Antibacterial, antifungal and deodorant functions of super fine powder of zinc oxides

Introduction

We have been selling zinc oxide super fine powder as UV shielding for various applications. Recently, we have confirmed they also have antibacterial, antifungal and deodorant functions. Most of conventional antibacterial, antifungal or deodorant agents are organic and problems have been pointed out such as bleeding out from resin and continuity of the effects. They also have safety problems when they are used in the conditions contacting a human body or foods. On the other hand, zinc oxide super fine powder does not have the problem of bleeding out due to the mechanism of dispersing within the resin, and the effects last long and stable because it is inorganic. Moreover, zinc oxide is an inorganic compound highly safe to a human body, whose use is allowed in the standards for cosmetic raw materials and in the Japanese pharmacopoeia.

As the diameter of a particle of zinc oxide super fine powder manufactured by our unique production method is very small, the total surface area of particles is far larger than that of the conventional zinc oxide particles. Due to this characteristic, our zinc oxide super fine powder has stronger antibacterial, antifungal and odor eliminating power. In addition, since the particles are very small, they hardly absorb visible light. When it is used in a highly dispersed condition, it maintains transparency, thus does not change the original tone of the color of the material. It can be used for a wide range of applications. Further, unlike certain inorganic antibacterial/antifungal/deodorant agents that contain silver and the like, it is easy to use as it does not stain as time elapses.

Accordingly, super fine powder of zinc oxide is expected to be used for a wide range of applications for its transparency, no-staining property, safety and other properties.

The following pages mainly present test data of our zinc oxide super fine powder. As a result of these data, it was proved that zinc oxide super fine powder has antibacterial, antifungal and deodorant properties effectively. We will continue to collect data, expanding the types of bacteria and fungi and sources of order to be tested. We are also conducting evaluation tests of films and other materials to which zinc oxide is applied or incorporated. As soon as data are collected and analyzed, we can present another report.

Thank you for your continuous and future patronage to our super fine powder of zinc oxide.

Outline of the test results

The antibacterial, antifungal and deodorant properties of zinc oxide are known popularly by other literatures. However, it was confirmed that the zinc oxides we produce have greater effect than conventional ones because they are super fine particles. This report presents mainly basic data that prove these characteristics of our super fine powder of zinc oxides. For comparison, a silver-based inorganic material of other manufacturer is tested at the same time.

Test (1) and Test (2) were conducted to confirm the antibacterial property of the zinc oxide super fine particles themselves by the halo testing and minimum inhibitory concentration (MIC). The halo test was conducted for 6 types of bacteria. An impeding halo was observed in all cases. In the MIC test, excellent antibacterial effect was observed in the use of low concentration of zinc oxide super fine powder. Especially, compared with Product A, a silver-based inorganic antibacterial agent of other manufacturer, zinc oxide had a very small impeding halo and showed stronger antibacterial property in the MIC test. Accordingly, it was confirmed that zinc oxide super fine powder is an excellent antibacterial agent that does not bleed out, thus it is safe and its effect lasts long.

Test (3) and Test (4) show the results of the testing of antibacterial property of films to which zinc oxide super fine powder is applied or incorporated.

In the antifungal test (Test (5)), it was found that zinc oxide super fine powder was especially effective to prevent the growth of black mold. A certain level of effect was also confirmed with three other fungi. Good antifungal effect was also observed with trichophytin in a similar test. The results of the MIC measurement are also included although they are only for ZnO-100.

The deodorant power of zinc oxide was confirmed in Test (6). Our zinc oxide showed quicker deodorant effect with isovalerate, that is the main cause of the foot odor, than grains of activated carbon. Good effect was also observed with elimination of the ammonia odor. In Test (7), the film to which zinc oxide super fine powder is incorporate eliminated odors quicker than Product A, which is a silver-based other manufacture's product. These results show that generation of odors was prevented by zinc oxide super fine powder that controlled the growth of bacteria, the cause of odors, and at the same time, zinc oxide super fine powder is effective to eliminate other odors not attributable to bacteria, such as the ammonia odor.

(1) Antibacterial property test of zinc oxide super fine powder (halo test)

Test materials : ZnO-100, ZnO-200, ZnO-200 treated with 2% chitosan
Test bacteria : Staphylococcus aureus, colibacilli, salmonellae, pneumobacillus, and Pseudomonas aeruginosa
Culture medium : Ordinary agar, ordinary broth
Test method : Each of the test bacteria was cultivated on an ordinary broth culture medium for 24 hours, and diluted to 102. 0.1ml of the diluted bacteria liquid was planted on an ordinary agar culture medium. Each of the test materials was placed on the ordinary agar to which the bacteria were planted. After 48 hours of cultivation, the width of inhibitory halo observed around each test material was measured.

