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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 nucléique

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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]

Table 2 *(continued)*

Characteristics	Measurement method	Relevant standards
Mass specific surface area	Gas adsorption method [46],[47]	ISO 9277 [48] ISO 18757[49]
Size of primary magnetic nanoparticles	TEM	ISO 21363[27]
Surface functional group density	Conductometric titration	

5.2 Descriptions of characteristics and their measurement methods

5.2.1 Bead mass concentration

The bead mass concentration of a sample of magnetic beads in suspension or in powder form is the ratio of the mass of the magnetic beads to the total volume of the sample.

The mass of the beads after drying shall be measured by the oven drying method. For oven drying of magnetic beads in suspension, a certain volume of the suspension shall be washed by deionized water and separated by magnetic separation several times to remove any soluble ingredients in the dispersing medium. This makes sure that the soluble content in the dispersing medium in the bead suspension is neglectable.

Then, the sample in powder or suspension form shall be dried until a constant mass is reached which is determined by weighing. The temperature used for drying shall induce the evaporation of the liquid compartments of the sample but not lead to decomposition of the beads. For water-based suspensions, the drying temperature is typically 105 °C ± 2 °C.

The bead mass concentration is expressed in the unit kg/l.

NOTE 1 The bead mass concentration is correctly measured when solid materials other than the magnetic beads are negligible in the sample. Otherwise, the measurement result includes the mass of the other solid materials.

NOTE 2 If the mass of the dried beads is divided by the total sample mass measured before drying, the result is called dry matter content and it is expressed in the unit kg/kg.

5.2.2 Bead size distribution

The bead size distribution can have an impact on their extraction performance, which makes the measurement of the size distribution necessary. The bead size distribution shall be measured by an appropriate measurement method. The recommended methods are DLS, SEM, TEM, ultrasonic attenuation spectroscopy and electrical sensing zone method. The measurement results are expressed in the unit of nm or µm.

The bead size distribution of magnetic beads for nucleic acid extraction should be performed according to the procedures described in the relevant ISO documents mentioned in Table 1. These documents explain the measurement, the data analysis and the expression of results in detail.

5.2.3 Nucleic acid binding capacity

For the measurement of nucleic acid binding capacity, a binding experiment between a magnetic beads sample and a reference sample of suspended nucleic acid is performed. Due to the large variety of magnetic beads for nucleic acid extraction and their different application scenarios, it is not possible to define a harmonized protocol for the binding experiment. The bead manufacturer should establish its own specified protocol to perform the binding experiment. Bead users and other interested parties can also develop their own protocol to perform the binding experiment. It is also possible that the details of the binding experiment are established by negotiations between bead manufacturer and bead user or other interested parties.

The purpose of the reference sample is to provide target nucleic acid under known conditions for binding by the magnetic beads. It should be made sure that the reference sample contains only target nucleic acids and no proteins or other confounding compartments.

The magnetic beads are mixed with the reference sample and the nucleic acid will bind to the magnetic beads. The established protocol shall contain procedures on how to perform the mixing and how to handle the mixed suspension including the time of the binding phase and the temperature.

After the binding phase, the magnetic beads are fixated on a solid surface by a magnetic field gradient and washed to remove unbound nucleic acid from the suspension medium. In a second washing step, the bound nucleic acids are separated from the magnetic bead surfaces according to the established protocol. The magnetic beads and the suspension containing now only the previously bound nucleic acid are then separated by magnetic separation. The mass of previously bound nucleic acid in the suspension shall be quantitatively determined by an appropriate measurement method.

The possible measurement methods include UV-Vis spectrometry, agarose gel electrophoresis, and real-time PCR. The principles of these measurement methods are:

a) UV-Vis spectrometry: ultraviolet and visible light is absorbed by the sample. The concentration of nucleic acids in solution is derived from the absorptivity and the optical length of the sample.

b) Agarose gel electrophoresis: nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose. After electrophoresis, the nucleic acid will be concentrated in bands that are characteristic for their length and charge. The light absorption of these bands is compared to that of a reference sample with known concentration of the same nucleic acid type and thus the amount of nucleic acid can be quantified.

c) Real-time PCR: nucleic acid molecules are stained by ethidiumbromide which enhances their fluorescence signal. Then the nucleic acid molecules are amplified in a polymerase chain reaction and the fluorescence is measured over different cycles. The intensity of the fluorescence signal is proportional to the amount of reaction products. Thus, the initial amount of nucleic acid can be calculated.

ISO 21571[35] specifies procedures of those measurement methods for foodstuffs applications which can also be applied here.

The magnetic beads which have been involved in the experiment are washed again and dried, and their mass is measured by weighing.

The nucleic acid binding capacity is determined by the ratio of the mass of the bound nucleic acid and the mass of magnetic beads.

