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Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antifungal activity of semiconducting photocatalytic materials

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National foreword

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Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antifungal activity of semiconducting photocatalytic materials

Céramiques techniques — Méthode d'essai pour l'activité antifongique des matériaux photocatalytiques semiconducteurs

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 13125 was prepared by Technical Committee ISO/TC 206, *Fine ceramics*.

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Introduction

Under the illumination of ultraviolet (UV) light, photocatalysts show diverse functions, such as the decomposition of air and water contaminants, as well as deodorization, self-cleaning, antifogging, antibacterial and antifungal actions. These functions of photocatalysts are generally based on the action of active oxygen species such as hydroxyl (OH) radicals formed on the surface of photocatalysts. The energy- and labour-saving nature of photocatalysis has attracted keen interest when the photocatalyst is activated by sunlight (or artificial lighting).

Practical applications of photocatalysts for both indoor and outdoor use have rapidly expanded in recent years. Many kinds of photocatalytic materials have been proposed or are already commercialized, based on ceramics, glass, concrete, plastics, paper, etc. Such materials have been proposed by either coating or mixing of a photocatalyst; in most cases, titanium dioxide (TiO_2).

However, the effect of photocatalysis is not easily inspected visually, and no appropriate and standardized evaluation methods have been available to date. Some confusion has thus arisen as photocatalytic materials have been introduced. Furthermore, the above-mentioned diverse functions of photocatalysts cannot be evaluated with a single method: thus it is required to provide different evaluation methods for air purification, water decontamination, and self-cleaning.

This International Standard applies to testing the antifungal activity of photocatalytic ceramics and other materials.

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Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antifungal activity of semiconducting photocatalytic materials

WARNING — Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence. Only personnel trained in microbiological techniques should carry out the test.

1 Scope

This International Standard specifies a test method covering the determination of the antifungal activity of materials that contain a photocatalyst or have photocatalytic films on their surface, by counting the number of pre-incubated fungal spores that survive exposure to ultraviolet (UV-A) light.

This International Standard provides for the assessment of different kinds on materials used in various applications, such as construction materials in flat coating, sheet, board or plate form, etc. Powder, granular, fibrous or porous photocatalytic materials are not included.

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

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

ISO 4892-3, *Plastics — Methods of exposure to laboratory light sources — Part 3: Fluorescent UV lamps*

IEC 60068-2-10, *Environmental testing — Part 2-10: Test J and guidance: Mould growth*

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 UV irradiation, including decomposition and removal of air and water contaminants, deodorization, antibacterial, antifungal, self-cleaning and antifogging

3.2
photoirradiation
irradiation to ultraviolet (UV-A) light at wavelength 300 nm to 400 nm

3.3
photocatalytic materials
surface or material to which a photocatalyst has been applied with the intention of making it photocatalytically active; photocatalytic treated materials, samples and pieces are included

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3.4
antifungal activity
inhibition of germination or inactivation of fungal spores

3.5
antifungal activity value in irradiation condition L
common logarithm of the ratio of the number of surviving fungal spores on a photocatalytic non-treated piece after UV irradiation condition L for a given period of time to the number of surviving spores on a treated piece after the same UV irradiation condition for the same period

3.6
antifungal activity value with UV irradiation by removing the effect in the dark
difference value between antifungal activity value in irradiation condition L and common logarithm of the ratio of the number of surviving fungal spores on a photocatalytic non-treated piece in the dark to the number of surviving spores on a treated piece stored in the dark for the same period of time

4 Symbols

S concentration of fungal spores

K average of colony numbers

D dilution factor

F number of surviving spores

V volume of recovery solution

L ultraviolet exposure

A average of colony numbers of photocatalytic non-treated pieces following inoculation

B_L average of surviving spore numbers of photocatalytic non-treated pieces at exposure L after several hours

C_L average of surviving spore numbers of photocatalytic non-treated pieces at exposure L after several hours

R_L antifungal activity value in irradiation condition L

C_D average of surviving spore numbers of photocatalytic treated test pieces in the dark after several hours

B_D average of surviving spore numbers of photocatalytic non-treated test pieces in the dark after several hours

ΔR antifungal activity value with UV irradiation by removing the effect in the dark

NOTE The term “several hours” means the period of time between 3 h (minimum) and 24 h (maximum).

