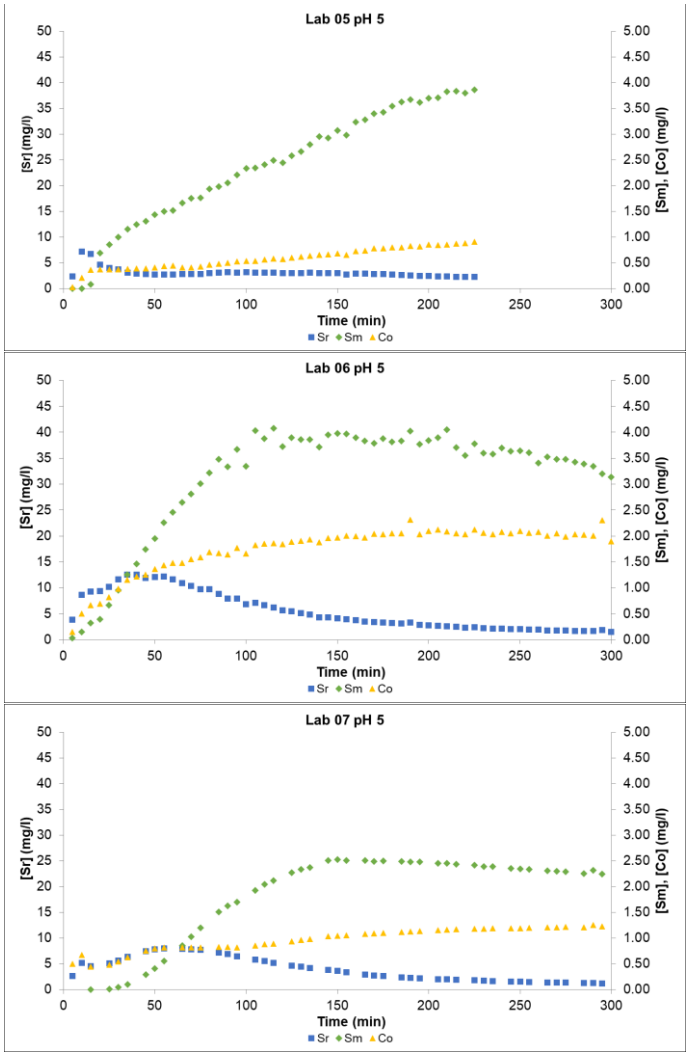
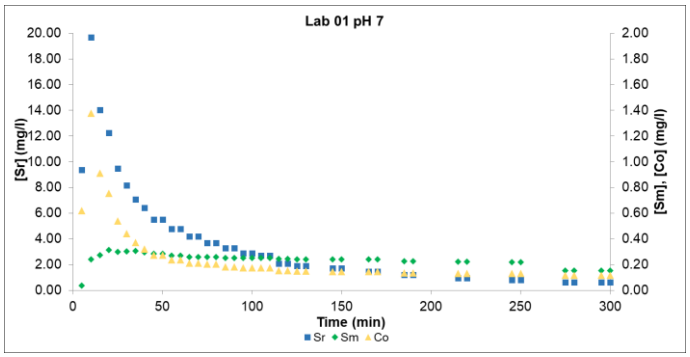


Figure 40: Results for part C1 with mixed oxide for pH 5, for labs 01, 05, 06 and 07. Lab 01 provided all data in triplicates. Only one of three reported data sets shown for lab 01.



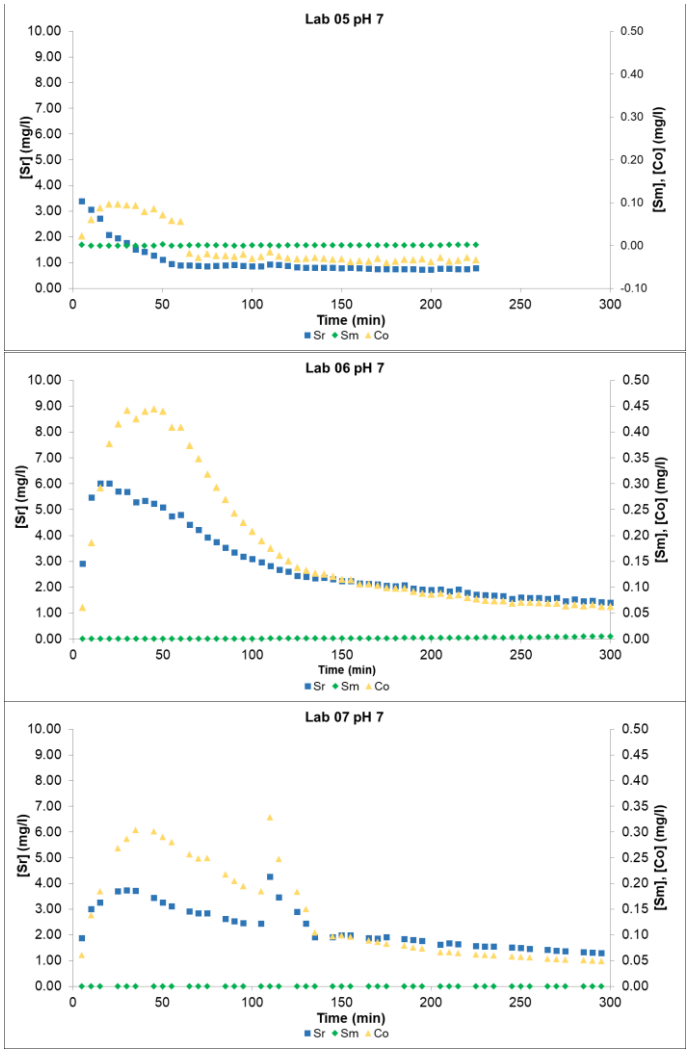
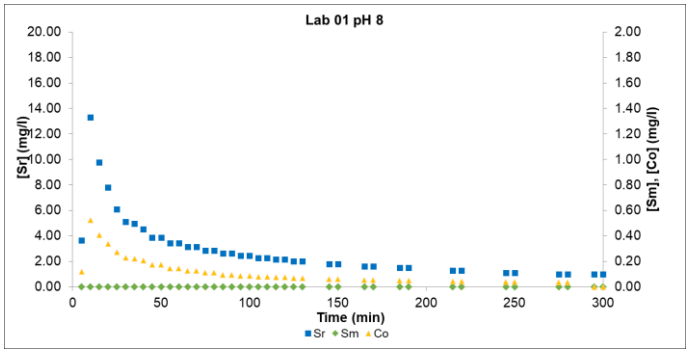


Figure 41: Results for part C1 with mixed oxide for pH 7, for labs 01, 05, 06 and 07. Only one of three reported data sets shown for lab 01. Please note the different scaling for lab 01.



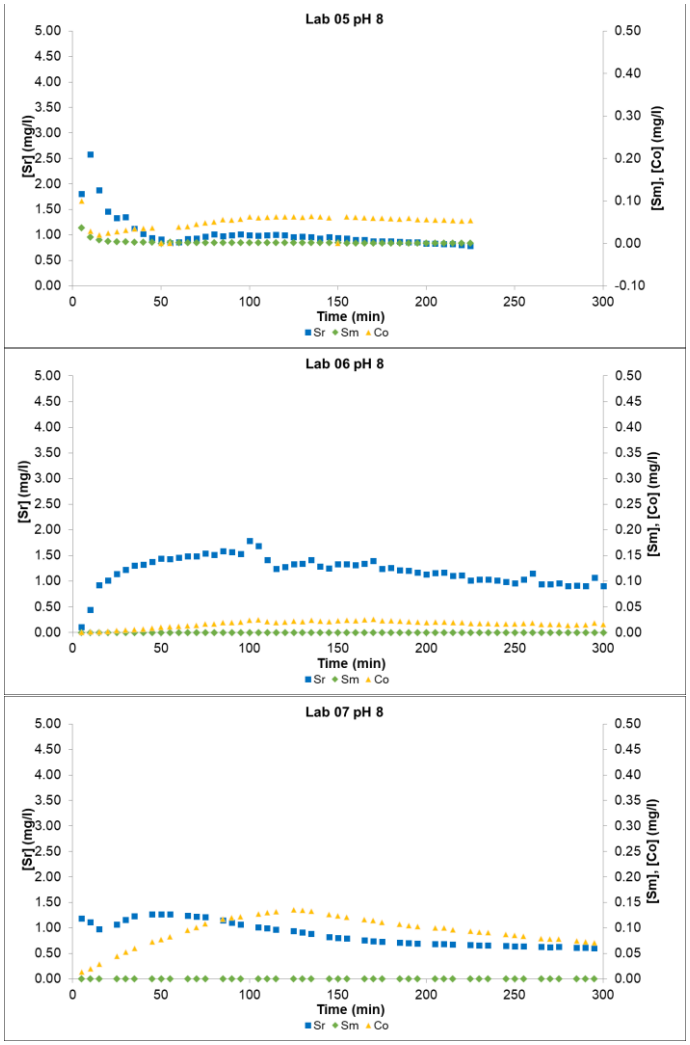


Figure 42: Results for part C1 with mixed oxide for pH 8, for labs 01, 05, 06 and 07. Only one of three reported data sets shown for lab 01. Please note the different scaling for lab 01.