Ordinary agar

Meat extract	3g
Peptone	10g
NaCl	5g
Agar	15g
pH7.0	1 liter

Ordinary broth

Meat extract	3g
Peptone	10g
NaCl	5g
pH7.0	1 liter

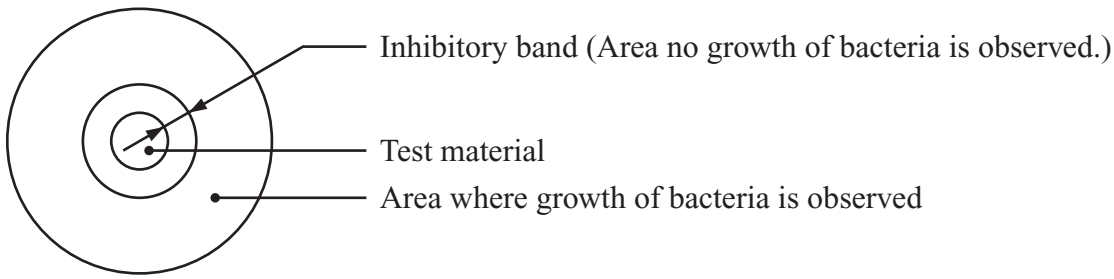

Table 1. Antibacterial property test of zinc oxide super fine powder (halo test)

unit : mm

Classification \ Type / Bacteria		ZnO-100	ZnO-200	ZnO-200 treated with chitosan	Product of other manufacturer (Product A)
Gram-positive bacteria	S.aureus	1.4	0.9	0.6	3.4
	B.subtilis	6.7	6.7	6.0	5.5
Gram-negative bacteria	E.coli	2.0	1.6	1.5	4.2
	Sal.typhi	2.0	1.8	1.5	3.6
	K.pneumo	2.4	1.6	1.5	3.4
	P.aerugi	△	△	△	5.9

* △ : Although no inhibitory band was observed, bacteria did not grown on the contacting border.

(2) Antibacterial property test of zinc oxide super fine powder

To evaluate the antibacterial property of our zinc oxide super fine powder, the minimum inhibitory concentration (MIC) was measured with general bacteria. For comparison, the measurement was also made for a silver-based other manufacturer's product at the same time.

Outline of the test : A culture medium added with zinc oxide super fine powder of a given concentration was inoculated with test bacteria and cultivated. The lowest concentration by which the growth of bacteria was impeded was taken as the minimum inhibitory concentration.

Table 2. Minimum inhibitory concentration (MIC) of zinc oxide super fine powder

Unit : ppm

Bacteria \ Type	ZnO-100	ZnO-200	ZnO-200 treated with chitosan	Product of other manufacturer (Product A)
S.aureus	125	125	125	500
B.subtilis	62	125	125	500
E.coli	500	1000	1000	500
Sal.typhi.	500	1000	1000	500
K.pneumo.	125	250	250	500
P.aerugi	>2000	>2000	>2000	500

(3) Antibacterial property test of films applied with zinc oxide super fine powder

Test material	: Film applied with zinc oxide super fine powder	: Base	: PET film
		: Application method	: bar coater
		: Thickness of application after drying	: 4μm
	:Application samples	: Acrylic binder (25% ZnO in the solid ingredients)	
		: Acrylic binder (75% ZnO in the solid ingredients)	
		: Polyvinyl chloride binder (25% ZnO in the solid ingredients)	
		: Polyvinyl chloride binder (50% ZnO in the solid ingredients)	
Test bacteria	: Staphylococcus aureus		
Culture medium	: Ordinary agar, ordinary broth, and physiological buffer solution added with phosphoric acid		
Test method	: The bacteria solution was cultivated on an ordinary broth culture medium for 24 hours, and diluted with physiological buffer solution added with phosphoric acid to make test bacterial liquid. A strip of film applied with ZnO super fine powder (4cm × 4cm) was put in an Eiken screw cup, to which 0.1ml of the test bacterial liquid was added. Immediately after the addition, and after 24 hours maintained at 37 ℃, it was rinsed with aseptic physiological saline and the number of live bacteria was measured.		

