



**NSAI**  
Standards

Irish Standard Recommendation  
S.R. CEN ISO/TS 21362:2021

# Nanotechnologies - Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation (ISO/TS 21362:2018)

**S.R. CEN ISO/TS 21362:2021**

*Incorporating amendments/corrigenda/National Annexes issued since publication:*

The National Standards Authority of Ireland (NSAI) produces the following categories of formal documents:

I.S. xxx: Irish Standard — national specification based on the consensus of an expert panel and subject to public consultation.

S.R. xxx: Standard Recommendation — recommendation based on the consensus of an expert panel and subject to public consultation.

SWiFT xxx: A rapidly developed recommendatory document based on the consensus of the participants of an NSAI workshop.

*This document replaces/revises/consolidates the NSAI adoption of the document(s) indicated on the CEN/CENELEC cover/Foreword and the following National document(s):*

*NOTE: The date of any NSAI previous adoption may not match the date of its original CEN/CENELEC document.*

*This document is based on:*

CEN ISO/TS 21362:2021

*Published:*

2021-04-07

*This document was published  
under the authority of the NSAI  
and comes into effect on:*

2021-04-25

ICS number:

07.120

NOTE: If blank see CEN/CENELEC cover page

NSAI  
1 Swift Square,  
Northwood, Santry  
Dublin 9

T +353 1 807 3800  
F +353 1 807 3838  
E standards@nsai.ie  
W NSAI.ie

Sales:  
T +353 1 857 6730  
F +353 1 857 6729  
W standards.ie

Údarás um Chaighdeáin Náisiúnta na hÉireann

## National Foreword

S.R. CEN ISO/TS 21362:2021 is the adopted Irish version of the European Document CEN ISO/TS 21362:2021, Nanotechnologies - Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation (ISO/TS 21362:2018)

This document does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

For relationships with other publications refer to the NSAI web store.

**Compliance with this document does not of itself confer immunity from legal obligations.**

*In line with international standards practice the decimal point is shown as a comma (,) throughout this document.*

This page is intentionally left blank

TECHNICAL SPECIFICATION  
SPÉCIFICATION TECHNIQUE  
TECHNISCHE SPEZIFIKATION

**CEN ISO/TS 21362**

April 2021

ICS 07.120

English Version

**Nanotechnologies - Analysis of nano-objects using  
asymmetrical-flow and centrifugal field-flow fractionation  
(ISO/TS 21362:2018)**

Nanotechnologies - Analyse des nano-objets par  
fractionnement par couplage flux-force asymétrique et  
à force centrifuge (ISO/TS 21362:2018)

Nanotechnologien - Analyse von Nanoobjekten mit  
Hilfe von Asymmetrischer-Fluss-  
Feldflussfraktionierung und zentrifugaler  
Feldflussfraktionierung (ISO/TS 21362:2018)

This Technical Specification (CEN/TS) was approved by CEN on 19 March 2021 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

**CEN ISO/TS 21362:2021 (E)**

<b>Contents</b>	<b>Page</b>
<b>European foreword.....</b>	<b>3</b>

## **European foreword**

The text of ISO/TS 21362:2018 has been prepared by Technical Committee ISO/TC 229 "Nanotechnologies" of the International Organization for Standardization (ISO) and has been taken over as CEN ISO/TS 21362:2021 by Technical Committee CEN/TC 352 "Nanotechnologies" the secretariat of which is held by AFNOR.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## **Endorsement notice**

The text of ISO/TS 21362:2018 has been approved by CEN as CEN ISO/TS 21362:2021 without any modification.

This page is intentionally left blank



# TECHNICAL SPECIFICATION

**ISO/TS  
21362**

First edition  
2018-06

---

---

## **Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation**

*Nanotechnologies — Analyse des nano-objets par fractionnement flux asymétrique et flux force centrifuge*



Reference number  
ISO/TS 21362:2018(E)

© ISO 2018

**ISO/TS 21362:2018(E)**



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2018

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Fax: +41 22 749 09 47  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