2.3.4. Part C2

The following table summarize the measured concentrations in the receiving vessel after 180 min of test performance.

Table 18: Results Part C2 concentrations for all three tested materials at pH 5

Lab	Concentration Zn / [mg/l]	Concentration Ba / [mg/l]	Concentration Sm / [mg/l]	Concentration Sr / [mg/l]	Concentration Co / [mg/l]
01	39.74	4.18	6.05	4.64	3.54
05					
06	10.7	2.12	0.97	4.99	0.76
07	29.54	3.89	1.31	4.89	0.87

Table 19: Results Part C2 concentrations for all three tested materials at pH 7

Lab	Concentration Zn / [mg/l]	Concentration Ba / [mg/l]	Concentration Sm / [mg/l]	Concentration Sr / [mg/l]	Concentration Co / [mg/l]
01	13.56	2.09	0.34	3.50	0.25
05					
06	0.88	2.00	<0.001	1.96	0.09
07	8.35	3.17	<0.001	2.42	0.19

Table 20: Results Part C2 concentrations for all three tested materials at pH 8

Lab	Concentration Zn / [mg/l]	Concentration Ba / [mg/l]	Concentration Sm / [mg/l]	Concentration Sr / [mg/l]	Concentration Co / [mg/l]
01	No data reported	No data reported	0.01	1.65	0.09
05					
06	0.14	1.96	0.001	0.95	0.16
07	0.20	2.48	<0.001	1.00	0.08

Appendix

A.1 Standard operating procedure (SOP) for the round robin test / validation plan

Standard Operating Procedures

– Nanomaterial solubility and dissolution rate –

In support of OECD guideline development

Frank von der Kammer

Boris Meisterjahn

Nicola Schröder

Kathrin Schwirn

Doris Völker



Part A: Solubility Quick Test

This test delivers the apparent solubility of a NM after 24h in a simple medium buffered at pH7

Materials:

ICP-OES spectrometer or equivalent with LoQ ≤ 100 µg/L of the target substance(s)

Precision balance (1.00000 g)

pH-meter and pH electrode (calibrated at pH 7 and 10)

horizontal shaker (200 rpm capable)

Centrifuge with swing-out rotor for 15 mL Falcon-type tubes (min. 3000 g)

Vortexer

1 L PE or PP bottle

500 mL volumetric flask

125 mL bottles with cap (min. 4 pcs), PTFE, FEP or PFA preferred but PMP and PE also accepted.

5 mL pipette

20 Centrifugal Ultrafiltration tubes 10.000 g/mol MWCO, 6 mL (similar geometry to 15 mL Falcon centrifuge tubes) (e.g. Thermo Scientific™ Pierce™ Protein Concentrators PES, 10k MWCO, 2-6 mL)

Chemicals:

MOPS buffer (e.g. Thermo Scientific Chemicals MOPS, 1.0M buffer sol., pH 7.0)

$NaNO_3$ analytical grade p.a.

HNO_3 65% high purity (suprapur) grade

HNO_3 0.1M analytical grade p.a.

NaOH 0.1M analytical grade p.a.

100 ppm $CuNO_3$ solution, prepared in 10 mM MOPS buffer solution, pH ~ 7

100 ppm $AgNO_3$ solution, prepared in 10 mM MOPS buffer solution, pH ~ 7 (only in case that copper is a target element)

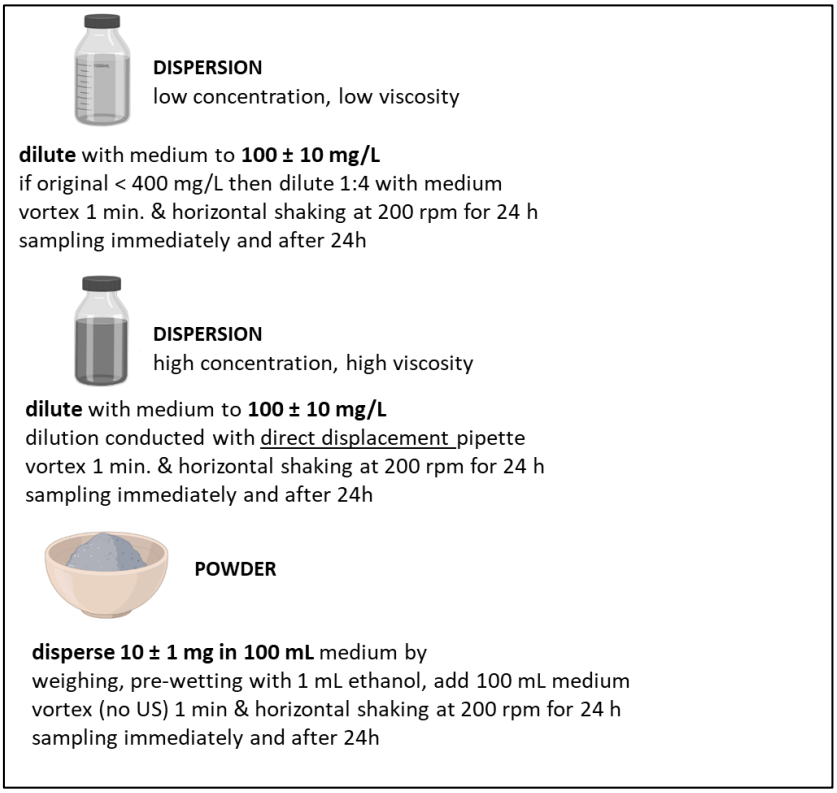
General Procedure

The test principle is a batch test based on:

1) the preparation of 100 mL nanomaterial (NM) dispersion with 100 mg/L initial concentration from a dispersion or powder. A good mixing is required but obtaining a fully stable dispersion is not required.
2) the continuous shaking of the dispersion on a horizontal shaker
3) sub-sampling at 0 h and 24 h and centrifugal ultrafiltration of the sample dispersion to obtain the dissolved fraction.
4) the determination of the concentration of the dissolved fraction of the main elements constituting the nanomaterial on a suitable device with an LoQ of ≤100 µg/L for the target element(s) (e.g. AAS, ICP-OES, ICP-MS). The selected elements should allow the calculation of the amount of dissolved nanomaterial (known stoichiometry). It should be considered that for NMs of medium to high solubility techniques with high sensitivity such as ICP-MS might require excessive dilution leading to possible errors.

