EFFECT OF PARTICLE SIZE AND HEATING TEMPERATURE OF CERAMIC POWDERS ON ANTIBACTERIAL ACTIVITY OF THEIR SLURRIES

JUN SAWAI, HIDEO IGARASHI,
ATSUSHI HASHIMOTO, TAKAO KOKUGAN AND
MASARU SHIMIZU
Department of Chemical Engineering, Division of Applied Chemistry, Tokyo University of Agriculture and Technology

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Theeffects of particlesize and heating temperature of magnesium oxide (MgO), calcium oxide (CaO), and zinc oxide (ZnO) powders on the antibacterial activities of their slurries were studied by the conductance method. An increase in particle size of the powders reduced their antibacterial activity. The variation of activity against *Staphylococcus aureus* was smaller than that against *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis*. This difference depended on the shape of the bacteria, but not their character, such as the structure of the cell walls. The antibacterial activity of these powder slurries decreased with an increase in heating temperature of the powders. For the ZnO powder slurry, the decrease in antibacterial activity were much larger than those of the MgO and CaO powder slurries. It was suggested that these decreases in the antibacterial activity was concerned with stabilization of the surface of the powders heated at high temperature.

Introduction

Recently, microbial pollution and degradation caused by microorganisms has created serious problems in various industrial fields (Kusaka and Takagi, 1972; Sato, 1993; Tsunoda *et al.*, 1992). Much attention has been paid to the use of ceramic materials with growth inhibitory effects on bacteria (antibacterial activity) as a new antibacterial technology (Kourai, 1993; Oya *et al.*, 1988; Tomioka *et al.*, 1993). However, it is poorly understood how ceramics affect bacteria (Hattori, 1965; Morisaki, 1983). Fundamental studies on the antibacterial activity of ceramics are required.

We have already reported the evaluation of antibacterial activity of ceramic powder slurry by the conductance method. As microorganisms metabolize, they create new end products in the medium. Generally, uncharged or weakly charged substrates are transformed into highly charged end products, for example proteins are metabolized to amino acids, carbohydrates to lactate and lipids to acetate, which increase the conductivity of the medium. The conductance method can detect bacterial growth as a variation of electrical conductance (Firstenberg-Eden and Eden, 1984). The conductance method could provide a quantitative and simple evaluation of the antibacterial activity of ceramic powder slurries. It was found that the magnesium oxide (MgO) and calcium oxide (CaO) powder slurries exhibited a bactericidal action and that the zinc oxide (ZnO) powder slurry exhibited a bacteriostatic action against the test bacteria (Sawai *et al.*, 1995a). These three powders have efficacy against spores of *Bacillus subtilis*, which have high resistivity to various stresses such as heat treatment and ultraviolet irradiation (Sawai *et al.*, 1995b).

In the previous study (Sawai *et al.*, 1995a), it was suggested that the antibacterial activity of the MgO, CaO, and ZnO powder slurries appeared very near the surface, or on the surface. If that is so, does the particle size of ceramic powders affect the antibacterial activity of their slurries? Also, ceramics such as MgO, CaO, and ZnO have been used as catalysts for various reactions including isomerization of olefins (Baird and Lunsford, 1972), dehydrogenation (Hattori *et al.*, 1975; Iizuka *et al.*, 1971; Tanaka *et al.*, 1976) and hydrogenation (Dent and Kokes, 1969a,b). It was reported that these powders showed the maximum activity by heating at specified temperatures. The heating temperatures required to show the maximum activity for each reaction are different. Thus, in this study, to get a better understanding of the antibacterial activity of ceramic powder slurries, we have focused on the particle sizes and heating temperatures of the ceramic powders, and studied the effect of these factors on the antibacterial activity by the conductance method.

1. Materials and Methods

1.1 Test Organism.

Escherichia coli 745, *Salmonella typhimurium* TSA-2121, *Staphylococcus aureus* 9779, and *Bacillus subtilis*

* Received on August 3, 1995. Correspondence concerning this article should be addressed to J. Sawai. J. Sawai is now at Dept. of Chem. Eng., Kanagawa Institute of Tecnology, Atsugi 243-02. H. Igarashi is at Dept. of Microbiol., Tokyo Metropolitan Research Laboratory of Public Health, Tokyo 169. A. Hashimoto is at Dept. of Bioproduc. & Machine., Mie University, Tsu 514.

