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Nanotechnologies — Evaluation of the antimicrobial performance of textiles containing manufactured nanomaterials

Nanotechnologies — Evaluation de la performance antimicrobienne des textiles contenant des nanomatériaux manufacturés

PROOF/ÉPREUVE

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Foreword

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

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.

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Introduction

The utilization of nanotechnology in textile industry has presented novel functions such as antimicrobial activity, stain resistance, flame retardancy, mechanical strength enhancement, UV resistance, and wrinkle resistance into the conventional textiles without significant loss or change of the original properties. According to the nanodatabase website[1] there are already over 400 Textiles Containing Manufactured Nanomaterials (TCMNMs), making them the second largest market among other nanoproducts.

The rapid and continued growth of TCMNMs is increasing the need to develop international standards specific for manufactured nanomaterials (MNMs) in textiles and testing processes guidelines. It is a dual need of industry and consumer.

TCMNMs can be classified into three groups based on how nanomaterials are integrated into the textiles including nanofinished, nanocomposite, and nanofibrous textiles[2]:

a) Nanofinished textiles: The textiles that the applied nanoscale property is added after the textile fabrication through post-manufacture treatments and coatings to create nanostructured surfaces on fibre media. Most nanotextiles on the consumer market belong to this category.

b) Nanocomposite textiles: The textiles composed of fibres containing one or more nanostructured or nanoscale components produced by pre-manufacture integration of nanoscale properties into fibrous components.

c) Nanofibrous textiles: The textiles made of nanofibres which have a nanoscale cross-sectional area and may or may not have a nanoscale length.

Natural and manufactured textile fibres can be treated with different nanomaterials and chemicals to provide enhanced antimicrobial properties. The antimicrobial activities of TCMNMs include activities against bacteria, fungal, viruses, and other microorganisms. Also, the antimicrobial activities can help to impart anti-odour property as the consequence of the reduced microbial activity. For antimicrobial TCMNMs, various metals, mainly silver and copper, and metal oxides such as copper oxide (CuO), titanium dioxide (TiO_2) and zinc oxide (ZnO) are normally used.

Several characteristics of MNMs have great impacts on their antimicrobial performance including size, shape, surface area, chemical composition, surface chemistry and surface charge. The size and shape of MNMs have important impacts on their antimicrobial property due to their association to their surface area. Generally, the antibacterial properties of nanoparticles are size-dependent. Smaller particles with higher surface area to volume ratio have more contact with bacteria and/or fungi cells leading to improve the bactericidal and/or fungicidal effectiveness[3]. Therefore, when they incorporate in textiles even at low concentrations they show noticeable antimicrobial activity compared to their micro-and macro scale counterparts.[4]-[6] The shape of MNMs remarkably influences the rate of interaction and uptake by microbial cells. For instance, spherical-shaped of gold nanoparticles demonstrated higher cellular uptake than nanorod shaped particles[7]. Surface charge of MNMs is another important characteristic that can be measured by Zeta potential method. The antimicrobial effect of MNMs is triggered by the electrostatic interaction between the positively charged MNMs and the negatively charged microbial cell membranes ultimately leading to cell damage and inhibition of their growth and reproduction. Surface chemistry of MNMs has an important effect on their antimicrobial activity. The presence of functional groups, capping agents or biomolecules on the surface of nanomaterials has also potential influence in their antibacterial activities. Surface functionalization of antimicrobial nanoparticles such as silver nanoparticles with bioactive molecules exhibited enhanced antibacterial activity compared to the bare ones[8]. The above mentioned inter-relationship highlights the important effect of physiochemical characteristics on antimicrobial performance of TCMNMs.

