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Nanotechnologies — Magnetic nanomaterials —

Part 2: Specification of characteristics and measurement methods for nanostructured magnetic beads for nucleic acid extraction

Nanotechnologies — Nanomatériaux magnétiques —

Partie 2: Spécification des caractéristiques et des méthodes de mesure pour les billes magnétiques nanostructurées pour l'extraction d'acide nucléïque

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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 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 by Technical Committee ISO/TC 229, *Nanotechnologies*.

A list of all parts in the ISO/TS 19807 series can be found on the ISO website.

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

Magnetic beads are composed of a large number of magnetic nanoparticles immobilized within a nonmagnetic matrix with a size range between tens of nanometres and hundreds of micrometres (see Annex A). The immobilization matrix is typically based on silica or organic polymers. The beads are commonly supplied while dispersed in a liquid suspension, for example, ethanol, isopropanol, sodium azide solutions, pure water. Magnetic beads in liquid suspension have become one of the most widely used nanomaterials in the biological and chemical fields, due to their unique magnetic properties and interactions with applied magnetic fields.

When the size of a magnetic object is small enough, it will form a single magnetic domain, behaving as a single large macrospin. At yet smaller sizes (for iron oxide, typically less than 30nm[1]), the thermal energy of the object can be sufficient to result in frequent reorientations of the magnetization direction of the object. If the timescale of these reorientations is shorter than the timescale of the measurement, the term 'superparamagnetism' is used to describe this behaviour and the magnetic nano-objects are said to be superparamagnetic. In large non-interacting ensembles of such particles, the thermally induced switching events will result in the average magnetization of the ensembles being zero in the absence of an applied magnetic field. In the presence of an applied large field, the ensemble of magnetic nano-objects is observed to acquire a large net magnetization, as the magnetic field overcomes the thermal fluctuations and aligns the macrospins of the individual magnetic nano-objects within the ensemble. Beads, if incorporating a large fraction of magnetic nano-objects which exhibit this behaviour, are often referred to as "superparamagnetic beads". However, as the beads may not themselves be superparamagnetic, they are referred to as "magnetic beads" herein.

Magnetic beads have been applied in many fields, especially in biosensing applications[2] such as *in vitro* diagnostics, targeted drug delivery[3]-[5], magnetic resonance imaging[6], bioseparation[7], and genetic engineering[8], among others. For example, nucleic acids, which carry genetic information, can be extracted or isolated from blood, saliva, faeces, urine, leaves, viral lysates, using suitably functionalized magnetic beads.

The nucleic acids (DNA) and ribonucleic acid (RNA) carry the key information that organisms use to build or maintain their biostructures. Correctly identifying DNA offers immensely valuable information on health. In recent years, in the human blood stream, scientists have not only found circulating cell free DNA (cfDNA), but also circulating tumour DNA (ctDNA). Now ctDNA extraction is one of the most widely used liquid-biopsy methods to determine cancer or track cancer development. However, the content of ctDNA is only 1 % or less of the total cfDNA amount. The concentration of cfDNA is very low, generally 5 ng/ml blood to 30 ng/ml blood. Therefore, the development of reliable methods for extracting the ctDNA is critical. The proper description of physicochemical characteristics of magnetic beads for DNA extraction is both valuable for developers of extraction kits and for users applying them for DNA analysis.

Nucleic acid binding to magnetic beads relies on electrostatic interactions, hydrophobic interactions, hydrogen bonding or specific binding mechanisms to the bead surface. Once DNA or RNA from cell or tissue lysate is released into the solution, then nucleic acids can bind to surface-modified magnetic beads to form a "nucleic acid-magnetic bead complex".[9]-[19]

Then, the complex can be separated under a proper combination of magnetic field and magnetic field gradient. The eluate can wash away the residual impurities. Finally, the nucleic acids to be extracted can be obtained from the beads after desalination and purification.[9]-[19]