	Page
<b>Foreword</b> .....	<b>v</b>
<b>Introduction</b> .....	<b>vi</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Symbols and abbreviated terms</b> .....	<b>7</b>
<b>5 Principles of operation</b> .....	<b>8</b>
5.1 Field-flow fractionation (General).....	8
5.2 Specific applications by applied field.....	10
5.2.1 Flow field.....	10
5.2.2 Centrifugal field.....	11
<b>6 Method development for AF4</b> .....	<b>13</b>
6.1 General.....	13
6.2 Sample specifications.....	14
6.3 Mobile phase specifications.....	14
6.4 Fractionation.....	15
6.4.1 Channel and membrane selection.....	15
6.4.2 Injection and relaxation.....	17
6.4.3 Optimizing flow conditions.....	18
6.4.4 Elution programme.....	18
6.4.5 Using FFF theory to select initial flow settings.....	19
<b>7 Method development for CF3</b> .....	<b>19</b>
7.1 General.....	19
7.2 Choice of mobile phase.....	20
7.3 Field strength selection.....	20
7.4 Field decay programme.....	20
7.5 Channel flow rate selection.....	21
7.6 Calculation of the relaxation time.....	21
7.7 Calculation of sample injection delay.....	21
<b>8 Analysis of nano-objects</b> .....	<b>21</b>
8.1 General.....	21
8.2 Online size analysis.....	22
8.3 Online concentration analysis.....	23
8.3.1 General.....	23
8.3.2 Mass-based methods.....	23
8.3.3 Number-based methods.....	24
8.4 Online material identification or composition.....	25
8.5 Off-line analysis (Fraction collection).....	26
<b>9 Qualification, performance criteria and measurement uncertainty</b> .....	<b>26</b>
9.1 System qualification and quality control.....	26
9.1.1 Basic system qualification.....	26
9.1.2 Focusing performance.....	27
9.1.3 Flow rate of the carrier liquid.....	28
9.1.4 Separation field.....	28
9.2 Method performance criteria.....	28
9.2.1 Recovery.....	28
9.2.2 Selectivity.....	29
9.2.3 Retention.....	29
9.2.4 Resolution.....	29
9.3 Method precision and measurement uncertainty.....	29

## ISO/TS 21362:2018(E)

<b>10</b>	<b>General procedures for measurement of samples</b>	<b>30</b>
10.1	General	30
10.2	Calibration of retention time for online size analysis	30
10.2.1	Calibration of the AF4 channel	30
10.2.2	Calibration of AF4 retention time for online size measurements	31
10.3	AF4 general measurement procedure	31
10.4	CF3 general measurement procedure	32
<b>11</b>	<b>Test report</b>	<b>33</b>
11.1	General	33
11.2	Apparatus and measurement parameters	33
11.2.1	AF4 recording/reporting specifications	33
11.2.2	CF3 recording/reporting specifications	34
11.3	Reporting test results	34
	<b>Bibliography</b>	<b>35</b>

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

## ISO/TS 21362:2018(E)

### Introduction

The capacity to isolate and analyse diverse populations of nano-objects and their agglomerates or aggregates, often suspended in, or extracted from, complex matrices, is critical for applications ranging from materials discovery and nanomanufacturing to regulatory oversight and environmental risk assessment. Furthermore, the ability to characterize these analytes with minimal perturbation of their natural or native state is highly desirable. The list of available techniques capable of achieving such objectives is relatively short, and while all techniques have advantages and disadvantages, and no single technique is solely adequate or appropriate for all possible applications and materials, a group of related separation techniques known collectively as field-flow fractionation (FFF), conceptually proposed by J. Calvin Giddings in 1966<sup>[1]</sup>, offers many advantages for nanotechnology applications. In FFF, the analyte, suspended in a liquid medium, is fractionated by the application of a field (e.g. flow, centrifugal, electric, thermal-gradient, magnetic) perpendicular to the direction of flow of the analyte and mobile phase eluting through a thin defined channel. Separation occurs when the analyte responds to the applied field, such that populations with different response sensitivities reach equilibrium positions (i.e. in equilibrium with diffusional forces) higher or lower in the laminar flow streamlines perpendicular to channel flow, thus eluting differentially.

