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Total Organic Carbon Analyzer TOC-L

Application News

TOC/TN_b Determination in Particle-Containing Samples – Performing the "Cellulose Test"

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User Benefits

- High Suspension mode ensures reliable, reproducible results of even heavily particle-laden samples.
- Delivers robust accuracy and proven compliance with ISO 20236, EN 1484, and ISO 8245.
- TOC-L simplifies the workflow and minimizes maintenance, letting users focus on secure, reliable results.

■ Introduction

Analyzing the total organic carbon (TOC) and total bound nitrogen (TN_b) content of water samples is a key requirement in environmental and industrial laboratories, especially for applications such as municipal and industrial wastewater treatment (Figure 1), where samples often contain particulate matter. TOC determination was originally specified in EN 1484 and ISO 8245 which addressed only carbon parameters, and is now more comprehensively defined in ISO 20236, which also includes nitrogen. This latest standard defines TOC as the sum of organically bound carbon, and TN_b as the sum of organically and inorganically bound nitrogen, present in a sample, regardless of whether these are dissolved or suspended. When required, the dissolved fractions (dissolved organic carbon, DOC, and dissolved bound nitrogen, DN_b) can be determined after membrane filtration (0,45 µm).

Fig. 1 Aerial view of a modern wastewater treatment plant.

Measuring TOC and TN_b in samples containing particulate material presents a unique analytical challenge. Accurate assessment of suspended material requires not only robust instrumentation, but also careful sample preparation and handling, as particles can settle, aggregate, or escape detection during analysis. One of the central challenges in such analyses is the reliable execution of the "particle processing control," more commonly known as the "cellulose test." Originally included in the standards EN 1484 and ISO 8245, and most recently detailed and mandated in ISO 20236, this test has become the primary benchmark for assessing instrument performance and operator proficiency in handling suspensions. Successfully passing the cellulose test is therefore required to demonstrate that both the system and laboratory procedures are fully fit for the analysis of real-world samples containing particulate material.

Challenges encountered in the "cellulose test"

Despite its apparent simplicity, the cellulose test is frequently cited as a source of frustration and stress in routine laboratory practice. Variability in recoveries or poor reproducibility often highlights hidden weaknesses in sample preparation, device configuration, or analytical technique. If left unresolved, these issues can jeopardize compliance with quality standards, method accreditation, or successful audits.

This application note is designed to provide both complete alignment with the latest ISO 20236 guidelines and practical, experience-based guidance for successfully performing the cellulose test using the Shimadzu TOC-L analyzer. By addressing both the normative framework and the day-to-day challenges observed in real laboratories, it aims to equip users with robust protocols and effective strategies. The goal is to ensure reliability, confidence, and compliance, even in the most demanding TOC/TN_b applications.

■ Cellulose test according to ISO 20236

The cellulose test, as specified in ISO 20236, is a comprehensive performance check designed to verify that both the analyzer and all laboratory procedures, including sampling and sample handling, are capable of reliably measuring water samples containing suspended particulate matter. By assessing the entire workflow, the test ensures that results for particle-containing samples are both reliable and compliant with the standard.

Preparation of the bulk cellulose suspension

The cellulose test requires the use of microcrystalline cellulose (MCC) as the standard particulate material. MCC serves as a model for suspended solids due to its defined particle properties and chemical consistency. The standard specifies a particle size range of 0,02-0,1 mm. However, MCC is produced for various industrial and pharmaceutical uses, so not all commercially available products match this requirement exactly. Particle size distribution can vary by manufacturer and batch, which may significantly affect test results. For reliable performance, laboratories should always select MCC with a documented and verified particle size.

Shimadzu recommends Merck microcrystalline cellulose*1 (product number 1.02331), which is supplied with batch-specific particle size certification.

The concentration of the test suspension should be selected to match the expected TOC concentration in the samples to be analyzed. For example, to prepare a 100 mg/l TOC test suspension, suspend 225 mg of MCC (assuming a carbon content of 44,45%) in a one-liter volumetric flask with ultrapure water. The suspension should be stirred overnight with a magnetic stirrer at approximately 400 rpm, ensuring complete homogenization before use.