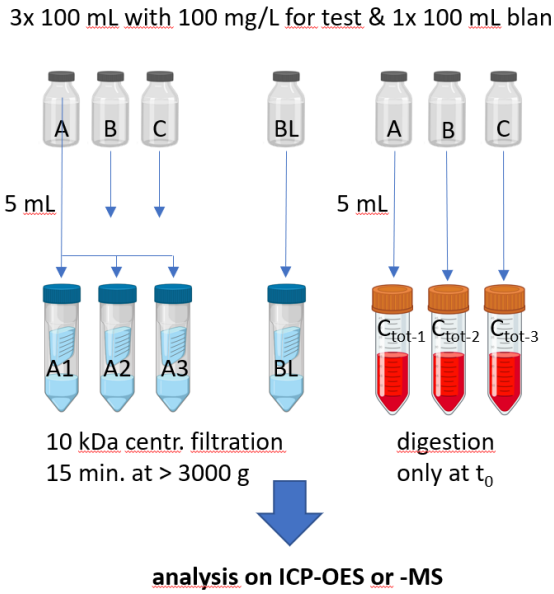
Workflow

While the dissolution test procedure is the same for NM delivered as dispersions or powders, the preparation of the NM test dispersion differs:



The experimental and analytical workflow is based on 125 mL screwcap bottles as batch reactors (PTFE or PFA preferred, PE is accepted), placed for 24 h lie-flat on a horizontal shaker, and pre-conditioned 6 mL centrifugal ultrafiltration units for solid/solute separation. Triplicates of dispersions, triplicates of filtrations of each dispersion plus a single blank are produced at two time-points (t_0 and t_{24}). Additionally, the total concentration of NM in the test dispersions is obtained after a digestion suitable for the NM under

consideration. This is shown in the following graph:



Also, the digested samples will be analyzed in triplicates. Thus, in total 20 samples must be analyzed by the analytical technique of choice (2 blanks, 3x3x2 filtrations). Plus, the 3 triplicates from the nanomaterial digests. The elemental analysis is performed according to the individual laboratory's method of choice. The chosen method, instrument type, settings, calibrations, and figures of merit must be reported.

Preparation of the test medium for the testing of a powder NM

The medium used for this test consists of ultrapure water, a biochemical buffer at pH 7 (MOPS, 25 mM) to keep the pH constant and sodium nitrate (10 mM) as a background electrolyte.

1) Weigh 425 mg $NaNO_3$ into a 500 mL volumetric flask.
2) Pipette 12.5 mL of a 1 M pH 7 MOPS buffer solution into the volumetric flask.
3) Add ca. 250 mL ultrapure water and shake the flask until the salt has been dissolved.
4) Add ultrapure water up to the marking to achieve the desired concentrations.
5) Transfer the prepared medium into a 1000 mL PE or PP bottle.
6) Determine the pH of the medium. If the pH is not at pH 7 ± 0.1 adjust the pH using small volumes of dilute sodium hydroxide solution or dilute nitric acid (e.g. 0.1 M) until pH 7 ± 0.1 is obtained.
7) Let the medium rest for 24 h allowing contact to the atmosphere (place the screwcap loosely on the bottle).
8) After 24 h determine pH and adjust as before. Try to meet the pH 7.00 as good as possible, ± 0.1 pH unit is the maximum acceptable.
9) After 48 h and/or directly before the start of the test, determine pH and adjust as before. Try to meet the pH 7.00 as good as possible, ± 0.1 pH unit is the maximum acceptable.

! The solubility limit of a NM can change by orders of magnitude per pH unit. Therefore, the setting of the pH is the greatest source of error in this experiment.

It was observed that the medium slowly changes pH even after 24 h. That is the reason why two pH adjustments at 24 h and ≥48 h after preparation of the medium are requested.

Preparation of the test dispersions and centrifugal ultrafiltration units

The preparation of the test dispersions is immediately followed by the first sampling (t_{0h}) and ultrafiltration. Therefore, the centrifugal ultrafiltration tubes need to be prepared and conditioned directly before the dispersions are prepared.

Conditioning of the centrifugal ultrafiltration tubes

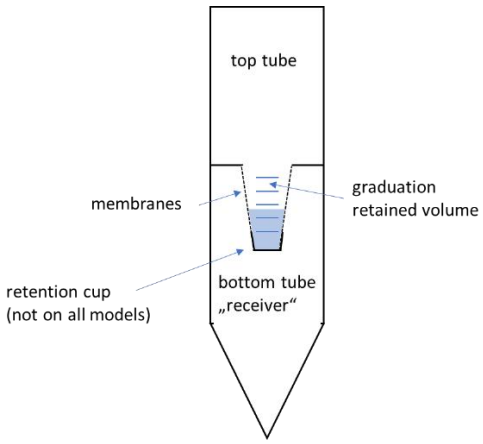
The pre-treatment of the ultrafiltration tubes (PES membrane, 10 kDa MWCO) is required to prevent losses of the dissolved ions to the membrane due to adsorption. A competing ion other than the target ion is applied at a high concentration to block possible adsorption sites on the membrane.

The pretreatment consists in the introduction of 5 ml of a 100 ppm copper nitrate ($CuNO_3$) or silver nitrate ($AgNO_3$), (if copper is among the target elements) solution. The tubes are then centrifuged at min. 3000 g for 15 min. The filtrated solution is discarded. Then, 5 ml of the medium is introduced into the tubes for the rinsing procedure and the tubes are centrifuged again at min. 3000 g for 15 min. The filtrated solution is again discarded. The bottom parts (receiver tube) should be dried with paper tissue or compressed air.

The tube´s membrane itself must not be left to dry before filtration of the samples, this would lead to membrane blocking. Therefore, the pretreatment must be finished maximum 30 min before sampling at t_{0h} and t_{24h}.

Remarks on centrifugal ultrafiltration:

The centrifugation tubes should be inspected for complete filtration. The tubes can be constructed to retain a certain volume (mostly around 200 µL).



If this is observed, the volume of the retained sample should be determined, either by inspecting the graduation present in some of these devices or by pipette extraction and weighing. If more than 500 µL are left in the top tube of the ultrafiltration tubes, g-force of the centrifuge might be too low or the membrane type applied in the tubes restricts the flow more than others. Increase the g-force in the centrifuge. If this cannot be achieved run the filtration for longer time and report this deviation from the SOP. The amount of possibly retained sample and the resulting dilution by 100 µL of nitric acid in the receiving bottom tubes must be accounted for. Alternatively, the receiving tubes can be weighted before and after filtration to determine the deviation from 5.00 mL.

As the preparation of the dispersions and the centrifugation require about 30 minutes in total, the first point (t_{0h}) is temporally “blurred” since dissolution is already proceeding during preparation and filtration.

Preparation of the dispersion and first sampling at t_{0h} – from NM Dispersions – 100 ppm batch

The 125 ml bottles (PTFE, FEP, PFA or PMP, PE with cap) are used for the 100 ppm batches. First, the volume of the original NM suspension, necessary to reach 100 ppm in 100 ml final volume, is pipetted into the Teflon bottles in triplicates (3 bottles). The volume of original NM dispersion required is based on the manufacturer value for its concentration.

> ***!*** *If the original dispersion has a NM concentration of less than 400 mg/L and the target concentration of 100 mg/l cannot be attained, a 1:4 dilution with medium is performed. This dilution is required to reduce possible effects of the NM dispersion medium on the pH and chemistry of the test medium.*
>
> ***!*** *When preparing the test dispersions by diluting an existing NM dispersion, the concentrations of buffer and salts in the medium must be increased to reflect the dilution by the NM dispersion. However, a dilution of the medium by up to 10 % is acceptable.*
>
> ***!*** *If the original NM dispersion has a high viscosity, preventing the use of normal air-cushion pipettes, a direct displacement pipette is required for dosing.*

Then the volume needed for 100 mL final volume of the medium is filled into the bottles from a graduated cylinder and the bottles are firmly closed.

A fourth bottle is filled with 100 mL medium without the NM (blank).

The batches are agitated manually and thoroughly (a vortexer can be used but no ultrasonication) for 1 minute.

The bottles are opened, and a 5 mL pipette is used to draw 5.00 mL subsamples from each replicate (A, B, C). The samples should be drawn from the center of the bottle and the bottle agitated in-between sampling.

Right before pipetting the sample into the top part of the tubes, the bottom part of the tubes is pre-loaded with 100 µl of 6M HNO_3 to acidify the samples after filtration and immediately stabilize the dissolved ions at low pH. This should be done right before loading the tubes with sample because otherwise acidic fumes of the acid could be absorbed in the wet membranes and alter the pH in the sample above the membrane. It is best practice to place the top and the bottom parts separately into a vial holder and fill them with acid and sample. Then quickly combine them and centrifuge them immediately.

For each dispersion replicate (A, B, C) three ultrafiltration tubes are filled with sample (A1, A2, A3; B1,…). From the “blank” bottle only one tube is filled (BL).

The samples are centrifuged at min. 3000 g for 15 minutes. After centrifugation the filtrate is transferred into suitable sample vials for later analysis and stored at 4°C in the dark.

During the centrifugation the pH of the three dispersions is determined and reported.