Currently, there are various antimicrobial TCMNMs products in the market such as underwear, shirts, socks, bed sheets/covers etc. The antimicrobial mechanism of action of nanomaterials can generally be described as one of three models: oxidative stress induction, metal ion release, or non-oxidative mechanisms, which can occur simultaneously as well[2]. The antimicrobial activity of TCMNMs can decline significantly after several washing cycles and exposure to body sweat due to the possible release of incorporated nanomaterials and also the chemical action of sweat and laundering solution on the nanocompounds. Currently, there is no ISO standard specific to TCMNM products. Therefore, the development of a standard to determine antimicrobial performance of TCMNMs subjected to washing process and body sweating can facilitate the trade and growth of market. It is worth mentioning that already published ISO standards are related to the assessment of antimicrobial properties of conventional textiles. Moreover, there is an ASTM standard document for detection and characterization of silver nanomaterials in textiles[9]. However, these documents do not address the potential release of nanomaterials/nanostructure from TCMNNs following washing or sweating and their possible consequence on the antimicrobial activity of these textiles.

This document does not address nano-safety and environmental impact of the release of nanomaterials from TCMNMs into the air, water and to landfill. Data related to the release of nanomaterials from the fabrics under different conditions such as sweating, mechanical stresses (repetitive abrasion) during washing process in a laundry machine, are considered as essential information for understanding the potential releases to the environment.

Artificial sweat solution is an appropriate candidate to use as a material to resemble the human skin sweat to determine the amount of release of nanomaterials from TCMNMs to human body. For many TCMNMs applications, such as human clothes, there is a high possibility of skin contact and interaction with incorporated nanomaterials[10]. In such condition, the involved interaction and release of the nanomaterial can also affect the antibacterial performance of TCMNMs.

Considering the effect of the release of nanomaterials from TCMNMs by washing process and human sweat, this document specifies the measurement methods of the released nanomaterials, the antimicrobial performance and the assessment method of TCMNMs. Further, from TCMNMs subjected to washing process and exposed to artificial human body sweat solution are specified.

Nanotechnologies — Evaluation of the antimicrobial performance of textiles containing manufactured nanomaterials

1 Scope

This document specifies the antimicrobial performance assessment method of textiles containing manufactured (metals/metal oxides) nanomaterials (TCMNMs). The textiles in this document include fabric, yarn and fibre in which manufactured nanomaterials are used during production or finishing process. Further, this document also specifies protocols to determine the quantity of nanomaterials released from textile following washing and/or exposure to artificial human body sweat.

This document only covers the antibacterial, antifungal, and the anti-odour performance assessment method of TCMNMs.

This document does not cover textiles that have therapeutic application as well as environment, health and safety (EHS) issues related to TCMNMs. Further, it does not cover the release of nanomaterials from TCMNMs as a result of aging, dry attrition and abrasion, although it is considered as an effective factor in releasing nanomaterials.

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 6330, *Textiles — Domestic washing and drying procedures for textile testing*

ISO 20743:2013, *Textiles — Determination of antibacterial activity of textile products*

ISO 13629-2, *Textiles — Determination of antifungal activity of textile products — Part 2: Plate count method*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6330, ISO/TS 80004-1 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
nanomaterial
material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.

[SOURCE: ISO/TS 80004-1:2015, 2.4, modified — Note 2 to entry has been deleted.]

3.2
textile
woven fabric, knitted fabric, etc., formed by the interlocking of fibres and yarns having certain cohesion and which is generally intended for clothing or furniture applications

Note 1 to entry: Textiles often include certain types of non-woven fabrics.

[SOURCE: ISO 16373-3:2014, 2.4]

3.3
antimicrobial activity
ability to kill/destroy/inactivate microorganisms, prevent their proliferation and/or prevent their pathogenic action

[SOURCE: ISO 18369-1:2017, 3.1.11.12]

3.4
antibacterial activity
activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

[SOURCE: ISO 20743:2013, 3.4]

3.5
antifungal activity
activity to prevent or mitigate the growth of fungus, expressed as the difference of growth value in logarithm of *ATP* (3.6) between the control and test sample

[SOURCE: ISO 13629-1:2012, 3.6]

3.6
ATP
adenosine triphosphate, a multifunctional nucleotide present in living fungi

[SOURCE: ISO 13629-1:2012, 3.5]