*1 As recommended in "LAWA AQS Merkblatt P-14" from May 1995 (Only available in German language)

Sampling bulk suspension: filling autosampler vials

After the suspension is prepared and thoroughly stirred overnight, it may be stored in the refrigerator for up to one month. Before sampling, the suspension should be re-homogenized by stirring on a magnetic stirrer for approximately 10 minutes at around 400 rpm. The stirrer bar must be properly sized for the vessel to ensure efficient mixing and to prevent spinout or the formation of dead zones.

For the cellulose test, analysis is performed in the same manner as routine TOC/TN_b sample measurement, using the ASI-L autosampler to supply each vial to the TOC-L analyzer. Representative aliquots of the bulk cellulose suspension must be transferred into standard 40 ml or 24 ml vials compatible with the ASI-L autosampler.

Aliquots are typically withdrawn using a pipette with sufficient length and a tip opening of about 1 mm (either a glass pipette or a direct displacement pipette). When drawing the sample, aim for the outer curved section of the flask bulb (the "belly," as shown in green in Figure 2).

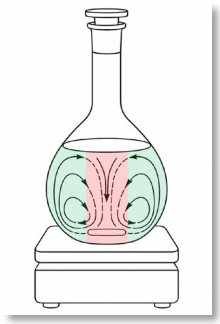

Fig. 2 Suspension sampling zones in a volumetric flask.

In this region, the upward flow created by the circulating suspension ensures the mixture is most homogenized and representative. It is best to avoid sampling near the center or base of the flask (the red zone in Figure 2), as these areas are affected by the vortex formed by the stirrer bar and may contain unevenly distributed particles. If accessing the 'belly' of the flask is difficult due to the narrow neck, the well-homogenized suspension can be carefully transferred to a wide beaker to allow easier and more accurate sampling from the optimal zone.

It is important to note that only about 70–80% of the total suspension volume in the flask can realistically be pipetted into vials. Once the liquid level drops below this threshold, almost all that remains is vortex and the space below the stirrer bar, making it impossible to withdraw further representative aliquots. Attempting to extract more may lead to strongly biased and non-representative results.

Alternatively, some users may pour the mixed suspension directly into sample vials. While pouring can provide representative aliquots, it requires considerable experience and a steady hand to avoid splashing, loss of material, or uneven particle distribution.

Each sample vial must be filled to the exact volume specified for its type and equipped with the recommended stirrer bar, as detailed in Table 1. Overfilling can lead to spillage and particle loss during subsequent handling or measurement. After filling and adding the stirrer bar, each vial is sealed immediately with a cap and septum.

Table 1 Sample volume for each ASI-L vial type

ASI-L vial type	Fill volume	Stirrer bar [PN]
24 ml	20 ml	10 mm [046-00617-02]
40 ml	35 ml	15 mm [046-00617-03]

Careful and consistent attention to homogenization, sampling technique, vial filling, and preparation is essential. Any deviation, such as interruption of stirring, inconsistent pipetting or pouring, sampling from a poorly mixed region, overfilling vials, or omission of the stirrer bar, can compromise the particle distribution between vials and jeopardize the validity of the cellulose test.

Cellulose test requirements

To comply with ISO 20236, laboratories must verify the instrument's particle processing performance using the cellulose test suspension on each day that solid-containing samples are analyzed. This involves preparing two vials of cellulose suspension and performing a minimum of three replicate injections from each vial, ensuring that sampling and injection occur while the suspension is magnetically stirred. Ultrasonic treatment is not permitted, as it reduces the particle size and invalidates the test.

For each vial:

- The mean value of three replicates must not deviate by more than ±10 % from the theoretical TOC value calculated. For example, for a 100 mg/l TOC suspension, the acceptable range is 90–110 mg/l.
- The repeatability expressed as coefficient of variation (CV %), must not exceed 10%.

Failure to meet these criteria indicates that either the instrument setup or aspects of the sampling, preparation, or handling are unsuitable for reliable TOC or TN_b determinations in particle-containing samples, and the particle processing control test is considered failed. For instruments designed for simultaneous TOC and TN_b analysis, checking particle processing for carbon using the cellulose test remains sufficient, as microcrystalline cellulose contains no nitrogen.

It is possible to run the cellulose test and successfully fulfill the requirements of ISO 8245, EN 1484, and ISO 20236 simultaneously. Table 2 summarizes the key points of comparison and compliance criteria for each standard.