5 Principle

This International Standard is for development, comparison, quality assurance, characterization, reliability, and design data generation of photocatalytic materials.[1] Photocatalyst is capable of decomposition of organic substances, including living cells such as fungal spores. A suspension of fungal spores on photocatalytic treated test piece is inactivated under photoirradiation. After the irradiation, fungal spores are recovered and cultivated on agar medium, and formed spore colonies are counted.

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Antifungal activity of the photocatalytic reaction is estimated as the decrease in surviving spore number on a test sample compared to a blank test of non-coated surface.

NOTE This International Standard is adapted from the common methodological concept for ISO 27447. Namely, same apparatus and test piece size, similar procedure and calculation are adapted between this International Standard and ISO 27447. Therefore, ISO 27447 is recommended to be used as a reference during the actual test of this International Standard.

6 Materials

6.1 Test fungi

a) *Aspergillus niger*

b) *Penicillium pinophilum*

The test fungi shall be obtained from national or international culture collections. The strains to be used are listed in Table 1. These strains are sorted by References[2],[3] and IEC 60068-2-10.

Table 1 — Fungal strains to be used in test

Fungal species and strains	WDCM code
Aspergillus niger	WDCM 00144 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00144
Penicillium pinophilum	WDCM 00194 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00194
NOTE Refer to WDCM and its website: http://refs.wdcm.org/search.htm . (Note that WDCM stands for World Data Centre for Microorganisms.)	

6.2 Chemicals and implements

6.2.1 Potato-Dextrose-Agar (PDA) Medium

Agar slants or agar plates used for the test shall be PDA medium with the following composition:

PDA medium

potato infusion	200 g
glucose	20 g
agar	20 g
purified water	1000 ml

PDA medium shall be sterilized at 121 °C ± 1 °C for 15 min - 20 min in an autoclave with an atmosphere saturated with steam (autoclaving).

NOTE PDA medium is able to use available preparation, for example Difco (Becton, Dickinson and company, USA), MERCK (Merck KGaA, Germany), etc.

6.2.2 Purified water

The water used for the preparation of all solutions and culture medium shall be distilled or deionized water.

NOTE Germination and growth of fungal spores might be inhibited by contained material (e.g. metal ions) of tap water.

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6.2.3 Sterilized water

Sterilized water is prepared from purified water by the autoclaving.

6.2.4 Solutions for suspension, recovery, and dilution of spores

For suspension, recovery, and dilution of spores, a solution of 0,005 % (mass %) dioctyl sodium sulfosuccinate and 0,9 % (mass %) sodium chloride in purified water shall be used. Solutions shall be sterilized by the autoclaving.

7 Apparatus

7.1 General

The following apparatus is required.

7.2 Irradiating equipment

The testing equipment enables a photocatalytic material to be examined for its antifungal activity by providing UV irradiation to active the photocatalyst. It consists of UV light source and the vessel with a test piece as described in ISO 27447. For example, the irradiating equipment with two black light fluorescent lamps (straight tube, 20 W BLB type, 580 mm in length) mounted in parallel with 200 mm between centres. A stand to support samples and a mechanism enabling the distance between sample and light source to be varied over 180 mm at the minimum (ca. 1,0 mW/cm^2 at the maximum) shall also be provided.

7.3 Black light blue lamp (BLB lamp)

Use a black light fluorescent lamp. The black light fluorescent lamp shall be one which has a peak wavelength of 351 nm, and emit light with a half bandwidth of 40 nm (a fluorescent lamp which uses $BaSi_2O_5$:Pb as the phosphor and, a visible light absorbing glass tube) as described in ISO 4892-3.

7.4 Ultraviolet light radiometer

A radiometer with a detector whose sensitivity peak is at 351 nm and provide limit, e.g. ±10 nm shall be used. The radiometer shall be calibrated for the light source to be used or corrected to ascertain sensitivity within the wavelength range to be absorbed by the photocatalytic test piece with suitable approach.

7.5 Adhesive film

Adhesive film shall be inert, non-water absorbent, good oxygen permeability and shall have a good sealing property with over 85 % transparency between 340 nm and 380 nm. A sheet shall be cut at 40 mm ± 2 mm square. The film shall be sterilized with wiping cotton transfused 80 % (volume %) ethanol solution before the test.

NOTE For example, polypropylene film is recommended as adhesive film.

