SLOVENSKI STANDARD
oSIST prEN ISO 21362:2024

01-november-2024

Nanotehnologije - Analiza nanoobjektov s frakcioniranjem asimetričnega in centrifugalnega pretoka skozi polje (ISO/DIS 21362:2024)

Nanotechnologies - Analysis of nano-objects using asymmetrical flow and centrifugal field-flow fractionation (ISO/DIS 21362:2024)

Nanotechnologien - Analyse von Nanoobjekten mit Hilfe von Asymmetrischer-Fluss-Feldflussfraktionierung und zentrifugaler Feldflussfraktionierung (ISO/DIS 21362:2024)

Nanotechnologies - Analyse des nano-objets par fractionnement flux asymétrique et flux force centrifuge (ISO/DIS 21362:2024)

Ta slovenski standard je istoveten z: **prEN ISO 21362**

ICS:

07.120	Nanotehnologije	Nanotechnologies

oSIST prEN ISO 21362:2024 **en,fr,de**

DRAFT International Standard

ISO/DIS 21362

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

ICS: 07.120

ISO/TC **229**

Secretariat: **BSI**

Voting begins on:
2024-09-02

Voting terminates on:
2024-11-25

Member bodies are requested to consult relevant national interests in IEC/TC 113 before casting their ballot to the e-Balloting application.

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 21362:2024(en)

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Published in Switzerland

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Foreword

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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).

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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.

This document was prepared jointly by Technical Committee ISO/TC 229, Nanotechnologies and Technical Committee IEC/TC 113, Nanotechnology standardization for electrical and electronic products and systems, and in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 352, Nanotechnologies, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). The draft was circulated for voting to the national bodies of both ISO and IEC.

This first edition cancels and replaces ISO/TS 21362:2018, which has been technically revised. The main changes compared to the previous edition are as follows:

— Additional section addressing alternative and emerging methods

— Minor technical revisions to update information to the current state of the art.

— Annex A summarizing an interlaboratory comparison conducted through VAMAS

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 characterise 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[1], 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 AsFlFFF) and centrifugal FFF (abbreviated as CF3, also called sedimentation FFF associated with the abbreviation 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[2], has been supplanted commercially by AF4, introduced in 1987[3], due to several advantages, including a simpler channel design, the ability to visualise the sample through a transparent top channel wall,[1)] and reduced analyte band width. The theory and application of CF3 as it is presently applied was described by Giddings and coworkers in 1974[4], although a centrifugal field-based FFF system was first developed and tested independently by Berg and Purcell in 1967[5]. Other FFF field variants, such as thermal, electrical and magnetic, provide unique capabilities, but are 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 estimate 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 characterise analytes over an extremely broad size range, from about 1 nm up to tens of micrometres, this document focuses primarily on materials in the nanoscale regime and their associative structures. However, the basic underlying principles, experimental approach, and hardware described here can be more broadly applied.

General references and further reading for FFF theory and practise, as well as AF4 and CF3 applications to nanotechnology, are listed in the Bibliography [6]-[18]. Annex A summarizes a VAMAS interlaboratory comparison conducted to evaluate the capacity of AF4 and CF3 techniques to separate and characterise components of a complex multimodal mixture of analytes reproducibly and with acceptable recovery

1) Bulging of the transparent "top" wall when the channel is pressurized could alter channel volume and flow. This could present a limitation under certain experimental conditions. Solid steel channel blocks offer an alternative that is rigid, but lacks transparency.

and resolution across laboratories using different commercial instrument platforms and instrument configurations.

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 80004-1, *Nanotechnologies – Vocabulary — Part 1: Core vocabulary*

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 80004-1 and 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 https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
nanoscale
length range approximately from 1 nm to 100 nm

[SOURCE: ISO 80004-1:2023, 3.1.1]

3.2
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

[SOURCE: ISO 80004-1:2023, 3.1.5]

3.3
nanoparticle
nano-object with all external dimensions in the nanoscale

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

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.4
field-flow fractionation
FFF
separation technique where a field is applied to a liquid suspension passing along a narrow channel in order to induce separation of the particles present in the liquid, dependent on their differing mobility under the force exerted by the field

Note 1 to entry: The field can be, for example, gravitational, centrifugal, liquid flow, electrical or magnetic.