Table 2 Comparison of normative requirements

	EN 1484	ISO 8245	ISO 20236
Analysis parameters	TOC, DOC		TOC, DOC, TN_b, DN_b
Instrument type	Combustion, wet chemical, UV oxidation		Catalytic combustion
Calibration substance	TOC: $C_8H_5KO_4$ („KHP")		TOC: $C_8H_5KO_4$ („KHP") TN_b: $(NH_4)_2SO_4$ / KNO_3
Control substance	Calibration standards or copper phthalocyanine		Nicotinic acid
Cellulose test	Yes, „informative part"		Yes, normative
- Control substance	MCC 20-100 µm, 100 mg/l TOC suspension		MCC 0,02-0,1 mm, suspension TOC conc. sample-dependent
- Conditions	Mean value of 3 injections, sampling during stirring, Recovery 90-110 mg/l, Repeatability CV <10%		Mean value of 3 injections of min. 2 suspensions, sampling during stirring, Recovery 90-110% of theor. value, CV ≤10%
- Restrictions	Ultrasonic „should not be used"; UV oxidation not suitable		Ultrasonic treatment may not be used
- Frequency	„Advisable" with each batch of samples with solid contents		Each day solid-containing samples are analyzed

Reliable results require strict adherence to the sampling and preparation procedures described above.

■ TOC-L settings

The cellulose test must be analyzed using the same instrument configuration, measurement methods, and autosampler setup as those applied to routine TOC/TN_b analysis of particle-containing samples. Only by mirroring the laboratory's standard workflow for sampling, handling, and measurement, does the test provide a realistic and comprehensive assessment of system performance under typical operating conditions. Consistent use of these instrument and method settings is essential for ensuring that the cellulose test provides valid and reproducible results, and for meeting regulatory and accreditation requirements.

Key aspects of method configuration and settings include:

- **Autosampler:**

The cellulose test requires an ASI-L autosampler with either a 24 ml or 40 ml tray, equipped with a magnetic stirrer and an external sparge kit for integrated sample preparation.

- **Acidification and sparging:**

All sample preparation steps, including acidification and sparging, are performed in the autosampler vial before injection to prevent particle settling after aliquotation.

- **Magnetic stirring:**

The ASI-L magnetic stirrer speed is set to about 500 rpm, which corresponds to the counterclockwise limit of the stirrer adjustment knob.

- **Particle sampling needle:**

For particle-containing suspensions, the 0,8 mm wide-bore particle needle is used in the ASI-L autosampler. This enables clog-free, high-throughput, and unattended analysis, even with demanding samples.

- **Vial placement:**

Sample vials should be placed in adjacent positions in the rotating tray. In the ASI-L autosampler, both the sampling and sparging positions are always actively stirred, which ensures optimal suspension stability throughout the analysis. On the 24 ml tray, only positions 1 to 85 are stirred.

- **Pre-sampling stirring time:**

A sparge duration of 10 minutes should be set. This ensures that each vial is stirred for at least 10 minutes before sampling. Stirring and sparging take place simultaneously and begin while the previous vial is being measured, so this step does not extend the analysis time.

- **Injection volume:**

ISO 20236 requires identical injection volumes for blanks, calibration, and samples. Use the software-suggested volume, applying the TN_b calibration volume to both TOC and TN_b in combined measurements..

- **Software settings:**

In the LabSolutions TOC PC control software, the instrument H/W setting “Measurement Method” is set to "High Suspension" *2. In this mode, sampling from the vial does not begin until the TOC-L instrument is ready for immediate measurement. Overlapping pre-processing in the main unit is also disabled in this mode.

■ Example ISO 20236-compliant method

A TOC-L analyzer was calibrated for both NPOC and TN_b analysis using the automatic dilution function. The instrument and autosampler were configured according to the requirements described in the previous section, ensuring full alignment with ISO 20236. All method and instrument settings mirrored those used for routine analysis of particle-containing water samples. For non-cellulose test measurements, ISO 20236 requires only the mean of two injections per vial, while for the cellulose test, a minimum of three replicate injections per vial is specifically required.

The detailed calibration and method parameters are summarized in Table 3 and Table 4, respectively. Figures 3 and 4 show the calibration results.