The different forms of magnetic beads and dispersing media for the extraction of nucleic acid will have different physicochemical characteristics such as specific surface area, bead concentration etc. All these characteristics will affect their performance to extract nucleic acid to varying extents. [9]-[19]

In common with other nanostructured materials, the manufacturing and material specification of composite magnetic beads are complex. Small variations in the synthesis conditions during bead manufacturing and functionalization can lead into dramatic shifts in the properties and binding capacities of the manufactured beads. This requires these products to have high manufacturing consistency. Currently, different manufacturers provide different characteristics and most of them

never provide the measurement methods, so it is difficult for consumers or regulators to compare different products or to verify the characteristics, which increases the difficulty of further development of the application. Universally accepted material specification and test reports for magnetic beads are a requirement in order to ensure customer confidence and the quality of the nucleic acid extraction products.

Nanotechnologies — Magnetic nanomaterials —

Part 2: Specification of characteristics and measurement methods for nanostructured magnetic beads for nucleic acid extraction

1 Scope

This document specifies characteristics to be measured of magnetic beads in suspension and powder forms for nucleic acid extraction applications. This document deals with magnetic beads that contain a substantial amount of magnetic nanoparticles (which can be superparamagnetic). Potential applicable measurement methods are listed for the individual characteristics. Specifically, this document lists critical characteristics of magnetic beads and suspensions, and additional characteristics to describe the magnetic beads and the suspension for nucleic acid extraction.

Health, safety and environmental aspects of magnetic beads are not within the scope of this document.

2 Normative references

The following referenced documents are indispensable for the application 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 80004-6, *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 80004-6 and the following apply.

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

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

— IEC Electropedia: available at http://www.electropedia.org/

3.1
bead mass concentration
ratio of the mass of *magnetic beads* (3.6) to the total volume of a magnetic beads sample in suspension or powder form

3.2
bead size
effective outer diameter of a *magnetic bead* (3.6) determined by using the specified measurement method

3.3
bead size distribution
distribution of beads as a function of *bead size* (3.2)

Note 1 to entry: Bead size distribution may be expressed as cumulative distribution or a distribution density (distribution of the fraction of beads in a size class, divided by the width of that class).

3.4
dispersing medium
liquid in which *magnetic beads* (3.6) are suspended

3.5
initial magnetic mass susceptibility
differential ratio of the change in mass magnetization of a material to the amplitude of a magnetic field change at a sufficiently small absolute magnetic field

Note 1 to entry: A *magnetic beads* (3.6) sample is assumed to be magnetically isotropic and its initial magnetic mass susceptibility is indicated as a scalar.

3.6
magnetic bead
small round piece containing a large number of magnetic nanoparticles which can be superparamagnetic and are immobilized within a non-magnetic matrix

Note 1 to entry: The size range of magnetic beads for DNA extraction spans from a few tens of nanometres to several micrometres.

3.7
mass-specific surface area
absolute surface area of the sample divided by sample mass

[SOURCE: ISO/TS 80004-6:2021, 4.6.1, modified — Note 1 to entry has been removed.]

3.8
nucleic acid
macromolecule that is the medium for genetic information or acts as an agent in expressing the information

Note 1 to entry: There are two types of nucleic acid, DNA and RNA.

[SOURCE: ISO 17822:2020, 3.32]

3.9
nucleic acid binding capacity
mass of *nucleic acid* (3.8) bound to the surfaces of *magnetic beads* (3.6) per unit mass of the magnetic beads under specified conditions

3.10
operational time
maximum time after the start of the extraction process where the suspension of *magnetic beads* (3.6) is ready for use to extract *nucleic acid* (3.8)

Note 1 to entry: the operational time is usually recommended by the manufacturer.

3.11
remanent mass magnetization
value of the mass magnetization remaining in a magnetized body when, in the absence of a self-demagnetizing field, the applied magnetic field strength is brought to zero

[SOURCE: IEC 60050:1990, 221-02-40, modified — "magnetization" has been changed to "mass magnetization".]

