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Nanotechnologies — Performance evaluation of nanosuspensions containing clay nanoplates for quorum quenching

Nanotechnologies – Évaluation des performances des nanosuspensions de nanofeuillets d'argile pour le quorum quenching

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Foreword

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

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Introduction

Among the abundant minerals in the earth's crust, the naturally occurring clays in the class of phyllosilicates such as smectite, talc and mica are layer silicates existed as laminated mass. The stacks of smectite can be exfoliated into individual clay nanoplates of high surface area and high charge density on the surface. Due to the presence of the surface charges, the clay nanoplate gives rise to a strong electrostatic and charge attraction on microbial surface. The clay nanoplates can be further modified by introducing various surfactants to enhance their functions for inhibiting bacterial growth through quorum quenching interactions. The clay nanoplate suspension in water is designed to inhibit the growth of pathogenic bacteria for crop protection from diseases. Moreover, as an additional benefit, harvesting yield increased.

The antibacterial efficacy is attributed to the unique combinations of chemical and physical properties including the nanoplate shape and size dimension, high surface area, ionic charge attraction and water dispersion stability. These combined characteristics in a single nanoplate enable for a long-term antibacterial effect. The inter-relation between clay nanoplate characteristics and antibacterial performance are described in Annex A. The quorum quenching ability depends on the interaction of clay nanoplates with bacterial signaling molecules and bacterial surfaces. It can be used as the standard for quality control for the clay nanoplate, and more importantly, the antimicrobial efficacy by using clay nanoplates can be measured and predicted. The correlation between quorum quenching ability and antibacterial performance are described in Annex B.

This document does not cover safety and environmental aspects. Some safety of clay nanoplate regarding the cytotoxicity and genotoxicity toward human cell, oral lethal dose (LD_{50}), and aquatic toxicity are described in Annex C.

Nanotechnologies — Performance evaluation of nanosuspensions containing clay nanoplates for quorum quenching

1 Scope

This document specifies the performance evaluation of nanosuspensions containing clay nanoplates for quorum quenching in crop production. This document does not cover safety and environmental aspects.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
nanosuspension
fluid nanodispersion where the dispersed phase is a solid

Note 1 to entry: The use of the term “nanosuspension” carries no implication regarding thermodynamic stability.

[SOURCE: ISO/TS 80004-4:2011, 3.5.1]

3.2
clay
naturally occurring or synthetically manufactured material composed primarily of fine-grained minerals, which is generally plastic at appropriate water contents and will harden when dried or fired

Note 1 to entry: Taken from Reference [1].

Note 2 to entry: Although clay usually contains phyllosilicates, it may contain other materials that impart plasticity and harden when dried or fired. Associated phases in clay may include materials that do not impart plasticity and organic matter. Different disciplines have uniquely defined the size of clay particles, and it is for this reason that “fine grained” is used in the definition rather than a precise value. However, because of these size variations from discipline to discipline, it is important that the particle size be specified in the context of the application.

[SOURCE: ISO/TS 21236-1:2019, 3.4]

3.3
nanoplate
nano-object with one external dimension in the nanoscale and the other two external dimensions significantly larger

[SOURCE: ISO/TS 80004-2:2015, 4.6]

3.4
clay nanoplate
nanoplate composed of clay

[SOURCE: ISO/TS 21236-2:2021, 3.3]

3.5
critical micelle concentration
concentration of a surfactant above which micelles will form

3.6
minimum inhibitory concentration
lowest concentration of the clay nanoplate that completely inhibits visible growth of the initial inoculum after incubation at 35 °C for 18 h

3.7
minimum bactericidal concentration
lowest concentration of the clay nanoplate that 99,9 % of the final inoculum is killed after incubation at 35 °C for 24 h

3.8
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-2: 2015, 2.2]

4 Abbreviations

AFM	Atomic force microscopy
BET	Brunauer–Emmett–Teller
CEC	Cationic exchange capacity
CMC	Critical micelle concentration
DLS	Dynamic light-scattering
ELS	Electrophoretic light-scattering
FB1	Fumonisin B1
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IEP	Isoelectric point
LD_{50}	Oral lethal dose, 50 %
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MMT	Montmorillonite
QQA	Quorum quenching ability