3.7
washing procedure
cycle of the washing action including water supplying, washing, and repeated rinsing, spinning and water supplying and ended by spinning as predetermined on the washing machine

[SOURCE: ISO 6330:2012, 3.7]

4 Symbols and abbreviated terms

AAS	Atomic absorption spectroscopy
AFM	Atomic force microscopy
AES	Auger electron spectroscopy
ATP	Adenosine triphosphate
BET	Brunauer, Emmett, and Teller
ELS	Electrophoretic light scattering
FESEM	Field emission scanning electron microscopy
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy

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ICP-MS	Inductively coupled plasma-mass spectrometry
MNM	Manufactured nanomaterial
TCMNM	Textiles containing manufactured nanomaterial
TEM	Transmission electron microscopy
SAED	Selected area (electron) diffraction
XPS	X-ray photoelectron spectroscopy

5 Characteristics of metal or metal oxide nanomaterials in TCMNMs

5.1 General

As was mentioned earlier in the introduction section, knowledge about the physicochemical characteristics of nanomaterials used in TCMNMs is important, considering their noticeable effects on their antimicrobial performance. Subject to the stakeholder agreement and the specific application, these characteristics as shown in Table 1 should be measured and reported.

A wide variety of analytical techniques are available for detection and characterization of nanomaterials in textiles. The selection of the appropriate techniques depends on capabilities, advantages and limitations of the techniques. Also, the cost and availability of the instrument need to be taken into account. There are no single techniques to both detect and characterize MNMs in textiles.

5.2 Physicochemical characteristics of metal/metal oxide nanomaterials

The commercially available techniques to measure the physiochemical characteristics of nanomaterials and definitions relevant to the characterization of them are available in ISO/TR 18196 and ISO/TS 80004-6, respectively. Also, the characteristics and measurement methods for powder or colloidal forms of silver nanoparticles applied as antibacterial agents are available in ISO/TS 20660.

These physicochemical characteristics include shape, size, surface charge, chemical composition, and surface chemistry of MNMs. Table 1 summarizes the list of physicochemical characteristics and their measurement methods for TCMNMs.

Table 1 — List of physicochemical characteristics of metallic or metal oxides nanomaterials used in TCMNMs

Characteristic/property	Measurement methods
Particle size, shape, and size distribution	FESEM, TEM, SEM, AFM
Zeta potential	ELS
Surface area	BET
Surface Chemistry	XPS, AES
Chemical Composition	AAS, ICP-AES, ICP-MS
Phase Identification	TEM/SAED

As mentioned before, nanomaterials utilized in textiles are either incorporated in the main fibre texture or applied as a coating onto the textiles by different methods. On the other hand, such fibres and textile fabrics made out of them can be further processed for different purposes. In some cases, there may be complexities for the characterization and detection of the nanomaterials used for antibacterial properties. This includes the possible elemental and chemical similarities of different chemical agents for various purposes (e.g. dyeing, printing) with those of the used nanomaterials. Therefore, to identify the latter from the former, care should be taken to choose a set of appropriate measurement techniques, since for such cases normally no single technique can be suitable to resolve the issue. In this respect, ASTM E3025-16[15] also explores some of the physicochemical characteristics measurement methods and the relevant detection challenges of textiles containing silver nanomaterials which can be considered[9].

5.3 Characterization methods

The brief description of the mentioned characterization methods of TCMNMs is presented in Annex A and Annex B. For chemical composition analysis, the sample shall be digested according to one of the procedures of acid digestion or microwave-assisted acid digestion presented in B.3. The goal of digestion is to completely decompose the solid matrix of TCMNMs to transfer the nanomaterials into the solution for the further determination step. The choice of the digestion method depends on the instrument availability and agreement between the concerned parties.

6 Measurement of the released metal or metal oxide nanomaterials

6.1 Principle

The nanomaterial released from textiles is measured during exposure to human perspiration and washing procedure as described in 6.2 and 6.3, respectively.