> *! Although the buffer should keep the pH constant, the (usually) consumption of protons during the dissolution of the NM will change the pH slightly. Especially well dissolving NM as ZnO will have an effect and at 100 mg/L initial loading a small shift cannot be prevented by the buffer. Therefore, reporting of the initial and final pH is mandatory.*

Analysis is performed in the filtrates for the tested nanomaterial constituting elements (see point 4 in General Procedure above). At least 3 triplicate (repetitions) measurements should be performed on the 5 mL of each filtrate.

Determination of C_{tot} in the test dispersions at t_{0h}

While the first samples are centrifuged the bottles are again vigorously agitated and from each dispersion replicate 5 mL are transferred into digestion vessels. The type of vessel and the digestion procedure applied must assure a complete digestion/dissolution of the type of NM in the test. Therefore, recovery experiments for the digestion procedure with the test item should be performed prior to the test. The obtained digests are determined for the target element(s) as the other samples. Dilution might be required. This gives the C_{tot}, the total amount of NM if stochiometry is known. If the stochiometry is not known, it gives the C_{tot} of the target element(s)to be expected from the dosed NM.

Then the bottles are wrapped in aluminum foil and placed on an orbital shaker at 200 rpm. The bottles are placed horizontally for better agitation.

After 24h the sampling of A1 to C3 and the blank is repeated as with t_{0h}. The pH in the test bottles is determined and recorded.

Preparation of the dispersion and first sampling at t_{0h} – from NM Powders – 100 ppm batch

The 125 ml bottles (PTFE, FEP, PFA or PE or PMP with cap) are used for the 100 ppm batches. First, 10 mg ± 1 mg is weighed and added into the Teflon bottles in triplicates. A small amount of pure ethanol ≤ 1 mL is dropped on the NM powder as a pre-wetting step as described in the NANoGENOTOX batch dispersion protocol, validated as part of the FP7 NANoREG project. Wait for ~ 5 minutes before adding the medium.

> *! If the NM powder or the bottles show electrostatic charging, a proper weighing might be prevented. In this case use anti-static measures as e.g. the ZeroStat3 anti-static pistol (Sigma Aldrich) or weighing into an anti-static weighing boat and transfer of the powder into the bottle with the 1 mL of ethanol.*

Then 100 mL of the medium is filled into the bottles from a graduated cylinder and the bottles are firmly closed.

A fourth bottle is filled with 100 mL medium without the NM (blank).

The batches are agitated manually and thoroughly (a vortexer can be used but no ultrasonication) for 1 minute.

The bottles are opened, and a 5 mL pipette is used to draw 5.00 mL subsamples from each replicate (A, B, C). The samples should be drawn from the center of the bottle and the bottle agitated right before sampling.

Right before placing the sample into the top part of the ultrafiltration tubes, the bottom part of the tubes is pre-loaded with 100 µl of 6 M HNO_3 to acidify the samples after filtration and immediately stabilize the dissolved ions at low pH. This should be done right before loading the tubes with sample because otherwise acidic fumes of the acid could be absorbed in the wet membranes and alter the pH in the sample above the membrane. It is best practice to place the top and the bottom parts separately into a vial holder and fill them with acid and sample. Then quickly combine them and centrifuge them immediately.

For each dispersion replicate three ultrafiltration tubes are filled with sample (A1, A2, A3; B1,…). From the “blank” bottle only one tube is filled (BL).

The samples are centrifuged at min. 3000 g for 15 minutes. After centrifugation the filtrate is transferred into suitable metal free sample vials for later analysis and stored at 4°C in the dark.

During the centrifugation the pH of the three dispersions is determined and reported.

! Although the buffer should keep the pH constant, the (usually) consumption of protons during the dissolution of the NM will change the pH slightly. Especially well dissolving NM as ZnO will have an effect and at 100 mg/L initial loading a small shift cannot be prevented by the buffer. Therefore, reporting of the initial and final pH is mandatory.

Analysis is performed in the filtrates for the tested nanomaterial constituting elements on ICP-OES or ICP-MS (see point 4 in General Procedure above). At least 3 triplicate (repetitions) measurements should be performed on the 5 mL of each filtrate.

Determination of C_{tot} in the test dispersions

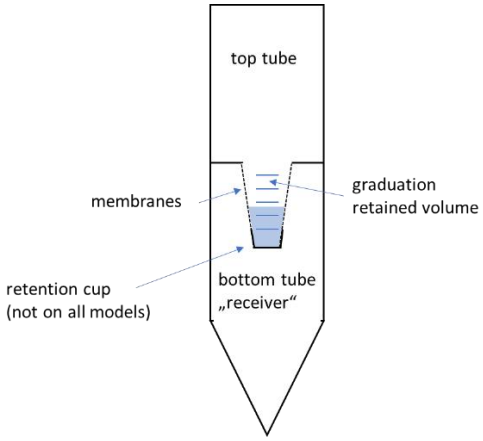
Since the dilution factor is relatively high, a representative sampling from the dispersions prepared from powders is difficult, especially with NMs which are not easily dispersed in the medium. Therefore, the original NM powder is digested directly. 10 mg ± 1 mg is weighed into digestion vessels in triplicate. Digestion procedure is performed according to the individual laboratory's method of choice e.g. microwave assisted digestion with aqua regia. The type of vessel and the digestion procedure applied must assure a complete digestion/dissolution of the type of NM in the test. The obtained digests are determined for the target element(s) as the other samples. Dilution might be required. This gives the C_{tot}, the total amount of NM if stoichiometry is known. If the stoichiometry is not known, it gives the C_{tot} of the target element(s)to be expected from the dosed NM.

Then the bottles are wrapped in aluminum foil and placed on an orbital shaker at 200 rpm. The bottles are placed horizontally for better agitation.

After 24 h the sampling of A1 to C3 and the blank is repeated as with t_{0h}, the pH in the test bottles is determined and recorded.

Remarks:

The centrifugation tubes should be inspected for complete filtration. The tubes can be constructed to retain a certain volume (mostly around 200 µL).



If this is observed, the volume of the retained sample should be determined, either by inspecting the graduation present in some of these devices or by pipette extraction and weighing. If more than 500 µL are left in the top tube of the ultrafiltration tubes, g-force of the centrifuge might be too low or the membrane type applied in the tubes restricts the flow more than others. Increase the g-force in the centrifuge. If this cannot be achieved run the filtration for longer time and report this deviation from the SOP. The amount of

possibly retained sample and the resulting dilution by 100 µL of nitric acid in the receiving bottom tubes must be accounted for. Alternatively, the receiving tubes can be weighted before and after filtration to determine the deviation from 5.00 mL.

As the preparation of the dispersions and the centrifugation require about 30 minutes in total, the first point (t_{0h}) is temporally “blurred” since dissolution is already proceeding during preparation and filtration.

Part B: Solubility and Dissolution Rate - Extended Test

This test delivers the apparent solubility of a NM after 96 h in a simple medium buffered at pH 5, 7 and 8 and information about the dissolution rate through time-resolved sampling.

Materials:

ICP-OES spectrometer or equivalent with LoQ ≤ 10 µg/L of the target substances

Precision balance (1.00000 g)

pH-meter and pH electrode, calibrated at pH 4 and 7 for experiments at pH 5 and 7 and calibrated at pH 7 and 10 for experiments at pH 8

horizontal shaker (200 rpm capable)

Centrifuge with swing-out rotor for 15 mL Falcon-type tubes (min. 3000 g)

Vortexer

1 x 1 L PE or PP bottle per pH condition

1 x 1000 mL volumetric flask per pH condition

250 mL bottles with cap (PTFE, FEP or PFA preferred, PE and PMP accepted) (min. 4 pcs, 12 when all pH should be tested in parallel)

5 mL pipette

74 (per pH condition) Centrifugal Ultrafiltration tubes 10.000 g/mol MWCO, 6 mL (similar geometry to 15 mL Falcon centrifuge tubes) (e.g. Thermo Scientific™ Pierce™ Protein Concentrators PES, 10K MWCO, 2-6 mL)

Chemicals:

MES and MOPS buffers (e.g. Thermo Scientific Chemicals MOPS, 1.0 M buffer sol., pH 7.0 and pH 8 & MES 1.0 M buffer sol., pH 5.0)

$NaNO_3$ analytical grade p.a.