Note 2 to entry: Using a suitable detector after or during separation allows determination of the mean size and size distribution of nano-object populations.

3.5
asymmetrical-flow field-flow fractionation
AF4
separation technique that uses a cross flow field applied perpendicular to the channel flow to achieve separation based on analyte diffusion coefficient or size

Note 1 to entry: Cross flow occurs by means of a semipermeable (accumulation) wall in the channel, while cross flow is zero at an opposing nonpermeable (depletion) wall.

Note 2 to entry: By comparison, in symmetrical flow, the cross flow enters through a permeable wall (frit) and exits through an opposing semipermeable wall and is generated separately from the channel flow.

Note 3 to entry: Nano-objects generally fractionate by the "normal" mode, where diffusion dominates and the smallest species elute first. In the micrometre size range, the "steric-hyperlayer" mode of fractionation is generally dominant, with the largest species eluting first. The transition from normal to steric-hyperlayer mode can be affected by material properties or measurement parameters, and therefore is not definitively identified; however, the transition can be defined explicitly for a given experimental set of conditions; typically, the transition occurs over a particle size range from about 0,5 µm to 2 µm.

Note 4 to entry: Including both normal and steric-hyperlayer modes, the technique has the capacity to separate particles ranging in size from approximately 1 nm to about 50 µm.

3.6
centrifugal field-flow fractionation
CF3
separation technique that uses a centrifugal field applied perpendicular to a circular channel that spins around its axis to achieve size separation of particles from roughly 10 nm to roughly 50 µm.

Note 1 to entry: Separation is governed by a combination of size and effective particle density.

Note 2 to entry: Applicable size range is dependent on and limited by the effective particle density.

3.7
channel
<field-flow fractionation> thin ribbon-like chamber with a parabolic flow profile required for separation under the influence of a field applied perpendicular to the channel flow

Note 1 to entry: Channel thickness can vary and is nominally determined by a spacer insert, while fixed channels have a predefined thickness and do not use inserts.

Note 2 to entry: In asymmetrical-flow field-flow fractionation, a trapezoidal channel is commonly used, typically with a maximum breadth of ca. 20 mm to 25 mm and length of ca. 100 mm to 300 mm.

Note 3 to entry: In asymmetrical-flow, one channel surface (depletion wall) is solid (impermeable) and the opposing surface (accumulation wall) consists of a semipermeable membrane on a porous frit.

Note 4 to entry: In centrifugal flow field-flow fractionation, both the inner and outer walls of the channel are solid (non-porous) and the channel is curved. A trapezoidal channel is commonly used, typically with a breadth of 10 mm to 20 mm and length of 300 mm to 550 mm.

3.8
spacer
<field-flow fractionation> thin plastic film with a cut-out that defines the thickness and lateral dimensions of the channel

Note 1 to entry: Trapezoidal or rectangular cut-outs are most commonly used in asymmetrical-flow field-flow fractionation.

Note 2 to entry: Typical spacer thickness used for separation of nano-objects ranges from 190 µm to 500 µm.

Note 3 to entry: Fixed channels do not use a spacer; in this case the channel shape and thickness are predefined.

3.9
channel thickness
<field-flow fractionation> nominal thickness as defined by the spacer or predefined in a fixed-height channel

3.10
effective channel thickness
<field-flow fractionation> thickness due to compressibility or swelling of the semipermeable membrane at the accumulation wall, the effective value of which can differ from the nominal value for a given spacer and is determined using a well-defined analyte of known diffusivity under the test conditions

Note 1 to entry: The measured effective channel thickness depends on other factors, such as interactions between the analyte and the membrane and variability in spacer manufacturing.