Table 1 Mean particle size of MgO, CaO, and ZnO powders

Powder	Mean particle size [μm]			
MgO	20	80	170	690
CaO	10	10	20	20
ZnO	30	70	180	560

Table 2 Mean particle size of heated MgO, CaO, ZnO powders

Powder	Mean particle size [μm]			
	523 K	777 K	1023 K	1273 K
MgO	90	190	190	180
CaO	80	90	80	80
ZnO	180	170	180	170

ATCC 6633 were used as test bacteria. These test bacteria were stored at Tokyo Metropolitan Research Laboratory of Public Health. The test bacteria were cultured in Brain Heart Infusion (BHI) broth (Difco) at 310 K for 24 h on a reciprocal shaker. The culture was then suspended in sterile physiological saline to yield a final bacterial concentration of approximately 10^3 CFU/ml.

1.2 Preparation of ceramic powder slurry.

MgO, CaO, and ZnO (Kishida Chemicals) were used as test materials. The initial mean particle size of the MgO, CaO and ZnO powders were 3.6, 2.7 and 2.6 μm, respectively. These powders were pressed at 10^5 kg/m^2, ground, and classified (less than 50, 50-100, 100-300, over 300 μm).

The powders were suspended with physiological saline to prepare a powder slurry concentration of 100 mg/ml. Then, serial twofold dilutions of the slurries were made with physiological saline. The diluted slurries were used for experiments.

The mean particle sizes of powders were measured by microscopic examination. The powders were suspended with physiological saline to give a concentration of 1.0 mg/ml. The slurry was put on a slide glass, and dried naturally at 298 K. Then, the arithmetic mean particle sizes of about a hundred particles were measured by microscopic examination. The results are shown in **Table 1**. Since the shape of ceramic powder is not perfect spherical, the longest length of a powder particle was employed as the particle size.

Samples of the powders with particle size of 100-300 μm were heated at 523, 773, 1023 and 1273 K for 2 h with programmed rate of 200 K/h, and passed through the same sieve again to prepare the test sample. After heating, the powders were stored in a desiccator and the arithmetic mean particle sizes were measured in a similar manner as above. The results are shown in **Table 2**.

1.3 Measurement of antibacterial activity

Bactometer microbial monitoring system model 64 (bioMérieux VITEK) was used as an apparatus for the measurement of conductance. This apparatus and its operation were the same as those in the previous studies (Sawai *et al.*, 1995a,b). Modified plate count agar (Difco) of 500 μl was used as the growth medium and poured into wells of a module for the Bactometer. A ceramic powder slurry of 100 μl was pipetted into the well containing the agar. Then, the bacterial suspension of 100 μl was dispensed into the well. The modules were set in incubator of the Bactometer, and the conductance change of the agar was monitored during incubation at 308 K for 48 h.

After incubation, a small quantity of the sample from the well was cultured in BHI broth at 310 K for 24 h. Based on the results, it was determined whether the growth inhibition of bacteria was due to bactericidal action or bacteriostatic action.

1.4 Measurement of pH value of ceramic powder slurry

The MgO, CaO, and ZnO powder slurries were prepared at 25 mg/ml, and left for 24 h. Then, the pH values of the slurry were measured by a pH meter with stirring.

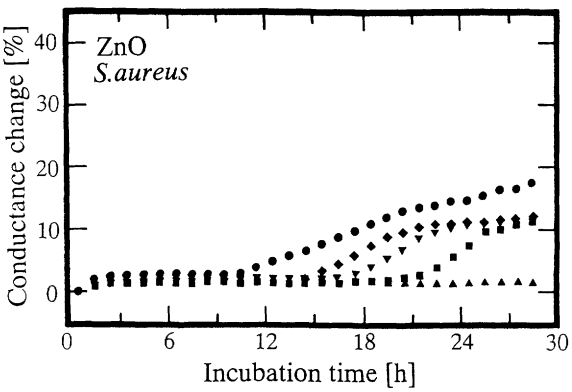


Fig. 1 Variation of DT values of *S.aureus* by ZnO powders having different particle size
(●: control, ▲: 30, ■: 70, ▼: 180, ◆:560 μm)

2. Results

2.1 Effect of particle size.

Figure 1 displays the conductance curves showing the antibacterial activity of the ZnO powder slurries against *S.aureus*. Percent conductance change of the growth medium is graphed against incubation time in hours. The concentrations of the ZnO powder slurries were 0.8 mg/ml.

The conductance method detects the change in conductance of growth medium caused by bacterial metabolism and growth. A detectable conductance change occurs when bacterial concentration reaches a specified concentration, where the bacterial concentration is approximately 10^7 viable cells per ml. The time required to described to detect the change is called "detection time" (DT). The variation of the DT value is used for the evaluation of antibacterial activity. The DT value of the control (powder concentration = 0 mg/ml) in Fig. 1 was about 10 h. This means that it took about 10 h for *S. aureus* to grow from 10^3 to 10^7 CFU/ml.