HNO_3 65% suprapur grade

HNO_3 0.1 M analytical grade p.a.

NaOH 0.1 M analytical grade p.a.

100 ppm $CuNO_3$ solution, prepared in 10 mM MOPS buffer solution, pH ~ 7

100 ppm $AgNO_3$ solution, prepared in 10 mM MOPS buffer solution, pH ~ 7 (only in case that copper is a

target element)

General Procedure

The test principle is a batch test based on:

1) the preparation of a nanomaterial (NM) dispersion with 10 mg/L initial concentration from a dispersion or powder. A good mixing is necessary but obtaining a fully stable dispersion is not required.
2) the continuous shaking of the dispersion on a horizontal shaker
3) sub-sampling at 0, 30, 60, 120, 240 min. and 24, 48, 96 h and centrifugal ultrafiltration of the dispersion to obtain the dissolved fraction. One blank is taken at the first sampling point and at 96 h.
4) the determination of the concentration of the dissolved fraction of the main elements constituting the nanomaterial on a suitable device with an LoQ of ≤ 10 µg/l for the target element(s) (e.g. AAS, ICP-OES, ICP-MS). The selected elements should allow the calculation of the amount of dissolved nanomaterial (known stoichiometry).

Workflow

While the dissolution test procedure is the same for NM delivered as dispersions or powders, the preparation of the NM test dispersion differs:



DISPERSION

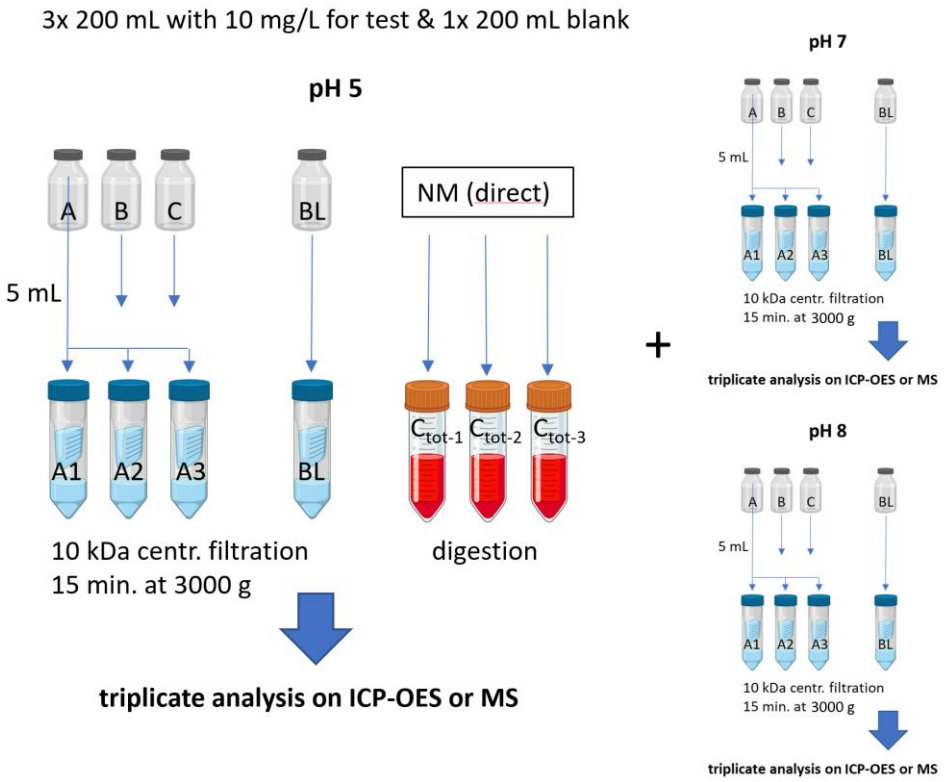
dilute with medium to **10 ± 1 mg/L**
For viscous NM dispersions dilution is conducted with direct displacement pipette
shake 1 min. & horizontal shaking at 200 rpm for 24 h
sampling immediately and after 24h



POWDER

disperse 2 ± 0.2 mg in 200 mL medium by weighing, pre-wetting with 1 mL ethanol, add 200 mL medium
shake (no US) 1 min & horizontal shaking at 200 rpm for 24 h
sampling immediately and after 24h

The experimental and analytical workflow uses 250 mL PTFE, FEP or PFA (PE and PMP also accepted) screwcap bottles as batch reactors, placed for 96 h lie-flat on a horizontal shaker and pre-conditioned 6 mL centrifugal ultrafiltration units for solid/solute separation. Triplicates of dispersions, triplicates of filtrations and triplicate analyses (repetitions) from each filtrate, plus a single blank, are performed.



Preparation of the test media

The media used for this test consists of ultrapure water, a biochemical buffer at pH 5 (MES 25 mM), pH 7 and pH 8 (MOPS 25 mM) and sodium nitrate (10 mM) as a background electrolyte.

The pH 5 medium:

1) Weigh 846 mg $NaNO_3$ into a 1000 mL volumetric flask.
2) Pipette 25 mL of a 1 M pH 5 MES buffer solution into the volumetric flask.
3) Add ca. 500 mL ultrapure water and shake the flask until the salts have been dissolved.
4) Add ultrapure water up to the marking to achieve the desired concentrations.
5) Transfer the prepared medium into a 1000 mL PE or PP bottle.
6) Determine the pH of the medium. If the pH is not at pH 5 ± 0.1 adjust the pH using small volumes of dilute sodium hydroxide solution or dilute nitric acid until pH 5 ± 0.1 is obtained. After re-adjustment fill up to the final volume.
7) Let the medium rest for 24 h allowing contact to the atmosphere (place the screwcap loosely on the bottle). After 24 h determine pH and adjust as before. Try to meet the pH 5.00 as good as possible.
8) After 48 h and/or directly before the start of the test, determine pH and adjust as before. Try to meet the pH 5.00 as good as possible.

! The solubility limit of a NM can change by orders of magnitude per pH unit. Therefore, the setting of the pH is the greatest source of error in this experiment.

It was observed that the medium slowly changes pH even after 24 h. That is the reason why two pH adjustments at 24 h and ≥48 h after preparation of the medium are requested.

The pH 7 medium:

1) Weigh 846 mg $NaNO_3$ into a 1000 mL volumetric flask.
2) Pipette 25 mL of a 1 M pH 7 MOPS buffer solution into the volumetric flask.
3) Add ca. 500 mL ultrapure water and shake the flask until the salts have been dissolved.
4) Add ultrapure water up to the marking to achieve the desired concentrations.
5) Transfer the prepared medium into a 1000 mL PE or PP bottle.
6) Determine the pH of the medium. If the pH is not at pH 7 ± 0.1 adjust the pH using small volumes of dilute sodium hydroxide solution or dilute nitric acid until pH 7 ± 0.1 is obtained. After re-adjustment fill up to the final volume.
9) Let the medium rest for 24 h allowing contact to the atmosphere (place the screwcap loosely on the bottle). After 24 h determine pH and adjust as before. Try to meet the pH 7.00 as good as possible.
7) After 48 h and/or directly before the start of the test, determine pH and adjust as before. Try to meet the pH 7.00 as good as possible.

! The solubility limit of a NM can change by orders of magnitude per pH unit. Therefore, the setting of the pH is the greatest source of error in this experiment.

It was observed that the medium slowly changes pH even after 24 h. That is the reason why two pH adjustments at 24 h and ≥48 h after preparation of the medium are requested.

The pH 8 medium:

1) Weigh 846 mg $NaNO_3$ into a 1000 mL volumetric flask.
2) Pipette 10 mL of a 1 M pH 8 MOPS buffer solution into the volumetric flask.
3) Add ca. 500 mL ultrapure water and shake the flask until the salts have been dissolved.
4) Add ultrapure water up to the marking to achieve the desired concentrations.
5) Transfer the prepared medium into a 1000 mL PE or PP bottle.
6) Determine the pH of the medium. If the pH is not at pH 8 ± 0.1 adjust the pH using small volumes of dilute sodium hydroxide solution or dilute nitric acid until pH 8 ± 0.1 is obtained. After re-adjustment fill up to the final volume.
10) Let the medium rest for 24 h allowing contact to the atmosphere (place the screwcap loosely on the bottle). After 24 h determine pH and adjust as before. Try to meet the pH 8.00 as good as possible.
7) After 48 h and/or directly before the start of the test, determine pH and adjust as before. Try to meet the pH 8.00 as good as possible.