Among the FFF variants, asymmetrical flow FFF (variously abbreviated in the literature as AF4, A4F, AFFFF, AfFFF or AsFFFF) and centrifugal FFF (abbreviated as CF3, also called sedimentation FFF and abbreviated as SdFFF), are available commercially and have been most widely adopted in the nanotechnology field (for convenience and simplicity, the abbreviations AF4 and CF3 are used throughout this document). AF4 is arguably the most versatile technique with respect to the wide range of applications, materials and particle sizes to which it has been applied. Symmetrical flow FFF (fFFF), the original “flow-based” technique as first described in 1976<sup>[2]</sup>, has been supplanted commercially by AF4, introduced in 1987<sup>[3]</sup>, due to several advantages, including a simpler channel design, the ability to visualize the sample through a transparent top channel wall, and reduced analyte band width. The theory and application of CF3 as it is presently applied was described by Giddings and coworkers in 1974<sup>[4]</sup>, although a centrifugal field-based FFF system was first developed and tested independently by Berg and Purcell in 1967<sup>[5]</sup>. Other FFF field variants, such as thermal, electrical and magnetic, provide unique capabilities, but have been limited in the scope of their applications vis-à-vis nanotechnology or commercial availability.

Where FFF was once predominantly the domain of specialists, these instruments are now commonly and increasingly utilized in government, industry and academic laboratories as part of the nano-characterization toolbox. Two factors are driving this increase in nanotechnology utilization: maturation of commercial instrumentation and versatility with respect to coupling a wide range of detectors to FFF systems. In the latter case, recent developments have led to the use of highly sensitive elemental detectors (e.g. inductively coupled plasma mass spectrometer or ICP-MS), which offer enhanced characterization and quantification for many materials. Additionally, traditional concentration or sizing detectors, such as ultraviolet-visible (UV-Vis) absorbance, fluorescence, multi-angle light scattering (MALS) and dynamic light scattering (DLS), yield online data for eluting populations, and theoretically provide more accurate information than obtainable using off-line measurements of unfractionated polydisperse systems. The measured retention time of an eluting peak can also be used to determine the hydrodynamic size by AF4 based on theoretical relationships or calibration with a known size standard. CF3 has the unique capacity to rapidly separate species of the same size but differing in density.

Although FFF based techniques have the capacity to separate and characterize analytes over an extremely broad size range, from about 1 nm up to tens of micrometers, this document focuses primarily on materials in the nanoscale regime and their associative structures. The basic underlying principles, experimental approach, and hardware described here can be more broadly applied.

While this specification includes the most common online detection schemes for nano-object analysis, other less common forms of detection have been utilized or reported in the literature, including differential refractometry (primarily used for macromolecular analysis), particle tracking analysis, graphite furnace atomic absorption spectrometry, single particle ICP-MS, and small-angle X-ray

scattering. This number is likely to grow in the future, as new techniques emerge and existing ones are modified and evaluated for coupling to FFF.

In order to develop and validate methods for application of FFF to the analysis of nano-objects and their agglomerates or aggregates, and to properly report experimental results and conditions in order to enable reproducibility across laboratories, it is critical to specify key parameters to be controlled and reported. These parameters encompass all aspects of FFF methodology, including sample/analyte, instrumentation, fractionation, calibration, qualification, performance specifications, measurement uncertainty, and data analysis. This document identifies the key parameters and lays out a general approach to method development for AF4 and CF3.

General references and further reading on FFF theory and practise, as well as AF4 and CF3 applications to nanotechnology, can be found in the Bibliography[6]-[18].





# Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation

## 1 Scope

This document identifies parameters and conditions, as part of an integrated measurement system, necessary to develop and validate methods for the application of asymmetrical-flow and centrifugal field-flow fractionation to the analysis of nano-objects and their aggregates and agglomerates dispersed in aqueous media. In addition to constituent fractionation, analysis can include size, size distribution, concentration and material identification using one or more suitable detectors. General guidelines and procedures are provided for application, and minimal reporting requirements necessary to reproduce a method and to convey critical aspects are specified.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1, ISO/TS 80004-2, ISO/TS 80004-6 and the following, apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

### 3.1

#### **nano-object**

discrete piece of material with one, two or three external dimensions in the nanoscale (from approximately 1 nm to 100 nm)

Note 1 to entry: Generic term for all discrete nanoscale objects.

[SOURCE: ISO/TS 80004-2:2015, 2.2, modified — In the definition, “(from approximately 1 nm to 100 nm)” has been added. Note 1 to entry has been changed.]

### 3.2

#### **nanoparticle**

nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than 3 times), terms such as nanofibre or nanoplate may be preferred to the term nanoparticle.

[SOURCE: ISO/TS 80004-2:2015, 4.4]

This is a free preview. Purchase the entire publication at the link below:

[Product Page](#)

- 
- Looking for additional Standards? Visit Intertek Inform Infostore
  - Learn about LexConnect, All Jurisdictions, Standards referenced in Australian legislation
-