As shown in Fig.1, delaying of DT values was observed by the addition of the ZnO powders. This indicated that the ZnO powder slurry had antibacterial activity

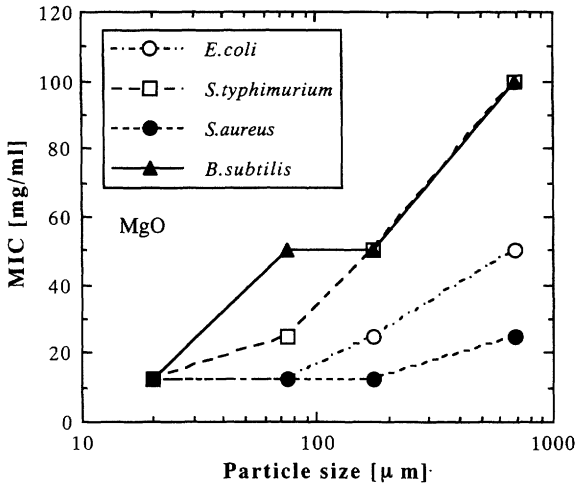


Fig. 2 Effect of particle size of MgO powder on MIC against test bacteria

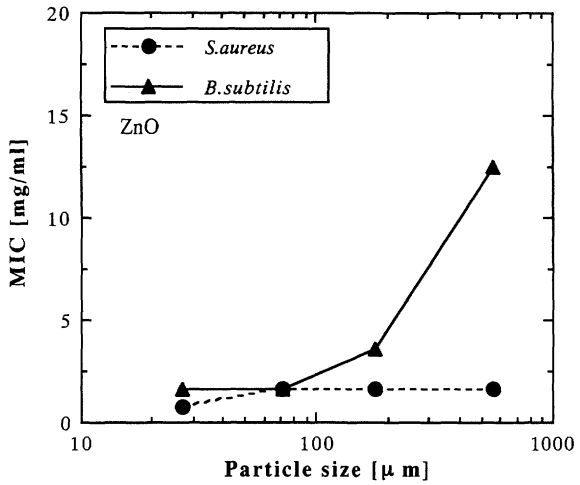


Fig. 3 Effect of particle size of ZnO powder on MIC against *S. aureus* and *B. subtilis*

against *S. aureus*. In the case of 30μm ZnO powders, the DT was not detected even after for incubation of 48 h. From the result of incubation in BHI broth, *S. aureus* was inhibited in a bacteriostatic manner. The delays of DT values decreased with increasing particle size, which meant a decrease in antibacterial activity of the ZnO powders against *S. aureus*.

Figure 2 shows the variation of minimum inhibitory concentration (MIC) of the MgO powder slurry against four kinds of tester strains. MIC presents the minimum concentration of powder slurry where the DT is not detectable with the measurement of conductance for 48 h. The MIC of the MgO powder slurry increased with increasing particle size. This increase in MIC means an increase in amount of powder required for inhibition of bacterial growth, namely, a decrease in antibacterial activity. However, the variation of MIC with particle size against *S. aureus* was smaller than those against other tester strains. Each MgO powder slurry exhibited a bactericidal action against four kinds of test bacteria over MIC.

For the CaO powders, the effect of particle size on

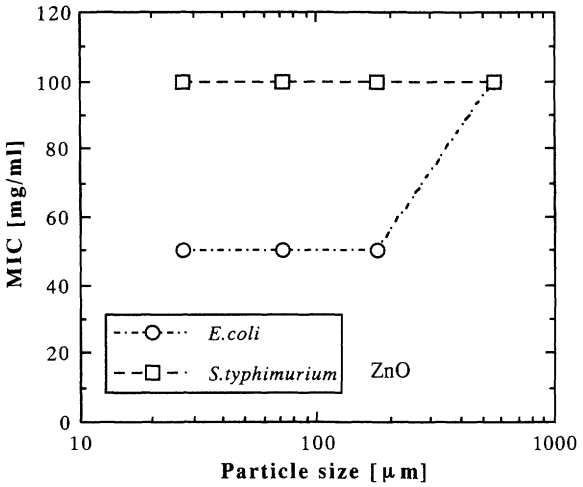


Fig. 4 Effect of particle size of MgO powder on MIC against *E. coli* and *S. typhimurium*