! The solubility limit of a NM can change by orders of magnitude per pH unit. Therefore, the setting of the pH is the greatest source of error in this experiment.

It was observed that the medium slowly changes pH even after 24 h. That is the reason why two pH adjustments at 24 h and ≥48 h after preparation of the medium are requested.

Preparation of the test dispersions

The preparation of the test dispersions is immediately followed by the first sampling (t_{0h}) and ultrafiltration. The blank(s) are also taken at this first sampling. The next samplings follow closely at 30, 60, 120 and 240 min. Therefore, the centrifugal ultrafiltration tubes at least for the first four samplings need to be prepared and conditioned directly before the test dispersions (mixtures) are prepared. Especially in the first 2 h there will be no time to condition the centrifugal ultrafiltration tubes in parallel to the sampling.

The three different pH batches may be executed simultaneously. However, the effort and time required for

sampling before filtration will introduce an additional "blurring" of the time points unless all three batches are sampled truly in parallel. If a true parallel sampling and processing of all three pH conditions is envisaged by the performing laboratory, adequate number of personal must be ensured.

Conditioning of the centrifugal ultrafiltration tubes

The pre-treatment of the ultrafiltration tubes (PES membrane, 10 kDa MWCO) is required to prevent losses of the dissolved ions to the membrane due to adsorption. A competing ion other than the target ion is applied at high concentration.

The pretreatment consists in the introduction of 5 ml of a 100 ppm copper nitrate ($CuNO_3$) or silver nitrate ($AgNO_3$), (if copper is among the target elements) solution. The tubes are then centrifuged at min. 3000 g for 15 min. The filtrated solution is discarded. Then, 5 ml of the medium that will be used is introduced into the tubes for the rinsing procedure and the tubes are centrifuged again at > 3000 g for 15 min. The filtrated solution is again discarded. The bottom parts (receiver tube) should be dry.

The tube´s membrane must not be left to dry before filtration of the samples, this would lead to membrane blocking. Therefore, the pretreatment must be finished maximum 30 min before sampling or the tubes. When preparing all tubes needed for the first four sampling points, it is good practice to dose 500 µl of the medium that will be used with the tubes into the top tube to keep the membranes wet. This liquid is then removed as completely as possible (e.g. by pipetting) before the filtration of the sample.

Preparation of the dispersion and first sampling at t_{0h} - NM Dispersions – 10 ppm batch

Teflon bottles (250 ml – preferably PTFE, FEP or PFA, PE or PMP is also accepted, with cap) are used for the 10 ppm batches. First, the volume of the original NM suspension, necessary to reach 10 ppm in 200 ml final volume, is pipetted into the Teflon bottles in triplicates. The amount of original NM dispersion required is based on the manufacturer value for its concentration.

> ***!*** *If the original dispersion has a NM concentration of less than 40 ppm, a 1:4 dilution with medium is performed.*
>
> ***!*** *If the original NM dispersion has a high viscosity, preventing the use of normal air-cushion pipettes, a direct displacement pipette is required.*
>
> ***!*** *When preparing the test dispersions by diluting an existing NM dispersion, the concentrations of buffer and salts in the medium must be increased to reflect the dilution by the NM dispersion. However, a dilution of the medium by up to 10 % is acceptable.*

Then the volume needed for 200 mL final volume of the medium is filled into the bottles from a graduated cylinder and the bottles are firmly closed.

A fourth bottle is filled with 200 mL medium without the NM (blank).

The batches are agitated manually and thoroughly (a vortexer can be used but no ultrasonication) for 1 minute.

The bottles are opened and a 5 mL pipette is used to draw 5.00 mL subsamples from each replicate (A, B, C). The samples should be drawn from the center of the bottle and the bottle agitated in-between sampling.

Right before placing the sample into the top part of the tubes, the bottom part of the tubes are pre-loaded with 100 µl of 6 M HNO_3 to acidify the samples after filtration and immediately stabilize the dissolved ions at low pH. This should be done right before loading the tubes with sample because otherwise acidic fumes

of the acid could be absorbed in the wet membranes and alter the pH in the sample above the membrane. It is best practice to place the top and the bottom parts separately into a vial holder and fill them with acid and sample. Then quickly combine them and centrifuge them immediately.

For each dispersion replicate three ultrafiltration tubes are filled with sample (A1, A2, A3; B1,…). From the blank bottle only one tube is filled (BL), the blank is only taken at the first time point and at 96 h.

The samples are centrifuged at > 3000 g for 15 minutes. After centrifugation the filtrate is transferred into suitable sample vials for later analysis and stored at 4°C in the dark.

During the first centrifugation the pH of the three dispersions and the blank is determined and reported. The pH is also measured and recorded for sampling points 60 min, 120 min, 240 min, 24 h, 48 h and 96 h in the dispersions and at 96 h also in the blank.

> *! Although the buffer should keep the pH constant, the (usually) consumption of protons during the dissolution of the NM will change the pH slightly. Especially well dissolving NM as ZnO will have an effect and at 100 mg/l initial loading a small shift cannot be prevented by the buffer. Therefore, reporting of the initial and final pH is mandatory.*

Analysis is performed in the filtrates for the tested nanomaterial constituting elements on ICP-OES or ICP-MS (see point 4 in General Procedure above). At least 3 triplicate (repetitions) measurements should be performed on the 5 mL of each filtrate.

Determination of C_{tot} in the test dispersions

Since the dilution factor is relatively high, a representative sampling from the prepared dispersions is difficult, especially with NMs which are not easily dispersed in the medium. Therefore, the original NM is digested directly. A suitable volume is pipetted into digestion vessels in triplicate. The type of vessel and the digestion procedure applied must assure a complete digestion/dissolution of the type of NM in the test. The obtained digests are determined for the target element(s) as the other samples. Dilution might be required. This gives the C_{tot}, the total amount of NM if stoichiometry is known. If the stoichiometry is not known, it gives the C_{tot} of the target element(s)to be expected from the dosed NM.

Then the bottles are wrapped in aluminum foil and placed on an orbital shaker at 200 rpm. The bottles are placed horizontally for better agitation.

After 30, 60, 120, 240 minutes and 24, 48, 96 h the sampling of A1 to C3 is repeated as with t_{0h}, the pH in the test bottles is determined and recorded for sampling points t_{0h} , 60 min, 120 min, 240 min, 24 h, 48 h and 96 h. A single blank sample is taken at the first time point and at 96 h.

0 min	30 min	60 min.	120 min.	240 min.	24 h	48 h	96 h
A1–C3 pH Blank	A1–C3	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH Blank

Preparation of the dispersion and first sampling at t_{0h} - NM Powders – 10 ppm batch

Teflon bottles Teflon bottles (250 ml – preferably PTFE, FEP or PFA, PMP or PE is also accepted, with cap) are used for the 10 ppm batches. First, 2 mg ± 0.2 mg is weighed and added into the bottles in triplicates. A small amount of ethanol ≤ 1 mL is dropped on the NM powder as a pre-wetting step (as

described in the NANoGENOTOX batch dispersion protocol validated as part of the FP7 NANoREG project). Wait for ~ 5 minutes before adding the medium.

> ***!*** *If the NM powder or the bottles show electrostatic charging, a proper weighing might be prevented. In this case use anti-static measures as e.g. the ZeroStat3 anti-static pistol (Sigma Aldrich) or weighing into an anti-static weighing boat and transfer of the powder into the bottle with the 1 mL of ethanol.*

Then 200 mL (graduated cylinder) of the medium is filled into the bottles and the bottles are firmly closed.

A fourth bottle is filled with 200 mL medium without the NM (blank).

The batches are agitated manually and thoroughly (a vortexer can be used but no ultrasonication) for 1 minute.

The bottles are opened and a 5 mL pipette is used to draw 5.00 mL subsamples from each replicate (A, B, C). The samples should be drawn from the center of the bottle and the bottle agitated in-between sampling.