6.2 Human perspiration solution preparation

6.2.1 General

Artificial perspiration solution shall be used to simulate human perspiration. Since perspiration varies widely from one person to another, it is not possible to design a method with universal validity. Generally, fresh human perspiration is weakly acidic. However, micro-organisms cause the pH to become weakly alkaline (pH 7,5 to pH 8,5). Therefore, two different artificial alkaline (pH 8) and acidic sweat solutions (pH 5,5) as specified in ISO 105-E04 shall be utilized as the natural perspiration source. The preparation of artificial alkaline and acidic sweat solutions shall be made according to ISO 105-E04.

6.2.2 Measurement method

The amounts of nanomaterials released from textiles are determined by measuring the concentration difference before and after they are being exposed to as-prepared artificial body sweat solution. The following formula can be applied to the calculation:

$$X = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where

X is the amount of nanomaterials released from the textile sample;

A_0 is the measured amount of nanomaterials (µg/l) in the textile sample solution before it is exposed to the artificial sweat solution;

A_1 is the amount of nanomaterials (µg/l) in the textile sample after it has been exposed to the artificial sweat solution (to be reported as a percentage).

A_0 and A_1 shall be measured after subjecting the samples to acid digestion or microwave-assisted acid digestion as explained in Annex B.

It should be mentioned that the measured release can be also due to the possible utilization of chemicals and or nano-sized particles as dyes. In such case, care must be taken to consider only the release of the nanomaterial(s) showing antimicrobial properties due to the limited available MNMs for such applications. Further, it is necessary that producer or manufacturer should declare the type of MNMs used in textiles.

6.3 Washing procedure

In this method, the released nanomaterials utilized in TCMNMs are measured after several washing cycles by regular domestic washing procedure. Considering the wide varieties of textiles covered by this document, the selection of specific procedure of washing, drying and type of detergent provided in ISO 6330 shall be done based on the instructions provided by textile manufacturer. The released amount of nanomaterials during the washing procedure is calculated similarly according to the Formula (1). However, here, A_0 and A_1 are referred to the number of nanomaterials in textile sample pre- and post-washing process, respectively. A_0 and A_1 shall be measured after subjecting the samples to microwave or acid digestion as explained in Annex B.

7 Determination of antimicrobial activities of TCMNMs

7.1 Principle

Antimicrobial activities of TCMNMs samples including antibacterial, antifungal and anti-odour shall be carried out on specimens, pre- and post-washing process and those before and after exposure to human sweat solution.

7.2 Antibacterial activity

The antibacterial activity of the TCMNMs shall be determined according to the ISO 20743:2013 standard method using Gram-positive *Staphylococcus aureus* and Gram-negative bacterium *Klebsiella pneumoniae* (AATCC 4352). A brief description of the method is given in C.1.

NOTE 1 Other bacteria can be used after appropriate validation.

NOTE 2 Refer to World Data Centre for Microorganisms (WDCM) and its website: http://refs.wdcm.org/search.htm.

7.3 Antifungal activity

The antifungal activity of TCMNM samples shall be determined according to ISO 13629-2. The test method determines the antifungal activity by measuring the intensity of luminescence produced by an enzymatic reaction (ATP method). A brief description of the method is given C.2.

The reference fungi to be used shall be selected from the following list:

— *Aspergillus niger*

— *Penicillium citrinum*

— *Cladosporium cladosporioides*

— *Trichophyton mentagrophytes*

NOTE 1 Other fungi can be used after appropriate validation.

NOTE 2 Refer to WDCM and its website: http://refs.wdcm.org/search.htm.

7.4 Anti-odour property

Sweat secreted by axillary glands is odourless. The human axillary malodour is mainly produced by bacteria flora found on axillary skin dominated by genus of Gram-positive *Corynebacteria*[11]. The antibacterial TCMNMs can reduce malodour by decreasing the number of *Corynebacteria* on axillary skin area. If the manufacturer claims that the product has the anti-odour property, this property of textile samples shall be determined according to ISO 20743 and carry out the test by using any strains of *Corynebacterium* such as *Corynebacterium xerosis*. A more detailed description is given in C.3.