The measurement results of nucleic acid binding capacity and measurement conditions shall be reported according to Clause 7 d); the type of nucleic acid used for the reference sample, concentration of the nucleic acids in the reference sample, concentration of magnetic beads in the mixture and measurement temperature.

The result of the measurement for nucleic acid binding capacity is expressed in the unit kg/kg.

NOTE The numeric value of the nucleic acid binding capacity is characteristic for the applied protocol of the binding experiment. It can be used for quality control of the magnetic beads and it gives an assessment of the binding performance of the beads under the specified conditions. The nucleic acid binding capacity obtained in a specific application can differ from the reported value, if the conditions of the application differ from the procedure which led to the reported value.

5.2.4 Remanent mass magnetization

The remanent mass magnetization of a magnetic bead for nucleic acid extraction is directly proportional to the mechanical force acting on the bead when it is in a magnetic gradient field at zero absolute magnetic field. Thus, remanent mass magnetization affects the speed and effectiveness of the extraction process, as well as the possible agglomeration of beads. When solid magnetizable surfaces

are present in the reaction environment, the remanent mass magnetization can also lead to unwanted accumulation of the beads at these surfaces in the absence of an external magnetic field.

For the measurement of the remanent mass magnetization, the magnetic beads shall be washed and dried in an oven. Their mass is determined by weighing. The magnetic moment of the dried beads sample is measured using a SQUID or VSM. During the measurement, the dried magnetic beads sample are first exposed to a high magnetic field in the range of the saturation magnetization field. Then, the magnetic field is monotonically brought to zero and the remaining magnetic moment of the sample is measured. The remanent mass magnetization is calculated as the ratio of the magnetic moment at zero field and the mass of the magnetic beads.

The result of the measurement is expressed in the unit $A \cdot m^2/kg$.

It is not trivial to obtain a zero field in precision magnetometry. Parts of the measurement setup can acquire a static magnetization which can interfere with the results. The device calibration should be considered.

NOTE There exists no internationally harmonized measurement protocol for the measurement of the remanent mass magnetization of magnetic beads.

5.2.5 Surface functional group type

The type of functional groups that are coated on the surfaces of magnetic beads is determined by the manufacturer of magnetic beads considering the downstream applications. For nucleic acid extraction purpose, silanol groups and carboxyl functional groups, leading to rapid binding of nucleic acid, are coated most frequently.

The type of coated functional groups on the bead surface shall be identified by an appropriate measurement method, which includes IR and XPS. In IR, the absorption spectrum of electromagnetic waves of the respective wavelength by the sample is measured. In XPS, the emission of electrons caused by X-ray radiation is measured. The chemical compounds present on the beads have specific spectral patterns and can thus be identified.

ISO 20903[43] specifies measurement protocols for XPS.

For the data analysis, the measured absorption spectrum (IR) or photoemission spectrum (XPS) is compared to reference spectra that are typical for the coated surface functional group. Such reference spectra are available in libraries or can be obtained by spectroscopic measurements of the material of the functional groups. The interpretation of the spectra of coated magnetic beads can benefit from comparison with those of uncoated magnetic beads of the same type if such spectra are available.

5.2.6 Saturation mass magnetization

The saturation mass magnetization of magnetic beads for nucleic acid extraction has an influence on the speed and effectiveness of the extraction process and on the possible agglomeration of beads during the extraction process. It is an important characteristic of the magnetic behaviour of the beads and can be used to monitor the quality of the beads.

For the measurement of the saturation mass magnetization, the magnetic beads shall be washed and dried in an oven. Their mass is determined by weighing. The magnetic moment of the dried beads sample is measured using a SQUID or VSM. During the measurement, the dried magnetic beads sample are susceptible to an increasing magnetic field until the value of the magnetic moment is no longer changing with field increase. At this field strength, the magnetic moment of the sample is measured. The saturation mass magnetization is calculated as the ratio of the measured magnetic moment and the mass of the magnetic beads.

The result of the measurement is expressed in the unit $A \cdot m^2/kg$.

NOTE There exists no internationally harmonized measurement protocol for the measurement of the saturation mass magnetization of magnetic beads.

5.2.7 Initial magnetic mass susceptibility

The initial magnetic mass susceptibility is an important characteristic of magnetic beads for nucleic acid extraction because it can have a significant influence on the extraction performance.

In the absence of an external magnetic field, the net magnetization of the magnetic beads is small due to random orientation of the magnetic moments of the nanoparticles inside the beads. However, when an external magnetic field is switched on, the magnetic moments of the nanoparticles inside the beads will acquire a preferential orientation and thus the magnetic beads will develop a net magnetization. The ratio between the change in magnetization and the corresponding change in magnetic field is called magnetic susceptibility. The susceptibility that is measured at a very small absolute magnetic field is called initial magnetic susceptibility.