Right before placing the sample into the top part of the tubes, the bottom part of the tubes are pre-loaded with 100 µl of 6 M HNO_3 to acidify the samples after filtration and immediately stabilize the dissolved ions at low pH. This should be done right before loading the tubes with sample because otherwise acidic fumes of the acid could be absorbed in the wet membranes and alter the pH in the sample above the membrane. It is best practice to place the top and the bottom parts separately into a vial holder and fill them with acid and sample. Then quickly combine them and centrifuge them immediately.

For each dispersion replicate three ultrafiltration tubes are filled with sample (A1, A2, A3; B1,…). From the "blank" bottle only one tube is filled (BL) and the blank is only taken at the first time point.

The samples are centrifuged at > 3000 g for 15 minutes. After centrifugation the filtrate is transferred into suitable sample vials for later analysis and stored at 4°C in the dark.

During the first centrifugation the pH of the three dispersions and the blank is determined and reported. The pH is also measured and recorded for sampling points 60 min, 120 min, 240 min, 24 h, 48 h and 96 h in the dispersions and at 96 h also in the blank.

> *! Although the buffer should keep the pH constant, the (usually) consumption of protons during the dissolution of the NM will change the pH slightly. Especially well dissolving NM as ZnO will have an effect and at 100 mg/l initial loading a small shift cannot be prevented by the buffer. Therefore, reporting of the initial and final pH is mandatory.*

Analysis is performed in the filtrates for the tested nanomaterial constituting elements on ICP-OES or ICP-MS (see point 4 in General Procedure above). At least 3 triplicate (repetitions) measurements should be performed on the 5 mL of each filtrate.

Determination of C_{tot} in the test dispersions

Since the dilution factor is relatively high, a representative sampling from the prepared dispersions is difficult, especially with NMs which are not easily dispersed in the medium. Therefore the original NM is digested directly. 10 mg ± 1 mg of the NM powder is weighed into a suitable digestion vessel. The type of vessel and the digestion procedure applied must assure a complete digestion/dissolution of the type of NM in the test. The obtained digests are determined for the target element(s) as the other samples. Dilution might be required. This gives the C_{tot}, the total amount of NM in the original NM dispersion and can be used to calculate the true C_{tot} concentration in the test vessels.

Then the bottles are wrapped in aluminum foil and placed on an orbital shaker at 200 rpm. The bottles are

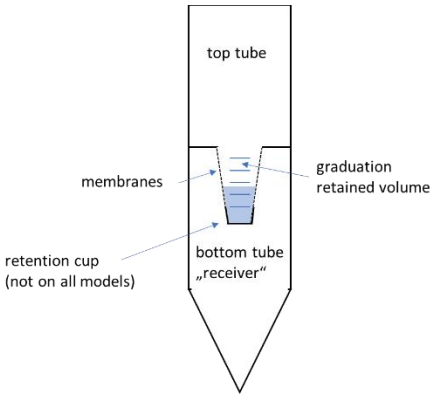
placed horizontally for better agitation.

After 30, 60, 120, 240 minutes and 24, 48, 96 h the sampling of A1 to C3 is repeated as with t_{0h}, the pH in the test bottles is determined and recorded for sampling points 60 min, 120 min, 240 min, 24 h, 48 h and 96 h. A blank sample is taken at the first time point and at 96 h.

0 min	30 min	60 min.	120 min.	240 min.	24 h	48 h	96 h
A1–C3 pH Blank	A1–C3	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH	A1–C3 pH Blank

Remarks:

The centrifugation tubes should be inspected for complete filtration. The tubes can be constructed to retain a certain volume (typically around 200 µL).



If this is observed, the volume of the retained sample should be determined, either by inspecting the graduation present in some of these devices or by pipette extraction and weighing. If more than 500 µL are left in the top tube of the ultrafiltration tubes, g-force of the centrifuge might be too low or the membrane type applied in the tubes restricts the flow more than others. Increase the g-force in the centrifuge. If this cannot be achieved run the filtration for longer time and record this deviation from the SOP.

The amount of possibly retained sample and the resulting dilution by 100 µL of nitric acid in the receiving bottom tubes must be accounted for.

As the preparation of the dispersions and the centrifugation require about 30 minutes in total, the first point (t_{0h}) is temporally “blurred” since dissolution is already proceeding during preparation and filtration.

Also, the following time points are “blurred” by ~ 15 minutes since the reaction continues during the filtration step.

Part C1: Dissolution Rate – Continuous-Flow Test – High Time Resolution

The test delivers the apparent dissolution rate of a NM in a medium under specified continuous-flow conditions at a given pH (here pH 7)

Materials:

ICP-MS spectrometer or equivalent with LoQ ≤ 0.1 µg/L of the target element or substance

Flow-through filter holder for ∅ 47 or 50 mm membrane filters

Peristaltic pump with tubing, delivering a flow rate of 0.1 to 10 mL/min

Programmable fraction collector capable to collect 60 x 5 mL samples, e.g. Omnicoll Atlas

60 metal-free sample collection vials, min. 5 mL each

Tubing and connectors required to connect the filter holder to the pump and fraction collector

Regenerated cellulose ultrafiltration membranes 10 kg/mol MWCO suitable for the chosen filter holder diameter

Regenerated cellulose filtration membrane 0.2 µm cut-off suitable for the chosen filter holder diameter

Glass-fiber filter membranes 1 or 1.5 µm cut-off suitable for the chosen filter holder diameter

Precision balance (1.00000 g)

pH-meter and pH electrode (calibrated at pH 4 and 7 for experiments at pH 5 and 7, calibrated at pH 7 and 10 for experiments at pH 8)

1 L PE or PP bottle

1000 mL volumetric flask

Chemicals:

MOPS buffers (e.g. Thermo Scientific Chemicals MOPS, 1.0 M buffer sol., pH 7.0)

$NaNO_3$ analytical grade p.a.

HNO_3 65% suprapur grade

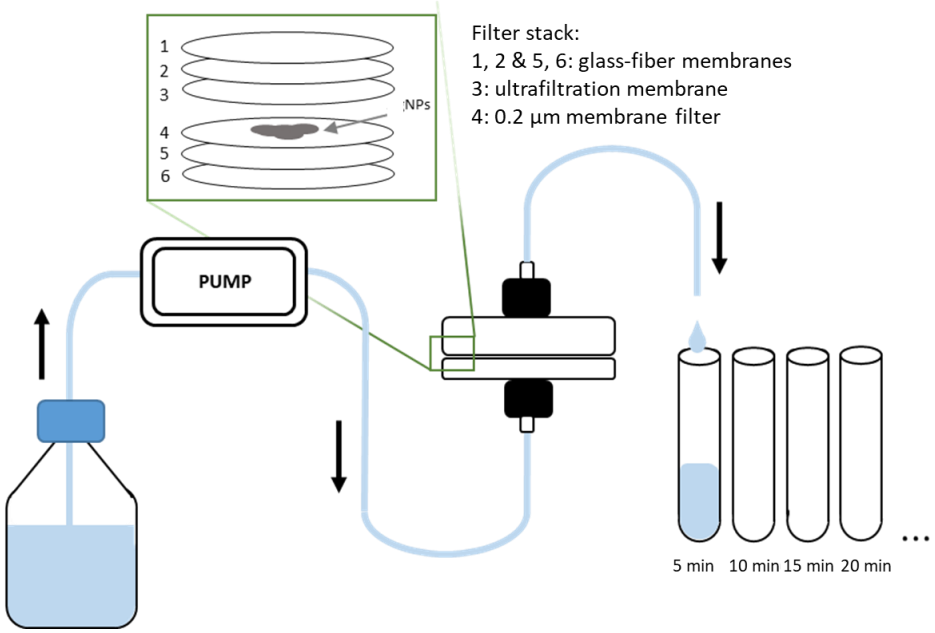
HNO_3 0.1M analytical grade p.a.

NaOH 0.1M analytical grade p.a.

General Procedure

The test principle is a flow-through or continuous-flow test based on:

1) placement of 10 mg nanomaterial (NM) between a sandwich of ultrafiltration membranes, supported by glass-fiber membranes and placement in a filter holder as a flow-through reactor.
2) the continuous flow of a medium through the reactor at flow rates between 0.1 and 10 ml/min for up to 5 hours. Here 1 mL/min is used.
3) sub-sampling of 5 mL samples in a fraction collector to monitor outflow concentrations of the target element and control of pH.
4) the determination of the time dependent outflow concentration of the dissolved element on a suitable device with an LoQ of ≤ 0.1 µg/l for the target element(s) (e.g. AAS, ICP-OES, ICP-MS).