7.6 Glass pane

A glass pane with a thickness of 1,1 mm ± 0,1 mm and greater than 85 % transmittance between 340 nm and 380 nm.

7.7 Test vessel

Sterile Petri dish or similar.

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8 Test piece

8.1 Preparation of test pieces

The flat sections of photocatalytic treated material and non-treated material shall be cut out in 50 mm ± 2 mm square (thickness: < 10 mm), respectively. Prepare 9 pieces of non-treated samples and 6 pieces of photocatalytic treated samples. When non-treated samples cannot be provided, use glass panes instead. Take great care to avoid microbial contamination and cross-contamination among samples.

NOTE When it is difficult or impossible to cut (50 ± 2) mm long (up to 10 mm thickness) square, it is acceptable to use a different sample size as long as the sample surface can be covered with a 400 mm^2 to 1600 mm^2 film.

8.2 Use of test pieces

Three treated pieces and 3 non-treated pieces shall be used in the irradiation test, and 3 treated pieces and 3 non-treated pieces also shall be used in the control test (in the dark). Other 3 non-treated pieces shall be used on the test for survivability of fungal spores following inoculation.

8.3 Cleaning of test pieces

For cleaning and sterilization, the surface of the test piece shall be wiped with soft cotton transfused 80 % ethanol solution and the test piece surface shall be irradiated with UV light over 0,8 mW/cm^2 within 24 h.

9 Procedure

9.1 Test temperature

All tests shall be carried out at 25 °C ± 5 °C.

9.2 Photoirradiation

9.2.1 Measurement of ultraviolet exposure and setting location of test vessel (Petri dish)

A ultraviolet light radiometer is set on the base of the test sample position, then the adhesion film and glass plate are placed on top of the sensor. The setting locations of a test vessel are fixed by counting ultraviolet exposure. BLB lamps shall be switched on 15 min before the measurement.

9.2.2 Ultraviolet exposure of test piece

The test piece in the test vessel is exposed in 0,8 mW/cm^2 at the maximum for several hours (over 3 h to 24 h at the maximum) in the irradiating equipment. The ultraviolet exposure shall be configured high-intensity in the range of 0,4 mW/cm^2 to 0,8 mW/cm^2 on the surface of the actual products and environment, because fungal spores are more tolerant of photocatalytic action than bacterial cell (see also ISO 27447). The control test pieces in the vessels are kept in the dark for same time at same temperature.

9.3 Preparation of fungal spore suspension

9.3.1 Stock strains and subculture

After incubation at 26 °C ± 2 °C (*Aspergillus niger*: 7 days, *Penicillium pinophilum*: 14 days) on PDA slants or plates, only well sporulating cultures shall be used. They shall be stored at a temperature between 5 °C and 10 °C for a maximum of three months and restocking of the culture shall be carried out within this time period.

The preparation and storage subcultures for the spore suspension shall be carried out in the same way as for stock cultures. Subcultures stored for more than 10 days shall not be used.

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9.3.2 Count of spore number

Count of the spore number before inoculation shall be carried out with a hemocytometer (improved Neubauer type) under a microscope.

9.3.3 Preparation of spore suspension

Pour adequate volume of suspension solution (6.2.4) into the subculture and gently scrape spores with bubbling by a Pasteur pipette, or inoculating loop. Filter the spore suspension through sterilized cotton or gauze, and then the filtrate is mixed again with the pipette or a vortex mixer. In the case of *Aspergillus niger*, disperse the spores by ultrasonic vibration (30 kHz - 50 kHz, 5 min).

Count of spores (9.3.2), and diluted to a concentration of 5,0 x 10^5 spores/ml. Store the suspension at a temperature of between 5 °C and 10 °C for a maximum of 24 h.

9.4 Estimation of the number of surviving spore

The number of surviving colonies is determined using the pour plate method using 10-fold serial dilutions of the original suspension. One ml of original recovery suspension is poured into a tube containing 9,0 ml ± 0,1 ml of dilution solution (6.2.4) herein, then the suspension is mixed. This procedure is repeated three times for each 10-fold dilution. One ml of original suspension and each dilution series poured into two Petri dishes, respectively. PDA medium (15 ml - 20 ml) at a temperature of 45 °C to 48 °C is poured into each Petri dishes, then the dishes are gently shaken to mix the contents. The agar medium shall be allowed to solidify for at least 15 min at room temperature. The plates are then incubated at 26 °C ± 2 °C for 7 days in an incubator.