3.12
saturation mass magnetization
limiting value of the mass magnetization of a liquid or dried sample with increasing applied magnetic field strength

Note 1 to entry: The saturation mass magnetization of *magnetic beads* (3.6) is indicated for the dried matter of a bead suspension sample or for the dried sample in the case of beads in powder form.

3.13
shelf life
recommended time period by manufacturer during which a product (suspension or powder) can be stored, throughout which the defined quality of specified characteristics of the product remains acceptable under expected (or specified) conditions of distribution, storage, display and usage

Note 1 to entry: Defined characteristics should be measured after fixed time intervals.

[SOURCE: ISO/TS 19807-1:2019, 3.37, modified — the manufacturer has been specified and the powder product has been added.]

3.14
surface functional group density
mass of surface functional groups per unit mass of *magnetic beads* (3.6)

3.15
surface functional group type
chemical type of substituents or moieties on the surface of *magnetic beads* (3.6) that are responsible for a specific chemical reaction

4 Abbreviations

For the purposes of this document, the following abbreviations apply:

BET method	Brunauer–Emmett–Teller method
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
IR	Infrared
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
SQUID	Superconducting quantum interference device
TEM	Transmission electron microscopy
UV-Vis spectrometry	Ultraviolet-visible spectrometry
VSM	Vibrating sample magnetometry
XPS	X-ray photoelectron spectroscopy

5 Characteristics to be measured and measurement methods

5.1 General

The critical characteristics listed in Table 1 of magnetic beads products supplied for nucleic acid extraction shall be measured. The additional characteristics listed in Table 2 are frequently measured in industrial communities depending on the application. However, whether to provide these additional characteristics is optional for the supplier. The selection criteria for the first table are the critical characteristics of magnetic beads and suspensions. They determine fundamentally the extraction performance, independent of the nucleic acid sample type or the subsequent extraction process. The additional characteristics can influence the extraction performance, depending on the specific application or process.

Measurement methods and relevant standards for these methods are listed in Tables 1 and 2. Listed measurement methods can be alternatively used. However, other measurement methods may also be used as agreed between supplier and purchaser. Any characteristic from these tables shall be reported by stating its value and the measurement method used. The listed ISO documents for measurements have been generally applied to measurements for characteristics of non-magnetic objects. However, it should be noted that these ISO documents have not yet been fully validated for the application to magnetic beads.

Tables 1 and 2 provide alternative measurement methods for some characteristics. It should be noted that the values of characteristics obtained by a measurement method can deviate to some extent from that obtained by another measurement method.

Table 1 — Critical characteristics of magnetic beads to be measured

Characteristics	Measurement method	Relevant standards
Bead mass concentration	Gravimetry and oven drying	ISO 11358-1[20]
Bead size distribution	DLS [21]-[24]	ISO 22412[25]
	SEM [21],[23]	ISO 19749[26]
	TEM [21]-[23]	ISO 21363[27]
	Ultrasonic attenuation spectroscopy [28]	ISO 20998-1[29] ISO 20998-3[30]
	Electrical sensing zone [31]	ISO 13319-1[32]
Nucleic acid binding capacity	UV-Vis spectrometry[33],[34]	ISO 21571[35]
	Real-time PCR[36],[37]	ISO 21571[35]
	Agarose gel electrophoresis[38]	ISO 21571[35]
Remanent mass magnetization	SQUID magnetometry [39],[40]	
	VSM [21],[22],[41]	
Surface functional group type	IR [21],[24],[42]	
	XPS	ISO 20903[43]
Saturation mass magnetization	SQUID magnetometry	
	VSM	

Table 2 — Additional characteristics of magnetic beads to be measured

Characteristics	Measurement method	Relevant standards
Initial magnetic mass susceptibility	VSM	
	SQUID magnetometry	
Iron ion concentration	ICP-OES [44]	ISO 11885[45]