SEM Scanning electron microscope

TEM Transmission electron microscopy

XRD X-ray Diffractometer

XRF X-ray Fluorescence Spectrometer

5 Characteristics and measurement methods

5.1 General

The characteristics and measurement methods of clay nanoplate are listed in Table 1. The essential characteristics of clay nanoplate can provide understanding of the potential quorum quenching ability and antibacterial performance. Characteristics can be determined using the methods listed in Table 1. The inter-relation between clay nanoplate characteristics and antibacterial performance are described in Annex A.

Table 1 — Characteristics of clay nanoplate to be measured

Characteristics	Units	Measurement method	Test specimen form	Relevant documents
Chemical composition	wt %	ICP-MS or XRF	Powder	ISO/TS 21236-1
Mineral composition	wt %	XRD	Powder	ISO/TS 21236-1
Specific surface area	m^2/g	BET	Powder	ISO 9277
Cation exchange capacity	meq/100 g	Ammonium acetate method	Powder	ISO 22171[a]
Hydrodynamic size	µm	DLS	Suspension	ISO 22412
		Laser diffraction method		ISO 13320
Zeta potential	V	ELS	Suspension	ISO 13099-2

[a] Under preparation. Stage at the time of publication: ISO/DIS 22171:2023.

5.2 Chemical composition

The chemical composition of clay nanoplate can be determined using ICP-MS, ICP-OES or XRF. The measurement results of the constituent oxides fractions are expressed as wt %.

5.3 Mineral composition

The mineral composition of clay nanoplate can be determined using XRD. The XRD pattern could provide the crystalline mineral phase with their corresponding d-spacing values, the measurement results are expressed as wt % to the mineral composition.

5.4 Specific surface area

The high surface area with the surface charge exposure in water enables the function of inhibiting microbial growth. The specific surface area of clay nanoplate can be determined according to ISO 9277. This standard specifies the determination of the overall specific external and internal surface area of samples by measuring the amount of physically adsorbed gas according to the BET method.

5.5 Cation exchange capacity

The generation of the negative surface charge of clay nanoplate is described by the isomorphous substitution of Si, Al or Mg in clay crystal and balanced by the adsorbed counter ions (cations). The cation exchange capacity indicates how many cations can be exchanged on the surface of silicates expressed as meq/100 g. The ammonium acetate at pH 7 method proposed by Schollenberger et al.[2] is widely used to determine the cation exchange capacity. ISO 22171[1)] specifies a method for the determination of cation exchange capacity and the content of exchangeable cations (Ca, K, Mg, Na) in soils using ammonium acetate solution at pH 7 as extractant.

5.6 Hydrodynamic size

Clay nanoplate samples are typically powders. Powder samples shall be well dispersed in the aqueous suspension for size measurement, a higher concentration of clay nanoplate can cause agglomeration. Suspensions often need to be diluted to minimize agglomeration prior to the size measurement. The hydrodynamic diameter of clay nanoplate generally is larger than the primary particle size observed in TEM, SEM or AFM images due to the additional hydration layer, or possible aggregation/agglomeration. The hydrodynamic size shall be measured using DLS or the laser diffraction method according to ISO 22412 or ISO 13320 respectively.

5.7 Zeta potential

Zeta potential reflects the surface charge characterization of a particle and its dispersion stability in aqueous suspension; a higher value of zeta potential (absolute value) indicates a high surface charge, leading to strong repulsion forces between charged particles to prevent aggregation. A low value of zeta potential increases the probability of aggregation due to van der Waals attraction.

The differences are lied on the exfoliated clays, that is silicate nanoplates, a surface totally exposed silicates from the pristine clay stacks. With a significantly different charge behaviour from the clay, the clay nanoplates usually exhibit an isoelectric point (IEP) and pH functions of zeta potential. An apparent coagulation can occur when the pH is below the IEP at high edge charge density.[9]

The pH value shall be reported along with the zeta potential. ISO 13099-2 specifies a method of measurement of electrophoretic mobility of particles suspended in a liquid for calculating zeta-potential.