Table 3 Measurement conditions TOC analysis

Analyzer	: TOC-L CPN
Catalyst	: TC/TN
Meas. Parameter	: NPOC (Direct method TOC=NPOC)
Calibration curve	: 6-point NPOC calibration using automatic dilution function in the range of 5 - 100 mg/l, 100 mg C/l KHP standard solution
Acidification	: HCl 1 mol/l, acid addition 3 %
Sparge settings	: 10 min. (external), 100 ml/min
Injection settings	: 40 µl, 2 / max. 3 injections

*2 Users of LabSolutions TOC ver. <1.20, all TOC-Control L or standalone control versions should contact their local Shimadzu representative about activating a similar legacy mode.

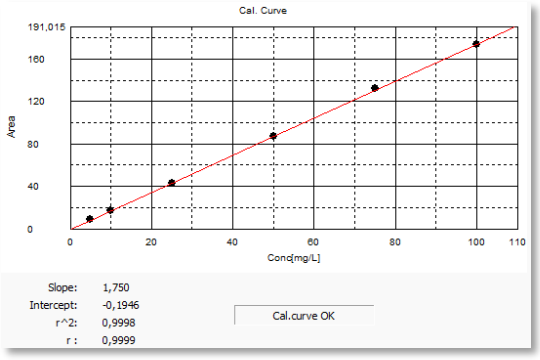

Fig. 3 Multi-point NPOC calibration with dilution function

Table 4 Measurement conditions TN_b analysis

Analyzer	: TOC-L CPN
Catalyst	: TC/TN
Meas. Parameter	: TN
Calibration curve	: 6-point TN calibration using automatic dilution function in the range of 1 - 20 mg/l, 20 mg N/l $(NH_4)_2SO_4$ / KNO_3 mix standard solution
Acidification	: HCl 1mol/l, acid addition 3 %
Injection settings	: 40 µl, 2 / max. 3 injections

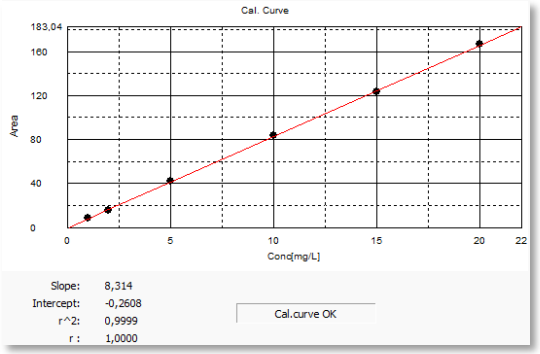

Fig. 4 Multi-point TN_B calibration with dilution function

■ Quality control

ISO 20236 requires a system check with solution control samples to verify calibration validity before the cellulose test. Nicotinic acid solution is used, providing both carbon and nitrogen for TOC and TN_b performance assessment.

System check determinations use at least two dilutions of the nicotinic acid solution (covering 20–80 % of the working range). For each, a deviation up to ±10 % or ±1 mg/l (whichever is greater) from the nominal value is permitted. Repeatability is evaluated from at least two injections per vial; the coefficient of variation (CV) must not exceed ±5 % or ±1 mg/l. For concentrations below 10 mg/l, individual values must not differ by more than 1 mg/l. Results that meet these criteria are considered a “pass.”

These solution controls are essential for confirming that the analyzer is functioning correctly before proceeding to the more demanding cellulose test.

Table 5 ISO 20236 nicotinic acid-based system check

Control parameter	Nominal value [mg/l]	Measured value [mg/l]	CV	Deviation	Result
TN_b 20 %	4	4,038	0,29 %	+0,95 %	PASS
TN_b 80 %	16	16,28	0,31 %	+1,75 %	PASS
NPOC 20 %	20,59	20,29	0,38 %	-1,46 %	PASS
NPOC 80 %	82,35	81,48	1,58 %	-1,06 %	PASS

■ Cellulose test results

To demonstrate method robustness and practical performance, the cellulose test was performed at multiple concentrations: 20, 40, and 100 mg/l—where the 20 mg/l measurement was carried out following the principle of the interlaboratory trials described in EN 1484 and ISO 8245. All measurements used the "High Suspension" method and TOC-L/ASI-L settings detailed previously. The measurement conditions and results of the cellulose test are summarized in Table 6 and Table 7, respectively.

