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

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[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 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[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 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 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]

3.3
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, a liquid flow, electrical or magnetic.

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

[SOURCE: ISO/TS 80004-6:2013, 4.4, modified — The term “field flow” has been changed to “field-flow”.]

3.4
asymmetrical-flow field-flow fractionation
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.

3.5
centrifugal field-flow fractionation
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.

3.6
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 defined by a spacer insert.

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 circular 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.

3.7
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.

3.8
channel thickness
<field flow fractionation> nominal thickness as defined by the spacer

3.9
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.

3.10
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.

3.11
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.

3.12
carrier liquid
eluent
mobile phase
liquid phase used to achieve separation and transport of analytes

Note 1 to entry: The eluent or mobile phase may 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.

3.13
elution
<field-flow fractionation> process by which analytes in the mobile phase, or eluent, are transported through, and exit from, the fractionation channel

3.14
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: During focusing outward flow occurs only through the permeable membrane at the accumulation wall.

3.15
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.

3.16
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.

3.17
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.

3.18
channel inlet flow
<field-flow fractionation> 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.

3.19
channel flow
<field-flow fractionation> eluent flow through the channel

Note 1 to entry: Channel flow is generally equivalent to the flow exiting the channel and entering the detectors under typical experimental conditions, but can differ if flow exiting the channel is split.

Note 2 to entry: In asymmetrical-flow field-flow fractionation, fluid loss through the permeable accumulation wall leads to a linearly decreasing channel-flow velocity. This gradient can be compensated using a trapezoidal channel design with decreasing channel breadth toward the outlet.

3.20
void volume
<field flow fractionation> fluid volume defined by the channel dimensions plus the volume between the channel exit and the first detector

3.21
void peak
<field-flow fractionation**>** a peak appearing in the fractogram that corresponds to unretained, typically small sample components that are not in equilibrium with the separation field

Note 1 to entry: The void peak travels at the average carrier velocity and elutes before retained components.

Note 2 to entry: In this context, unretained means components that are not separated by the field and elute with the void peak. Unretained has a different meaning in traditional enthalpic-based chromatographic separations.

3.22
void time
time between initiation of elution and detection of the void peak defined at its maximum signal intensity

3.23
retention time
time between initiation of elution and detection of an analyte peak defined at its maximum signal intensity

Note 1 to entry: For a Gaussian peak, the maximum and peak centre are equivalent.

3.24
retention parameter
<field flow fractionation> dimensionless parameter equal to the ratio of the analyte zone centre-of-mass distance (from the accumulation wall) to the channel thickness

Note 1 to entry: A measure of the strength of interaction between the applied field and the analyte.

3.25
retention ratio
<field flow fractionation> ratio of the mean velocity of the analyte zone to the mean velocity of the mobile phase in the channel during elution

Note 1 to entry: This can be calculated theoretically or determined empirically from the ratio of the retention times associated with the void and analyte peaks, and is directly related to the retention parameter.

3.26
selectivity
<field-flow fractionation> measure of the ability of a method to separate analytes of different diffusion coefficient or size; empirically, the slope of a double logarithmic plot of diffusion coefficient versus retention ratio for analytes of known size, where a high value reflects a large change in retention time with a small variation in analyte size

Note 1 to entry: In centrifugal field-flow fractionation, selectivity is also dependent on effective mass, but the empirical relationship is defined in the same manner as asymmetrical-flow field-flow fractionation.

3.27
resolution factor
fractionation power
ratio of the difference in retention time to the average of the peak widths measured as the full width at half maximum for two adjacent eluting analytes

Note 1 to entry: Measure of the degree of separation between neighbouring or overlapping peaks.

3.28
band broadening
overall dispersion or widening of an analyte band as the analyte passes through a separation system