6 Performance of quorum quenching

6.1 Quantification of quorum quenching ability by surfactants

The quorum quenching ability depends on the interaction of clay nanoplates with bacterial signaling molecules and bacterial surfaces. It is the measurement and indication of the non-covalent affinity of the clay nanoplate with polar organic molecules or the microbial cell surface. Three types of commonly used surfactants are selected as the representatives including cationic (dodecyltrimethylammonium bromide), nonionic (octylphenol ethoxylate) and anionic (sodium dodecyl sulfate) surfactants for measuring the quorum quenching ability.

The surface tension with the Wilhelmy plate method is measured by varying the quantity of clay nanoplate to individual surfactant at the original point of the CMC, indicating the quantitative effect on the intrinsic characteristics of the surfactant CMC. The alternation of the surface tension in the titration process of clay nanoplate adding to surfactant is measured. A sharp change in surface tension can be observed at the equivalent point of a titration.[10]

In the beginning of the titration process, the small volume of clay nanoplate solution in specified concentration is slowly added to the surfactant solution at the point of CMC. In general, during the measurements of surface tension drops, the time to reach an equilibrium is necessary before the final

1) Under preparation. Stage at the time of publication: ISO/DIS 22171:2023.

data reading. The completion of the reaction is monitored in reaching its equilibrium or no further change of surface tension reading. The added amount of the clay is plotted against the surface tension changes at the surfactant CMC.

The *QQA*, expressed in units of meq/100 g, is calculated at the equivalent point with Formula (1):

$$QQA = \frac{(Ws / Es)}{Wc \cdot 10^5} \qquad (1)$$

where

Wc	is the clay nanoplate concentration at the equivalent point, wt %;
Ws	is the surfactant concentration at the equivalent point, wt %;
Es	is the surfactant equivalent weight, in g/eq;
10^5	is the factor to convert eq/g to meq/100 g.

The change of surface tension at CMC actually represents the quorum quenching ability toward the specific surfactant. The cationic surfactant has a strong ionic exchange or formation of ionic bonds on the clay nanoplate surface and equivalent ratio at critical point. The nonionic surfactant is less affected due to the lacking of ionic exchange interaction but only by C-O bonding dipole-dipole interaction with the added clay nanoplate, it represents a weaker interaction than the charge species. The anionic surfactant has no noticeable interaction on the addition of clay nanoplate due to the absence of both charges and dipole- dipole interactions.

The quantitative strength of interaction between clay nanoplate and surfactant is dependent on the types of the surfactants, in general, the opposite charge interaction is the strongest for the quorum quenching ability or binding affinity.

6.2 Antibacterial activities

The MIC and MBC can provide information on antibacterial activities of clay nanoplate potentially being bacteriostatic or bactericidal.

6.2.1 Determination of MIC

The MIC of clay nanoplate can be determined with a broth microdilution method according to CLSI M07-11 or ISO 20776-1. The tests are performed in sterile 96-well plastic microdilution trays by making serial twofold dilutions of clay nanoplate, the final test bacterial concentration in each well should be approximately 5×10^5 CFU/ml ($2–8 \times 10^5$ CFU/ml). At least each tray should be included as a growth control well and an uninoculated negative control well. After incubating at (35 ± 2) °C for (18 ± 2) h, the MIC is determined by observing the lowest concentration of clay nanoplate that completely inhibits visible growth of the bacterium in the wells. The MIC tests should be performed in triplicate.

6.2.2 Determination of MBC

The MBC of clay nanoplate can be determined with a broth microdilution method according to CLSI M26-A. In general, the MBC is determined by first performing the microdilution test procedures for MIC, then, sub-culturing samples from the wells of MIC assay having no visible growth onto the agar plates and incubating the plates for 24 h. The MBC is measured as the lowest concentration of the clay nanoplate that 99,9 % of the final inoculum is killed.

The antimicrobial activity is nonspecific for a diverse range of bacterial species in relation to the physical capturing mechanism enabled by the clay nanoplate.[11]