Before the measurement, the magnetic beads sample is washed and dried in an oven and then the mass of the dried magnetic beads sample is determined by weighing.

For the measurement of the initial magnetic susceptibility, the dried magnetic beads sample should be demagnetized by a sufficiently small absolute magnetic field with vanishing amplitude over time. Then, the magnetic moment of the dried magnetic beads sample is measured using VSM or a SQUID at a small absolute magnetic field amplitude, where the relation between the magnetic moment of the sample and the magnetic field is still sufficiently linear.

The initial magnetic mass susceptibility of a magnetic beads sample is calculated by dividing the magnetic moment of the sample by the product of the applied magnetic field and the mass of the dried beads.

The result of the measurement is expressed in the unit m^3/kg.

5.2.8 Iron ion concentration

Iron ion concentration is a characteristic of the magnetic beads suspension. The iron ions can be introduced into the magnetic bead suspension during manufacturing processes and also can leak from the magnetic beads. The iron ion concentration can help to determine the quality of the coating of the magnetic beads.

For the measurement of the iron ion concentration of a magnetic beads suspension sample, first the magnetic beads need to be separated from the suspension sample. The easiest way to achieve this is to extract the magnetic beads using a magnetic tip that is introduced into the suspension sample or to immobilise the magnetic beads at the container wall by placing a strong magnet on the outside of the container wall. Then, the supernatant is removed from the container. The supernatant can contain the iron ions, as well as nanoparticles, which can be present in the suspension and which are too small to be attracted by the magnet. The nanoparticles are removed by another subsequent separation step applying ultracentrifugation to the supernatant.

Once a magnetic beads suspension sample is clean of magnetic beads and magnetic nanoparticles, the amount of iron ion in the supernatant is determined by ICP-OES. The iron ion concentration of the magnetic beads suspension sample is calculated by dividing the amount of iron ions in the supernatant by the volume of the suspension sample. ISO 11885[45] specifies measurement procedures for iron ion concentration in different types of water.

The result of the measurement is expressed in mol/l.

5.2.9 Mass specific surface area

The mass specific surface area is a measure for the total bead surface in an ensemble of beads and an important characteristic for assessing quality and functionality of the beads. It is measured by the gas adsorption method, often called BET method. In this method, the specific surface area is determined by measuring the amount of physical adsorbate required to cover the external and accessible internal pore surface of a solid with a complete monolayer of adsorbate. Any gas may be used provided it is physically adsorbed by weak bonds at the surface of the solid (Van der Waals forces) and can be desorbed by a

decrease in pressure at the same temperature. Due to practical reasons the adsorption of nitrogen at a temperature of 77 K (liquid nitrogen) has been established as the method for the determination of specific surface areas. By means of the BET equation, the amount of adsorbed gas, which builds up one monolayer on the surface, can be calculated from the measured isotherm. The amount of molecules in this monolayer multiplied by the required space of one molecule (0,162 nm^2 for N_2) and normalized to the mass of magnetic beads gives the mass specific surface area.

The BET method requires samples in powder form. For that reason, before the measurement, a sample of magnetic beads in suspension is washed and dried in an oven and the mass of magnetic beads is then determined by weighing.

Guidance on the details of the BET method can be found in ISO 18757[49] and ISO 9277[48].

The result of the measurement is expressed in m^2/kg.

For gel-like beads, the BET method is not suitable to measure the mass specific surface area, as swelling property of gel-like materials can cause the morphology to change. An approximate mass specific surface area of gel-like beads is estimated by the ratio of calculated spherical surface area to mass of the magnetic bead.

5.2.10 Size of primary magnetic nanoparticles

Primary magnetic nanoparticles are the magnetic compartments of the beads. The magnetic behaviour of magnetic nanoparticles depends strongly on their size and size distribution. Therefore, these parameters will also determine the overall magnetic performance of the beads. The size of the primary magnetic nanoparticles shall be measured by TEM. The measurements may be performed before or after the synthesis of magnetic beads.

The primary magnetic nanoparticles may not necessarily be spherical. Therefore, the area equivalent diameter of a magnetic nanoparticle in the two-dimensional TEM image is measured as the size.

The size of all particles in a TEM image should be measured. Clearly recognizable aggregates should be excluded from the analysis and only primary particles should be measured. It is suggested to measure more than 500 primary particles.

The measurement results should be displayed as a histogram of the number of magnetic nanoparticles versus size at the interval of 1 nm. Also, the average (median) of the size should be expressed in the unit of nm. It should be noted that the measurement results can exhibit an increased uncertainty when the observed microscopic images lack the representativeness of sample.

