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Fine Ceramics (Advanced Ceramics, Advanced Technical Ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials — Test method using bacteriophage Q-beta

Céramiques techniques — Détermination de l'activité antivirale des matériaux photocatalytiques semi-conducteurs — Méthode d'essai utilisant le bactériophage Q-beta

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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 WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 206, *Fine ceramics*.

Introduction

This International Standard applies to testing the antiviral activity of photocatalytic ceramics and other materials produced by either coating or mixing of a photocatalyst. The International Standard for testing the antibacterial activity that use photocatalytic materials has been published as ISO 27447. The International Standard for testing the antifungal activity that use photocatalytic materials has also been published as ISO 13125.

The test method for cloths or textiles is not included in this International Standard because of lack of photocatalytic cloths or textiles with antiviral activity using photocatalytic activity. When photocatalytic cloths or textiles with antiviral activity using photocatalytic activity have been developed, a test method for photocatalytic cloths or textiles will be proposed with the glass adhesion method in ISO 27447.

Fine Ceramics (Advanced Ceramics, Advanced Technical Ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials — Test method using bacteriophage Q-beta

WARNING — Only personnel trained in microbiological techniques should carry out tests.

1 Scope

The test method in this International Standard specifies the determination of the antiviral activity of materials that contain photocatalytic materials or have photocatalytic films on the surface, by enumerating the destruction of bacteriophage Q-beta after irradiation of ultraviolet light.

NOTE In this test method, the surrogate microbe is bacteriophage Q-beta, intended as a model for Influenza viruses.

The test method in this International Standard is intended for use with different kinds of semiconducting photocatalytic materials used in construction materials, in flat sheet, board, or plate shape that are the basic forms of materials for various applications. It does not include powder, granular, or porous photocatalytic materials.

The test method in this International Standard is applicable to photocatalytic materials produced for an antiviral application. Other types of performance of photocatalytic materials, i.e. antibacterial activity, antifungal activity, decomposition of water contaminants, self-cleaning, antifogging, and air purification, are not determined by this test method.

The values expressed in this International Standard are in accordance with the International System of Units (SI).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10677, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Ultraviolet light source for testing semiconducting photocatalytic materials*

ISO 27447, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials*

ISO 80000-1, *Quantities and units — Part 1: General*

3 Terms and definitions

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

3.1
photocatalyst
substance that carries out many functions based on oxidization and reduction reactions under photoirradiation, including decomposition and removal of air and water contaminants, deodorization, and antiviral, antibacterial, antifungal, self-cleaning, and antifogging actions

3.2
photocatalytic materials
materials in which, or on which, the photocatalyst is added by coating, impregnation, mixing, etc.

Note 1 to entry: Photocatalytic materials are used for building and road construction materials to obtain the functions mentioned in 3.1.

3.3
antiviral
condition decreasing the infectivity of viruses on the surface of materials

3.4
bacteriophage
type of virus which infects bacteria

Note 1 to entry: The bacteriophage used in this test method is Q-beta that is one of F-specific RNA phages. The bacteriophage Q-beta is not pathogenic to humans and animals but serves to simulate Influenza viruses that are pathogenic to humans.

Note 2 to entry: Example of test results with Influenza virus and bacteriophage Q-beta are given in Annex A.

3.5
plaque
visible, clear area which is theoretically the result of infection and lysis of host cells by a single viable bacteriophage

3.6
photocatalyst antiviral activity value
difference value between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after UV irradiation and on non-treated materials after UV irradiation

Note 1 to entry: This value includes the decrease of number of bacteriophage plaques without UV irradiation.