Table 3. Antibacterial property test of film applied with ZnO super fine powder

Film	Number of live bacteria (cell/ml)	Rate of reduction (%)
Film without application of ZnO (0 hours)	1.55×10^6	
Film without application of ZnO (24 hours later)	1.96×10^6	
Acrylic 25% (24 hours later)	2.29×10^4	30.7%
Acrylic 75% (24 hours later)	1.02×10^2	68.1%
PVC 25% (24 hours later)	3.30×10^4	28.2%
PVC 50% (24 hours later)	5.10×10^2	57.0%

* Rate of reduction (%)
= (log number of live bacteria for comparison- log number of live bacteria in the tested sample)/ log number of live bacteria for comparison × 100 effective > 26%

(4) Antibacterial property test of films incorporated with zinc oxide super fine powder

Test materials : Film incorporated with ZnO super fine powder
LLD-PE (3% of ZnO-100 added)
LLD-PE (5% of ZnO-100 added)
LLD-PE (3% of inorganic Product A of other manufacturer added)

Test bacteria : Staphylococcus aureus

Culture medium : Ordinary agar, ordinary broth, and physiological buffer solution added with phosphoric acid

Test method : The bacteria solution was cultivated on an ordinary broth culture medium for 24 hours, and diluted with physiological buffer solution added with phosphoric acid to make test bacteria liquid in the order of 106 cell/ml. 10 strips of each of the above-mentioned films (2cm × 2cm) were put in a sterilized 100 ml Erlenmeyer flask, to which 10ml of the test bacteria liquid was added, then, the flask was agitated for 20 hours at 27 ℃ After the agitation, the test bacteria liquid was diluted appropriately to measure the number of live bacteria.

Table 4. Antibacterial property test of films incorporated with ZnO super fine powder

Film	Number of live bacteria (cell/ml)	Rate of reduction (%)
LLD-PE (no addition : 0 hours)	2.62×10^6	
LLD-PE (no addition : 24 hours later)	1.98×10^5	
LLD-PE (3% of ZnO : 24 hours later)	4.23×10^4	12.7%
LLD-PE (5% of ZnO : 24 hours later)	4.13×10^4	12.9%
LLD-PE (3% of other manufacturer's product : 24 hours later)	5.18×10^4	11.0%

* Rate of reduction (%)
= (log number of live bacteria for comparison- log number of live bacteria in the tested sample)/log number of live bacteria for comparison × 100 effective > 26%

(5) Antifungal property test of zinc oxide super fine powder

Test materials : ZnO-100, ZnO-200, ZnO-200 treated with 2% chitosan, Product A of other manufacturer

Test bacteria : Asp. nigger, Clad. cladospo., Cheatomium sp., Trichoderma sp., Trichophyton ment., Candida albicans, Saccha. serevisiae

Culture medium : Potato dextrose agar + CM, Sabouraud's agar

Test method : The test bacteria was pre-cultivated on a PDA culture medium for 14 days, and spores were suspended in physiological saline added with phosphoric acid (0.02% TWEEN80) to make spore suspended liquid. The test material of a given concentration (1%, 2.5%, 5% and 10%) was mixed to a PDA (or Sabouraud's agar) culture medium, and one loop of spore suspended liquid was inoculated to the culture medium. Candida albicans and Saccha. serevisiae were observed 3 days later, Trichophyton ment. was observed 7 days later, and Asp. nigger, Clad. cladospo., Cheatomium sp. and Trichoderma sp. were observed 3, 7, 10 and 14 days later (the table shows the results of 14 days later).

Remarks : The figures in ( ) indicate the results of the MIC measurement for reference (in ppm).

Table 5. Antifungal property test of ZnO super fine powder (2.5% concentration)

Type / Bacteria	ZnO-100	ZnO-200	ZnO-200 treated with chitosan	Product of other manufacturer (Product A)
Asp. nigger	± (>2000)	±	±	±
Clad. cladospo	– (2000)	–	–	–
Cheatomium sp.	± (500)	±	±	–
Trichoderma sp.	± (500)	±	±	–
Trichophyton	– (1000)	–	–	–
Candida albicans	± (2000)	±	±	–
Saccha. serevisiae	– (1000)	–	–	–
Green mold	(>2000)			
Red mold	(>2000)			
rhizopus	(>2000)			

– : no growth of fungi observed
± : slight growth of fungi observed
+ : growth of fungi observed

(6) Deodorant property test of zinc oxide super fine powder

1. Deodorant property test with isovalerate

Test material : Zinc oxide super fine powder ZnO-200
Grains of activated carbon for comparison

Reagents and instruments : Odor bags (Alex Shokai)
Isovalerate (Tokyo Kasei Kogyo)
Gas detecting tube (Gas Tech)

Test method : 1 gram of the test material was put in the odor bag (25cm × 25cm) and the bag was heat-sealed. Then, 1 liter of nitrogen-based isovalerate (approx. 50 ppm) was inserted into the bag. The bag was left in the room temperature and the change of the residual gas concentration within the bag with time was measured by the gas detecting tube. The test of a bag to which no test material was put was also conducted in the same procedures.