5.2.11 Surface functional group density

The surface functional group density of magnetic beads for nucleic acid extraction is a characteristic of the reactive surface of magnetic beads. The surface functional group density strongly influences the binding of nucleic acids to the magnetic beads. However, it is not equivalent to the nucleic acid binding capacity described in 5.2.7, which depends on more parameters.

The surface functional group density in a dried magnetic beads sample is measured most frequently by conductometric titration. When the sample is provided in suspension form, a test specimen in powder form is prepared by washing magnetic beads and drying them in an oven. Their mass is determined by weighing and then the beads are resuspended and treated with a known amount of acid. The excess acid is back titrated using a standardized base solution. During titration, the conductivity is measured. The conductivity in dependence on consumed base has a minimum from which the number of surface charges on the bead surface and thus the mass of functional groups on the bead surface can be calculated. The surface functional group density of magnetic beads is then the ratio of the mass of functional groups to the mass of the magnetic beads.

The result of the measurement is expressed in the unit kg/kg.

NOTE There exists no internationally harmonized measurement protocol for the measurement of the surface functional group density of magnetic beads.

6 Sample preparation

The sample subjected to a measurement shall be chosen as representative of the parent population of the sample. Sampling of powders and suspension should be performed according to the instructions in ISO 14488[50].

As many nano-objects are reactive, their physical and chemical properties can be affected by the sampling and their storage environment. Consequently, the supplier and purchaser should agree the sampling point and storage of the samples for comparability of results.

7 Test report

7.1 The test report shall contain, but not be limited to, the following information:

a) a reference to this document (i.e. ISO/TS 19807-2:2021);

b) details generally necessary to identify the product tested (product name, lot number, chemical name of bead components (nanoparticles and matrix) and dispersing medium, operational time);

c) sample description: for example, state (suspension or powder);

d) measurement results of characteristics listed in Table 1 with their name and the measurement methods as well as the measurement conditions;

e) the date of test, name of testing laboratory, and statement on the quality system of testing laboratory;

f) shelf life guaranteed by supplier and the method how it was determined;

g) any special information supporting the reliability of measurement results;

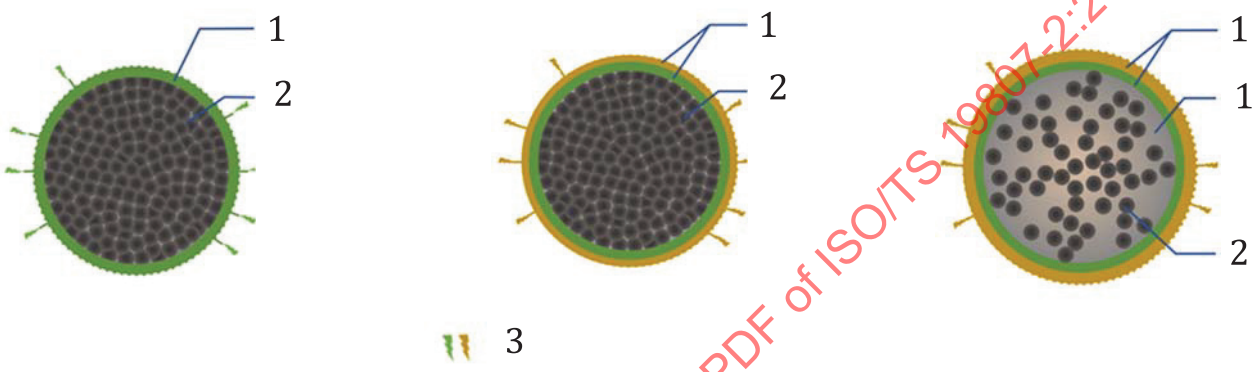
h) any deviations from the procedure.

7.2 The test report should contain, but not be limited to, the following information: measurement results of characteristics listed in Table 2 with their name, the measurement methods and measurement conditions.

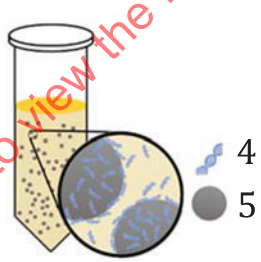
Annex A
(informative)

Schematics of magnetic beads

Figure A.1 shows schematic diagrams of magnetic bead structures. They include the most popular three kinds of magnetic beads in the market for nucleic acid extraction.



a) Schematic cross-sectional views of three kinds of magnetic bead for nucleic acid extraction



b) Schematic for magnetic beads with target nucleic acid in dispersing medium

Key

1 non-magnetic matrix
2 primary magnetic nanoparticles
3 surface functionality of magnetic beads
4 nucleic acid
5 magnetic beads

Figure A.1 — Schematic diagrams of magnetic bead structures and interaction with target nucleic acid