3.11
accumulation wall
surface of a field-flow fractionation channel toward which sample components are forced by the applied field acting perpendicular to the channel flow

Note 1 to entry: In asymmetrical-flow field-flow fractionation, the accumulation wall is flat and consists of a semipermeable membrane on a porous frit substrate.

Note 2 to entry: In centrifugal field-flow fractionation, the accumulation wall is impermeable and curved, and is located farther from the axis of rotation relative to the depletion wall. In the rare case that the particles have a lower density than the aqueous medium, the depletion and accumulation walls are reversed.

3.12
depletion wall
surface of a field-flow fractionation channel opposite the accumulation wall, which is depleted in analyte due to the movement of analyte toward the accumulation wall in the applied field

Note 1 to entry: In asymmetrical-flow field-flow fractionation, the depletion wall is flat and impermeable.

Note 2 to entry: In centrifugal field-flow fractionation, the depletion wall is impermeable and curved, and located closer to the axis of rotation relative to the accumulation wall. When the effective particle density is lower than the density of the medium, the depletion and accumulation walls are reversed.

3.13
carrier liquid
eluent
mobile phase
liquid phase used to achieve separation and transport of analytes

Note 1 to entry: The eluent or mobile phase can contain salts, surfactants, and/or other chemical constituents that are required for optimized separation and recovery of an analyte.

Note 2 to entry: In this document, only aqueous phases are relevant, but organic solvents can also be used if equipment and channel are compatible.

3.14
elution
<field-flow fractionation> process by which analytes in the mobile phase, or eluent, are transported through, and exit from, the fractionation channel

Note 1 to entry: Elution begins after injection, focusing and other pre-elution steps have completed.

Note 2 to entry: Elution can occur with or without an applied field.

3.15
elution time
<field-flow fractionation> elapsed time after initiation of elution and excluding preliminary steps such as injection, focusing or other transitions

Note 1 to entry: Elution and retention share the same timeline and can be use interchangeably.

Note 2 to entry: The horizontal (time) axis of a fractogram is generally expressed as elution time.

3.16
focusing
<asymmetrical-flow field-flow fractionation**>** process by which, during and after sample injection a counter-balanced flow entering from opposite ends of the channel (inlet and outlet) is applied to focus the sample components into a thin band close to the inlet port and near the accumulation wall

Note 1 to entry: This step is necessary to minimize band broadening and to allow components to achieve an equilibrium localization (relaxation) within the channel.

Note 2 to entry: Focusing is not used during frit-inlet injection.

3.17
relaxation
<field-flow fractionation**>** process by which the sample components assume their equilibrium state with respect to the opposing forces of diffusion and the applied field before elution is initiated

Note 1 to entry: In flow field-flow fractionation there are two means to achieve relaxation: normal focusing relaxation and frit inlet or hydrodynamic relaxation.

Note 2 to entry: In centrifugal field-flow fractionation, stop-flow is used to achieve relaxation.

3.18
injection flow
<field-flow fractionation> flow that drives the sample out of the injection loop and into the fractionation channel

Note 1 to entry: Depending on instrument design, injection can occur via a separate injection port or through the channel inlet port.

3.19
cross flow
<flow field-flow fractionation> flow field applied perpendicular to the channel flow to achieve separation of analytes

Note 1 to entry: In asymmetrical-flow field-flow fractionation, cross flow is created by the pressure differential across a permeable membrane at the accumulation wall, which results in a downward force that decreases with increasing distance from the accumulation wall.

Note 2 to entry: Cross flow is generated by using a flow controller combined with a single pump or by use of a second dedicated pump.

3.20
channel inlet flow
<field-flow fractionation> mobile phase, or eluent, that enters the channel at the front end (upstream)

Note 1 to entry: In asymmetrical-flow field-flow fractionation, inlet flow is split between cross flow and channel flow during elution.