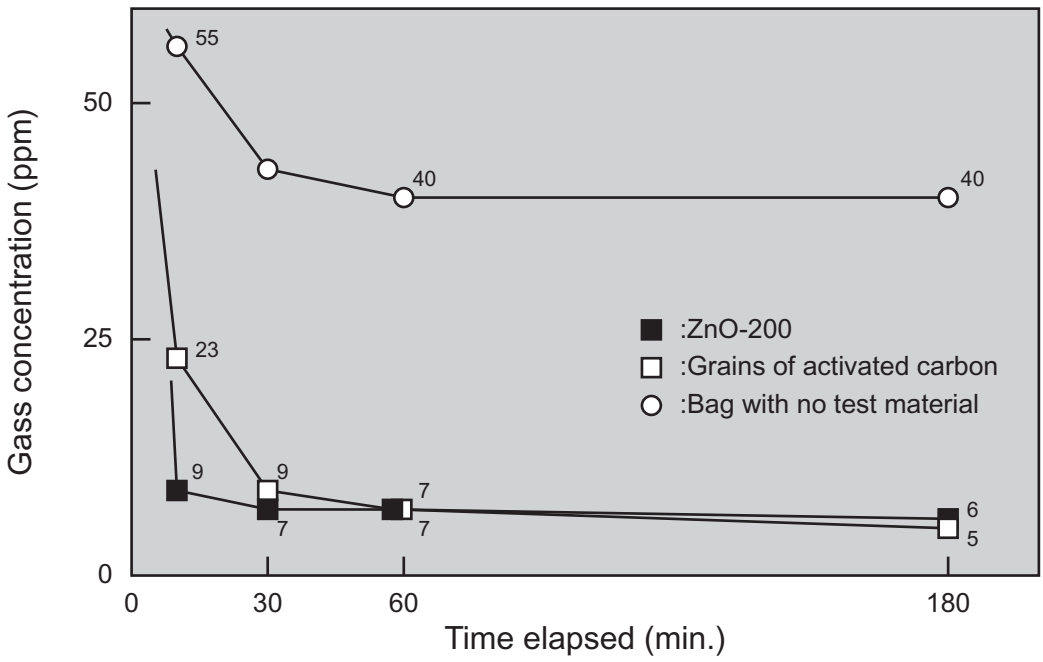

Fig.1 Deodorant property test of ZnO super fine powder with isovalerate

(6) Deodorant property test of zinc oxide super fine powder

2. Deodorant property test with ammonia

Test material : Zinc oxide super fine powder ZnO-200

Reagents and instruments : Odor bags (Alex Shokai)
Ammonia water (Koso Kagaku Yakuhin, super high grade)
Gas detecting tube (Gas Tech)

Test method : 1 gram of the test material was put in the odor bag (25cm × 25cm) and the bag was heat-sealed. Then, nitrogen-based ammonia (approx. 500 ppm) was inserted into the bag. The bag was left in the room temperature and the change of the residual gas concentration within the bag with time was measured by the gas detecting tube. The test of a bag to which no test material was put was also conducted in the same procedures.