3.7
photocatalyst antiviral activity value for UV irradiation
difference between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after UV irradiation and on photocatalytic treated materials kept in the dark

4 Symbols

A	average of titre of bacteriophage on non-treated specimens, just after inoculation
B_D	average of titre of bacteriophage on non-treated specimens, after being kept in a dark place
B_L	average of titre of bacteriophage on non-treated specimens, after UV irradiation of intensity L
C_D	average of titre of bacteriophage on photocatalytic treated specimens, after being kept in a dark place
C_L	average of titre of bacteriophage on photocatalytic treated specimens, after UV irradiation of intensity L
D_F	dilution factor
L	UV irradiation intensity
Log_{max}	maximum logarithmic value of titre of bacteriophage
Log_{mean}	average logarithmic value of titre of bacteriophage for three non-treated specimens

Log_{min} minimum logarithmic value of titre of bacteriophage

N titre of bacteriophage (plaque forming unit)

V_D antiviral activity value without photocatalyst, after being kept in a dark place on a testing material

V_L photocatalyst antiviral activity value, after irradiation at a constant intensity (L) on a photocatalytic testing material

ΔV photocatalyst antiviral activity value with UV irradiation

Z average number of plaques in two Petri dishes

5 Principle

This test method is suitable for use in development, comparison, quality assurance, characterization, reliability, and design data generation of photocatalytic materials. The method is used to obtain the antiviral activity of photocatalytic materials by the contact of a specimen with bacteriophage under UV light irradiation. The method is suitable for use with flat sheet, board, or plate-shaped materials.

The specimen of photocatalytic-treated material is inoculated with bacteriophage suspension and exposed to UV radiation of known intensity for a specified period. Following exposure, the test suspension is removed and measured by the plaque-forming method with *Escherichia coli* which is sensitive to bacteriophage Q-bata. The results obtained are compared with those obtained from inoculated specimens of non-photocatalytic treated material exposed to UV radiation under identical conditions to the treated material, and to those obtained from inoculated specimens of both photocatalytic-treated and non-treated material kept in the dark for the same period of time.

6 Materials

6.1 Strains and preparation for tests

6.1.1 Strains

The strains to be used in the test shall be the same as or equivalent to those described in Table 1 and are supplied by an entity that is registered under the World Federation for Culture Collections or the Japan Society for Culture Collections. Aseptic manipulations using microorganisms can be performed in an appropriate safety cabinet.

Table 1 — Bacteriophage and bacteria strains to be used in test

Species	Strain number	Organization for the collection
Bacteriophage Q-beta	ATCC 23631-B1	American Type Culture Collection
	DSM 13768	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC20012	NITE Biological Resource Center
Escherichia coli	ATCC 23631	American Type Culture Collection
	DSM 5210	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 106373	NITE Biological Resource Center

NOTE ATCC23631-B1 and NBRC20012 are not strictly the same but they are from the same origin and their photocatalytic activity effects is equivalent, as shown in Annex B.

6.1.2 Bacteria preparation

a) Inoculate *E. coli* strain into a slant culture medium [6 ml to 10 ml of LB agar (see 6.2.6)], incubate for 16 h to 24 h at (37 ± 1) °C, and then store in refrigerator at 5 °C to 10 °C.

b) Repeat subcultures within 1 month by replicating this process.

c) The slant culture must not be used for further storing after 1 month.

d) The maximum number of subcultures from the original strain transferred by culture collection is 10.

NOTE In the case of bacteria stored in a deep freezer, the maximum number of subcultures from the original strain transferred by culture collection is 10.

6.1.3 Bacteriophage preparation

a) Introduce 25 ml of LB broth with calcium (see 6.2.4) into a conical flask of 300 ml and inoculate with E. coli strain.

b) Incubate for 18 h ± 2 h at (37 ± 1) °C while shaking at 110 min^{-1} ± 10 min^{-1}.

c) Pre-warm 25 ml of LB broth with calcium in a 300 ml conical flask to 35 °C to 37 °C and inoculate with 0,025 ml of the culture prepared under item b).

d) Incubate as above condition until a bacterial concentration will be reached at 2,0 ± 1,0 × 10^8 cfu/ml.

