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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

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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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This document was prepared jointly by Technical Committee ISO/TC 229, *Nanotechnologies* and Technical Committee IEC/TC 113, *Nanotechnology for electrotechnical products and systems*, 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 are as follows:

— addition of subclause 8.6 addressing alternative and emerging methods;

— revision of technical content to reflect the current state of the art;

— addition of Annex A summarizing an interlaboratory comparison conducted through VAMAS.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 in Reference [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 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 in 1974,[4] although a centrifugal field-based FFF system was first developed and tested independently 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 characterize 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.

For general references and further reading for FFF theory and practise, as well as AF4 and CF3 applications to nanotechnology, see References [6] to [18]. Annex A summarizes a Versailles Project on Advanced Materials and Standards (VAMAS) interlaboratory comparison conducted to evaluate the capacity of AF4 and CF3 techniques to separate and characterize components of a complex multimodal mixture of analytes reproducibly and with acceptable recovery 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 describes the general principles of field-flow fractionation and specifies parameters, conditions and minimal reporting requirements, as part of an integrated measurement system, required to develop and validate methods for the application of asymmetrical flow and centrifugal field-flow fractionation in the analysis of nano-objects and their aggregates and agglomerates in aqueous media. General guidelines and procedures are provided to aid the user.

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 80004-1:2023, *Nanotechnologies – Vocabulary — Part 1: Core vocabulary*

ISO/TS 80004-6:2021, *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* (3.1)

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

3.3
nanoparticle
nano-object (3.2) with all external dimensions in the *nanoscale* (3.1)

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]