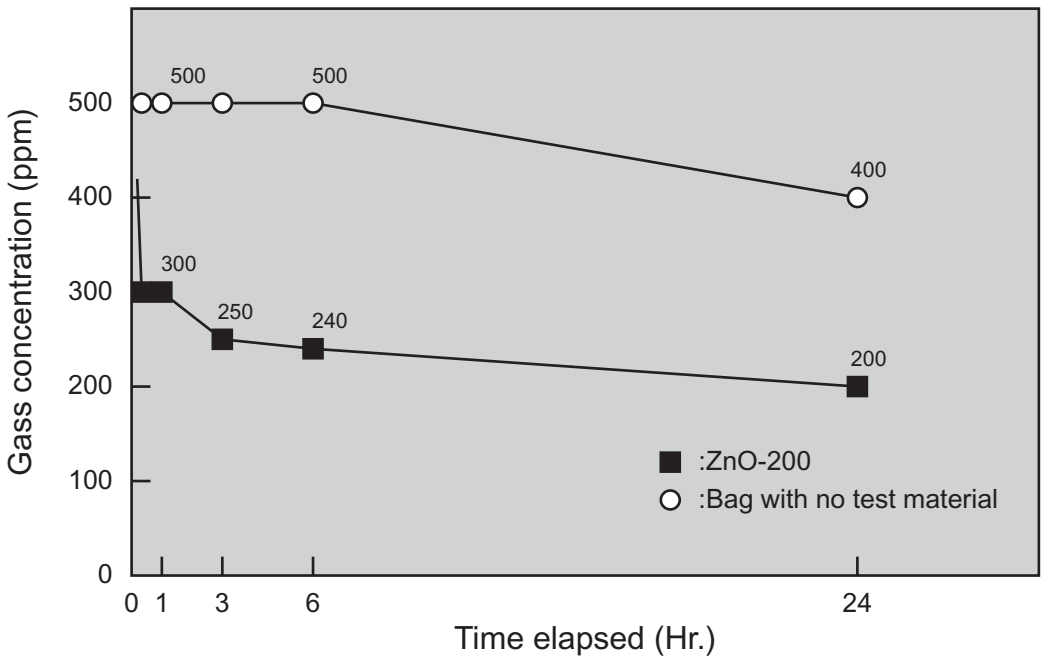

Fig.2 Deodorant property test of ZnO super fine powder with ammonia

(7) Deodorant property test of films incorporated with zinc oxide super fine powder (elimination of odor of isovalerate)

Test material : Film incorporated with ZnO super fine powder
: LLD-PE (3wt.% of ZnO-100 added)
Film incorporated with the inorganic antibacterial agent of other manufacturer
: LLD-PE (3wt.% of Product A of other manufacturer added)
Film not added with the test material
: LLD-PE (without additive)

Reagents and instruments : Odor bags (Alex Shokai)
Isovalerate (Tokyo Kasei Kogyo)
Gas detecting tube (Gas Tech)

Test method :Heat seal was applied to the test material to form it into a bag of 20 cm × 30 cm. Then, 1 liter of nitrogen-based isovalerate (approx. 50 ppm) was inserted into the bag. The bag was left in the room temperature and the change of the residual gas concentration within the bag with time was measured by the gas detecting tube. The test of an odor bag (20cm × 30cm) to which no test material was put was also conducted in the same procedures.

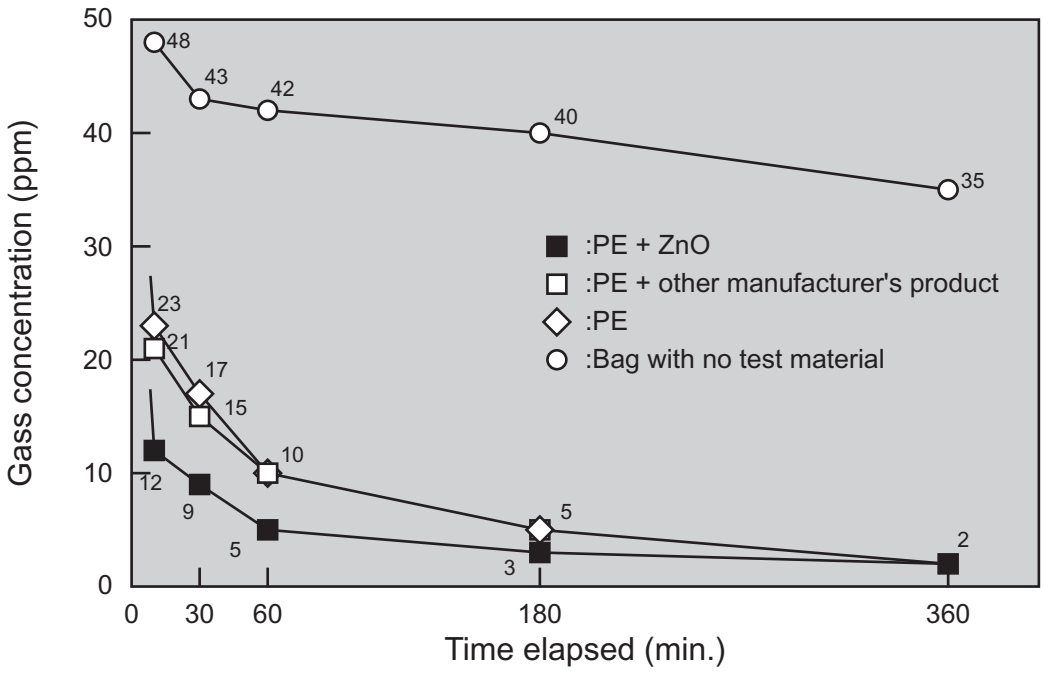

Fig.3 Deodorant property test of films incorporated with ZnO super fine powder