After incubation, Petri dishes that hold 10 colonies - 99 colonies are selected and then the number of colonies is counted. The dilution factor of the samples that are counted should be recorded.

9.5 UV Irradiation test

9.5.1 Inoculation of spore suspension

On the centre of the test piece, 0,1 ml (5,0 x 10^4 spores) of spore suspension shall be inoculated, and then, shall be covered with adhesion film (40 mm x 40 mm). By covering of the film, the suspension is spread between the test piece and the film. Care should be taken that none of culture is outside the adhesive film.

This type of inoculation is possible to apply only flat surface sample, because suspended spores on porous or rough surface will be not dispersed homogeneously.

9.5.2 Arrangement of test piece in a vessel

According to the Figure 1, a moisturizing filter paper is laid on the bottom of a vessel (Petri dish), adequate sterilized water (5-7 ml) is soaked into the filter paper, then glass tubes or rods are put on. Furthermore, the inoculated piece and film are laid on glass tubes or rods, and the vessel is closed up a glass pane. All glass equipment for a vessel except the test piece with film shall be sterilized by the autoclaving.

NOTE For convenience, U- or V-shaped glass tubes or rods are recommended.

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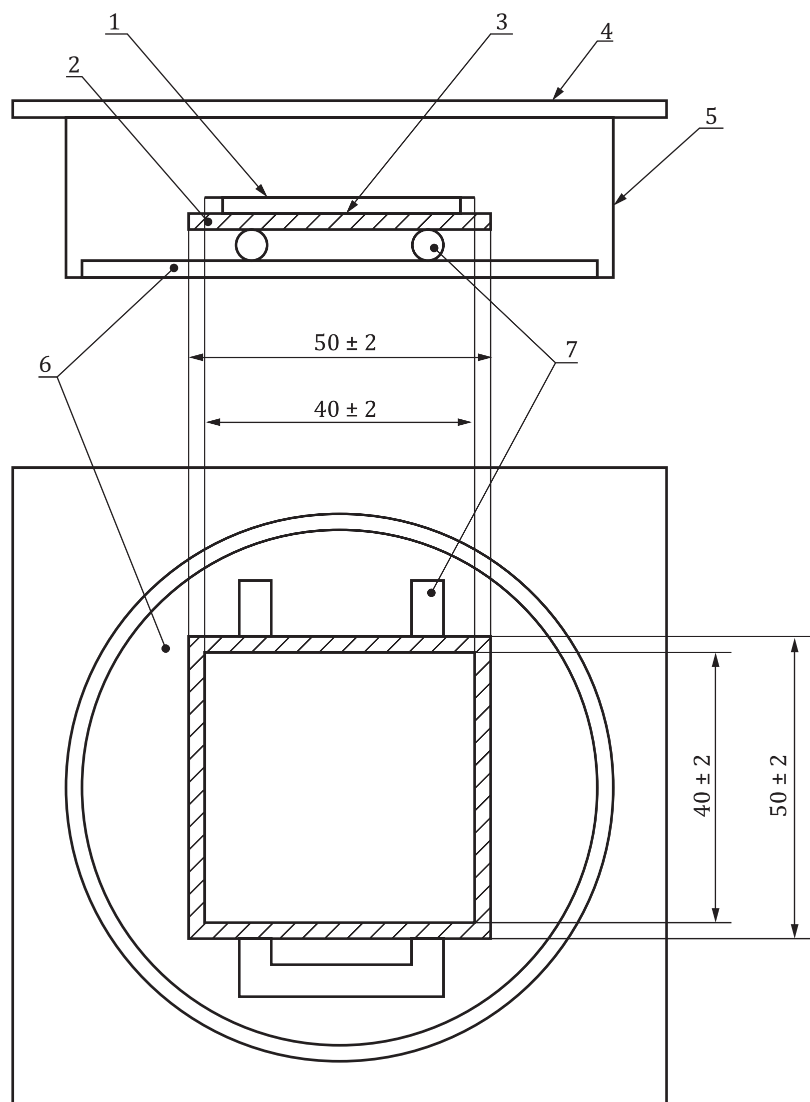
Unit: mm



Key

1 Adhesive film
2 Test piece
3 Spore suspension
4 Glass pane
5 Petri dish (vessel)
6 Moisturizing filter paper
7 Glass tube/rod

Figure 1 — Arrangement of test piece and vessel

9.5.3 Recovery of fungal spores from test piece

9.5.3.1 From test piece following spore inoculation

Three non-treated test pieces with the adhesion film are put into a stomaching bag using sterile forceps carefully. Ten ml of recovery solution (6.2.4) is poured into bag, then spores are resuspended from the

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surface by kneading the bag manually for 10 times at a minimum. This suspension is used to estimate the surviving spore concentration (see 9.4).