Table 6 Measurement conditions cellulose test

Analyzer	: TOC-L CPN, ASI-L 40 ml
Catalyst	: TC/TN
Meas. Parameter	: NPOC (Direct method TOC=NPOC)
Acidification	: HCl 1 mol/l, acid addition 3 %
Sparge settings	: 10 min. (external), 100 ml/min
Injection settings	: 40 µl, 3 / max. 5 injections, CV max. 5 %

Table 7 Cellulose test results at various concentrations

#	Nominal value [mg/l]	Measured value [mg/l]	CV	Deviation
1	100	101,8	1,18 %	+1,80 %
2	100	96,50	1,75 %	-3,50 %
3	100	100,1	2,60 %	+0,10 %
4	40	41,55	4,81 %	+3,88 %
5	40	41,71	3,47 %	+4,28 %
6	40	37,85	4,48 %	-5,38 %
7	20	20,04	5,81 %	+0,20 %
8	20	18,82	3,40 %	-5,90 %
9	20	16,86	0,43 %	-15,7 %
10*3	1200	1145	2,30 %	-4,58 %
11*3	1200	1141	0,82 %	-4,92 %
12*3	1200	1138	1,17 %	-5,17 %
13*3	10000	9753	0,08 %	-2,47 %
14*3	10000	9648	1,67 %	-3,52 %
15*3	10000	9450	1,40 %	-5,50 %

*3 The 1200 mg/l result was measured using a dedicated high-range calibration; the 10000 mg/l result was obtained with a 1:10 automatic dilution. Both values demonstrate the extended capability of the TOC-L system beyond standard requirements.

The limit of quantification (LOQ) for each parameter was determined as ten times the standard deviation of six injections of a pure water. Results are shown in Table 8.

Table 8 Limit of quantification (LOQ) for NPOC and TN_b

Parameter	Sample	Result [mg/l]	SD [mg/l]	LOQ [mg/l]
NPOC	Pure water blank (6 injections)	0,184	±0,044	0,44
TN_b		0,033	±0,003	0,03

This confirms that the method's quantification limits are far below any of the concentrations used for the cellulose test, ensuring that observed variability at low cellulose test levels is due to particle statistics and sample handling, not instrument sensitivity.

■ Performance beyond standards

The cellulose test results at 20, 40, 100, 1200, and 10000 mg/l illustrate both the robustness and versatility of the TOC-L method, while also highlighting practical considerations at lower concentrations. At higher concentrations, including 1200 mg/l and 10000 mg/l, the test is reliably passed, demonstrating superior instrument performance and stable sample handling. Even under the most demanding conditions, the TOC-L ensures confident compliance and consistent results.

In contrast, passing the cellulose test at lower concentrations, particularly at and below 20 mg/l, is more challenging. This is not due to instrument sensitivity, as confirmed by the method's low LOQ values for both NPOC and TN_b, but rather the statistical difficulty of evenly distributing a small number of particles between replicate aliquots. This effect is well documented in interlaboratory trial data from EN 1484 and ISO 8245, where the coefficient of variation for repeatability at 20 mg/l reached up to 27,3 %. Such high variability means results at very low concentrations are influenced primarily by particle statistics and sample preparation technique, emphasizing the importance of robust laboratory practices.

ISO 20236 requires the use of microcrystalline cellulose with a particle size of 20–100 µm for compliance, though smaller particles could improve uniformity at low concentrations.

Routine use of the cellulose test at 100 mg/l is therefore recommended. At this concentration, the test simulates a practical worst-case scenario in which most TOC is particulate and provides a stringent yet reproducible verification of both analytical and procedural performance. It ensures not only the instrument's high performance, but also confidence in essential processes of sample homogenization, handling, and aliquoting, delivering consistent, reliable TOC/TN_b analysis for particulate samples.

Lastly, the low LOQ values determined for both NPOC and TN_b are far below any cellulose test concentration performed, confirming that variability at low levels is due to particle statistics and not instrument sensitivity and further highlighting the TOC-L's outstanding capabilities for both standard and challenging applications.

■ Recommended analyzer configuration

TOC-L CPH or CPN

LabSolutions TOC software

ASI-L autosampler (24 or 40 ml), External sparge kit, magnetic stirrer, particle needle

Fig. 5 TOC-L with ASI-L autosampler

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