This procedure should be carried out several times to establish the relationship between turbidity measurements and colony counts. If sufficient data have been obtained, further work can be based on turbidity measurements only.

e) Inoculate the bacterial culture with Q-beta from a stock solution to a final concentration of approximately 2 × 10^7 pfu (plaque forming unit)/ml [multiplicity of infection (m.o.i.) is approximately 0,1].

f) Incubate the inoculated bacterial culture for 4 h as under b).

g) Store the culture overnight at 4 °C ± 2 °C.

h) Pour the culture into centrifuge tubes and centrifuge for 20 min at 4 °C ± 2 °C at 10 000 *g*.

i) Pipette the supernatant carefully to a sterilized tube.

j) Filter bacteriophage containing supernatant suspension through a sterilized syringe filter unit to purify the bacteriophage solution.

k) Determine the titre of the bacteriophage stock solution and store at 4 °C ± 2 °C.

l) To check bacterial contamination, mix 1 ml of the bacteriophage stock solution with LB agar (see 6.2.6) and incubate for 24 h at (37 ± 1) °C. Discard the bacteriophage stock solution if any colonies are detected.

m) Do not use the bacteriophage stock solution with less than 1,0 × 10^{10} pfu/ml or contaminated stock solution.

NOTE 2 The titre of the phage suspension should be above 1,0 × 10^{11} pfu/ml and might reach up to 1,0 × 10^{13} pfu/ml.

NOTE 3 The titre of the phage stock suspension will slowly decrease over time.

6.2 Media

6.2.1 General

Commercial media of same components described below can be used.

Volume of prepared media can be adjusted in accordance with the number of specimens.

6.2.2 1/500 Nutrient broth (1/500 NB)

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone, and 5,0 g of sodium chloride, put them in a flask, and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. Dilute this medium by 500 times using purified water, and set the pH to (7,0 ± 0,2) at 25 °C using hydrochloric acid solution or sodium hydroxide solution. Sterilize in an autoclave (at 121 °C ± 2 °C for at least 15 min). After preparation, if 1/500 nutrient broth is not used immediately, store at 5 °C to 10 °C. Do not use 1/500 nutrient broth made more than a week ago.

6.2.3 Calcium solution

For 100 ml of purified water, take 3,0 g of calcium chloride dihydrate, put it in a flask, and dissolve it thoroughly. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if calcium solution is not used immediately, store at 5 °C to 10 °C. Do not use the calcium solution made more than 6 months ago.

6.2.4 LB broth with calcium

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract, and 10,0 g of sodium chloride, put them in a flask, and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After autoclaving, add 10 ml of calcium solution to medium and mix well. After preparation, if LB broth with calcium is not used immediately, store at 5 °C to 10 °C. Do not use the broth made more than 1 month ago.

6.2.5 Agar powder

Use agar powder for which the gel strength of 1,5 % agar is from 400 g/cm^2 to 600 g/cm^2.

6.2.6 LB agar

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract, and 10,0 g of sodium chloride and 10,0 g of agar powder (see 6.2.5), put them in a flask, and mix. Heat the flask in boiling water to dissolve the contents thoroughly. Use a 0,1 mol/l solution of sodium hydroxide to bring the pH to (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if nutrient agar is not used immediately, store at 5 °C to 10 °C. Do not use nutrient agar made more than 1 month ago.

6.2.7 Bottom agar plate (LB agar plate with calcium)

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract, and 10,0 g of sodium chloride and 15,0 g of agar powder (see 6.2.5), put them in a flask, and mix. Heat the flask in boiling water to dissolve the contents thoroughly. Use a 0,1 mol/l solution of sodium hydroxide to bring the pH to (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After autoclaving, add 10 ml of calcium solution to medium and mix well. After preparation, pour 15 ml to 20 ml of medium into 90 mm diameter Petri dish, store at 5 °C to 10 °C. Do not use nutrient agar made more than 2 weeks ago.