NOTE Before the test, it is recommended to prove that all inoculated spores are extracted and recovered (over 90 %) from the same test piece and film.

9.5.3.2 From test piece following irradiation test

After the irradiation (9.2.2), surviving spores on test pieces are resuspended by the same procedure as mentioned above (9.5.3.1).

10 Calculation

The test results are calculated as follow. The calculated values are usually rounded to the second decimal place according to IEC 60068-2-10.

10.1 Surviving spore concentration of recovery solution

From the number of colonies (9.4), calculate the surviving spore concentration using Formula (1) below.

$$S = K \times D \tag{1}$$

where

S is the concentration of spores (spores/ml);

K is the average of colony numbers (cfu);

D is the dilution factor (fold).

10.2 Number of surviving spores

From the concentration of spores (10.1), calculate the number of surviving spores using Formula (2) below

$$F = S \times V \tag{2}$$

where

F is the number of surviving spores (spores);

S is the concentration of spore (spores/ml);

V is the volume of recovery solution (ml).

10.3 Validity of the test

The test may be considered effective when all of the following requirements are fulfilled,

a) In 9.3.2 and 9.3.3, fungal spores are dispersed adequately and homogeneously.

b) The average of colony numbers on non-treated test piece following inoculation is over 1,0 x 10^4 and under 1,0 x 10^5.

c) The number of surviving spores on all non-treated test pieces after UV irradiation is over 5,0 x 10^3.

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10.4 Antifungal activity value in irradiation condition *L*

After the validity of the test (10.3) is confirmed, the antifungal activity value in irradiation condition *L*, R_L shall be calculated using Formula (3).

NOTE It is useful to calculate values to two decimal places and record them to one decimal place.

$$R_L = \left[\log\left(B_L / A\right) - \log\left(C_L / A\right)\right] = \log(B_L / C_L) \quad (3)$$

where

R_L is the antifungal activity value in irradiation condition *L;*

L is the ultraviolet exposure (mW/cm^2);

A is the average of colony numbers of non-treated test piece following inoculation;

B_L is the average of surviving spore numbers of non-treated test piece at exposure L after several hours;

C_L is the average of surviving spore numbers of photocatalytic treated test piece at exposure L after several hours.

10.5 Antifungal activity value with UV irradiation by removing the effect in the dark

Antifungal activity value with UV irradiation by removing the effect in the dark is calculated as follows.

$$\Delta R = R_L - \log\left(B_D / C_D\right) \quad (4)$$

where

ΔR is the antifungal activity value with UV irradiation by removing the effect in the dark;

B_D is the average of surviving spore numbers of non-treated test piece in the dark after several hours;

C_D is the average of surviving spore numbers of photocatalytic treated test piece in the dark after several hours.

11 Test report

The test report shall include the following information:

a) the description of the photocatalytic treated test piece (materials, dimensions);

b) the description of the non-treated test piece (materials, dimensions);

c) the ultraviolet light source used (manufacturer, type);

d) the condition of preliminary irradiation (intensity, exposure duration);

e) the ultraviolet light radiometer used (manufacturer, type);

f) kind of the adhesion film used (manufacturer, type);

g) kind of the glass pane used (manufacturer, type);

h) the irradiation condition (intensity *L*, exposure duration);

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i) inoculated volume of spore suspension (ml);

j) the fungi used (fungal species, strain No.);

k) the results obtained:

1) average of colony numbers of the non-treated test piece following inoculation (A),

2) average of surviving spore numbers of the non-treated test piece at exposure L after several hours (B_L),

3) average of surviving spore numbers of the photocatalytic treated test piece at exposure L after several hours (C_L),

4) antifungal activity value in irradiation condition L (R_L),

5) antifungal activity value with UV irradiation by removing the effect in the dark (ΔR).

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