Performing the test

1) The medium is prepared as in Part A or B, required volume per test is flow rate x duration + 20%
2) Place 60 sampling vials in the fraction collector and dose 100 µL of 6 M HNO_3 in 3 vials, leave one out and then again in the next 3 vials, leave one out etc. The vial without acid addition is the one where the pH of the outflow will be monitored. Program the fraction collector to collect 5 min per vial (5 mL at 1 ml/min flowrate).
3) Place the two glass-fiber membranes in the bottom part of the filter holder, they prevent that the ultrafiltration membrane is damaged by the membrane support frit.

   Note: depending on the coarseness of the membrane support frit or mesh these glass-fiber membranes might not be needed or one will be sufficient. The support screen of the BOLA™ PTFE holders requires two filters for support.
4) Place the ultrafiltration membrane in the filter holder.
5) Put the filter holder on an analytical balance. If the tubing connector of the filter holder prevents a proper horizontal placement, use an empty 100 mL beaker and place the holder on the beaker.
6) 10 ± 1 mg of the NM is weighed onto the ultrafiltration membrane disc and distributed as equally as possible on the whole disc area. Leave a margin of 3 mm towards the filter edge free of NM. Electrostatic charge may be a problem, use an anti-electrostatic device, as e.g. ZeroStat3 anti-static pistol (Sigma Aldrich).
7) Place the 0.2 µm filter membrane on the ultrafiltration membrane to sandwich the powder in-between.
8) Place two more glass-fiber membranes on top of the stack.
9) Mount the filter holder in a way that the flow direction will be upwards.
10) Purge all tubes towards the filter holder with medium. Adjust the pump rate to 1 mL/min
11) Connect the filter holder to the pumps.

12) Fill the filter holder with medium. Stop the flow when medium appears at the filter holder outlet.
13) Connect the fraction collector and set the pump to the desired flow rate (typ. 1 mL/min)
14) When the first vial begins to fill, start the fraction collector sequence.
15) After 300 min. close all acidified sample vials and determine and record the pH in the not acidified vials.
16) Determine the concentration of the target element or substance in the samples with an ICP-MS or a suitable device with an LoQ of ≤ 0.1 µg/l for the target element(s).
17) Optional: collect the membranes from the filter holder and digest to retrieve a mass balance.

Part C2: Dissolution Rate – Continuous-Flow Test – integration method

The test delivers the **average** apparent dissolution rate of a NM in a medium under specified continuous-flow conditions and a given pH (here pH 7)

Materials:

ICP-MS spectrometer or equivalent with LoQ ≤ 0.1 µg/L of the target elements or substances

Flow-through filter holder for ∅ 47 or 50 mm membrane filters

Peristaltic pump with tubing delivering a flow rate of 0.1 to 10 mL/min

500 mL acid-cleaned PTFE or PFA bottle, PE is acceptable, for sampling

Tubing and connectors required to connect the filter holder to the pump

Regenerated cellulose ultrafiltration membranes 10 kg/mol MWCO suitable for the chosen filter holder

Regenerated cellulose filtration membrane 0.2 µm cut-off suitable for the chosen filter holder

Glass-fiber filter membranes 1 or 1.5 µm cut-off suitable for the chosen filter holder

Precision balance (1.00000 g)

pH-meter and pH electrode (calibrated at pH 4 and 7 for experiments at pH 5 and 7, calibrated at pH 7 and 10 for experiments at pH 8)

1 L PE or PP bottle for the medium

1000 mL volumetric flask

Chemicals:

MES and MOPS buffers (e.g. Thermo Scientific Chemicals MOPS, 1.0M buffer sol., pH 7.0 and pH 8 & MES 1.0 M buffer sol., pH 5.0)

$NaNO_3$ analytical grade p.a.

HNO_3 65% suprapur grade

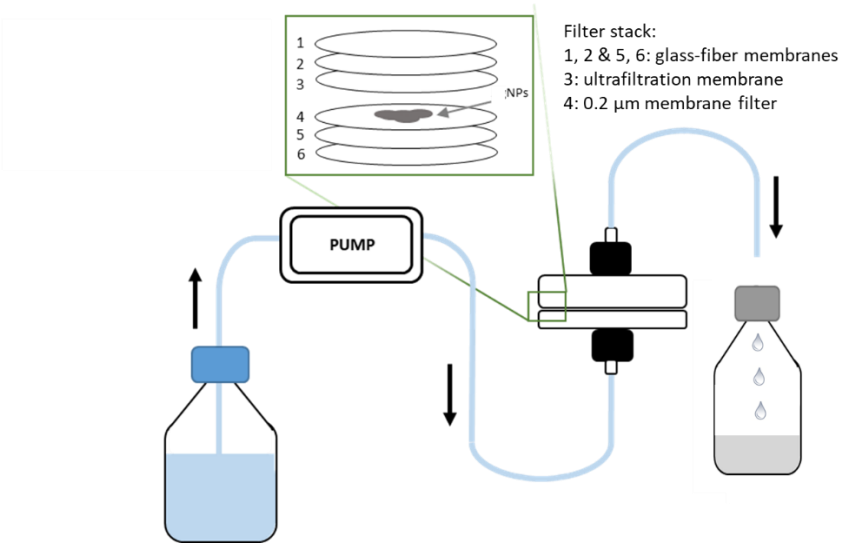
HNO_3 0.1 M analytical grade p.a.

NaOH 0.1 M analytical grade p.a.

General Procedure

The test principle is a flow-through or continuous-flow test based on:

1) placement of 10 mg nanomaterial (NM) between a sandwich of ultrafiltration membranes, supported by glass-fiber membranes and placement in a filter holder as a flow-through reactor.
2) the continuous flow of a medium through the reactor at flow rates between 0.1 and 10 ml/min for up to 5 hours, here 1 ml/min is used.
3) collection of the medium from the beginning until 180 minutes of flow (typical 180 mL) in a PTFE or PFA bottle (PE or PMP are also accepted).
4) the determination of the averaged outflow concentration of the dissolved element on a suitable device with an LoQ of ≤ 0.1 µg/l for the target element(s) (e.g. AAS, ICP-OES, ICP-MS).



Performing the test

1) The medium is prepared as in Part A or B, required volume per test is flow rate x duration + 20%
2) Place the two glass-fiber membranes in the bottom part of the filter holder, they prevent that the ultrafiltration membrane is damaged by the membrane support frit.

   Note: depending on the coarseness of the membrane support frit or mesh these glass-fiber membranes might not be needed or one will be sufficient. The support screen of the BOLA™ PTFE holders requires two filters for support.

3) Place the ultrafiltration membrane in the filter holder.
4) Put the filter holder on an analytical balance. If the tubing connector of the filter holder prevents a proper horizontal placement, use an empty 100 mL beaker and place the holder on the beaker.
5) 10 ± 1 mg of the NM is weighed onto the ultrafiltration membrane disc and distributed as equally as possible on the whole disc area. Leave a margin of 3 mm towards the filter edge free of NM. Electrostatic charge may be a problem, use an anti-electrostatic device, as e.g. ZeroStat3 anti-static pistol (Sigma Aldrich).
6) Place the 0.2 µm filter membrane on the ultrafiltration membrane to sandwich the powder in-between.
7) Place two more glass-fiber membranes on top of the stack.
8) Mount the filter holder in a way that the flow direction will be upwards.

9) Transfer 5 mL of 6 M HNO_3 into the collection bottle.
10) Purge all tubes towards the filter holder with medium. Adjust the pump rate to 1 mL/min
11) Connect the filter holder to the pumps.
12) Fill the filter holder with medium. Stop the flow when medium appears at the filter holder outlet.
13) Connect the outlet tube to the filter holder and the collection bottle and set the pump to the desired flow rate (here 1 mL/min).
14) After 60, 120 and 180 min. remove the tube from the collector bottle, let the flow from the outlet tube fill 5 mL into a clean 15 mL centrifuge tube (e.g. Falcon) and determine the pH. Add the 5 mL to the collection bottle and re-connect the tube.
15) Determine the concentration of the target ion in the collection bottle.
16) Optional: collect the membranes from the filter holder and digest to retrieve a mass balance.