MIC could not be examined. The CaO powders remain the same particle size corresponding to opening of the sieves before the preparation of the slurries. However, when the CaO powders were suspended with physiological saline to prepare the slurries, all powders became fine powders of 10 to 20 μm diameter as shown in Table 1. This is because the CaO powders are relatively more soluble than the other ceramics and readily absorb water in the slurries. It was reported that the CaO is cracked readily by absorption of water (Sato and Shimada, 1990)

Figure 3 shows the results of the ZnO powder slurries against gram-positive bacteria. The MIC of the ZnO powder slurry against *B. subtilis* markedly increased with an increase in particle size. There was a different tendency in variation of MIC between *S. aureus* and *B. subtilis*, although these bacteria were both gram-positive bacteria. For *S. aureus*, though the DT delayed with particle size as shown in Fig. 1, a remarkable decrease in antibacterial activity, such as an increase in MIC, was not observed.

Figure 4 shows the results of the ZnO powder slurries against gram-negative bacteria. An increase in MIC of the ZnO powder slurry against *E. coli* was observed. However, there was no change in MIC against *S. typhimurium*. Because the ZnO powder slurry acted upon gram-positive bacteria stronger than upon gram-negative bacteria (Sawai *et al.*, 1995a), higher slurry concentration was required to inhibit growth of gram-negative bacteria. In this study, serial two-fold dilutions of 100 mg/ml of powder slurry were made for MIC measurement (100, 50, 25...0.1, 0.05 mg/ml). The difference in powder concentration in a step becomes larger at higher concentration in serial two-fold dilutions. Hence, the variation of MIC of the ZnO powder slurry against *S. typhimurium* did not appear.

2.2 Effect of heating temperature

Using powders of about the same mean particle size as shown in Table 2, how heating temperature of ceramic powders influenced their antibacterial activity was examined. **Figure 5** shows the effect of heating temperature of

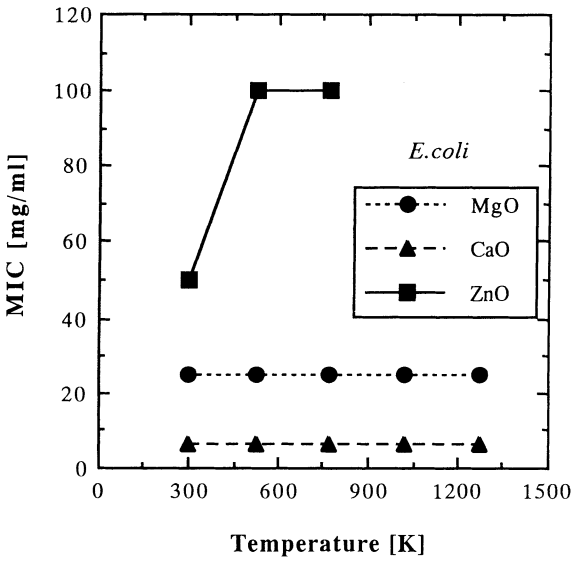


Fig. 5 Effect of heating temperature on MIC of ceramic powder slurry against *E. coli*

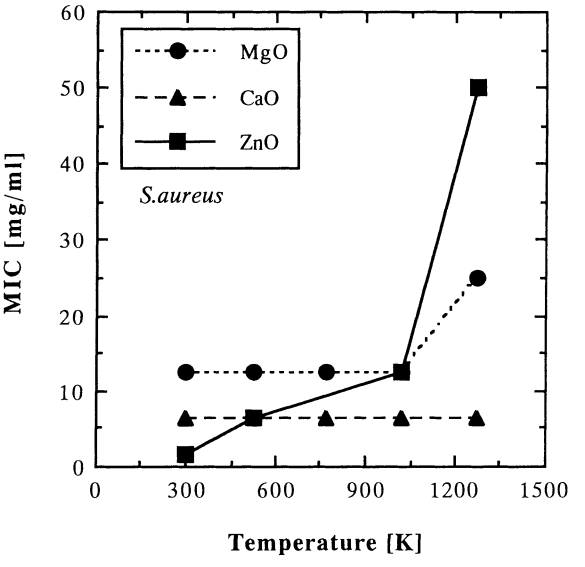


Fig. 6 Effect of heating temperature on MIC of ceramic powder slurry against *S. aureus*

the MgO, CaO, and ZnO powders on MIC of their slurries against *E. coli*. The CaO powders heated at different temperatures remained their mean particle size corresponding to the sieve opening from 100 to 300 μm. However, the heated CaO powders in slurries reduced into fine powders of 80 to 90 μm diameter just like the unheated CaO powders did into fine powders of 10 to 20 μm diameter.

For the MgO and CaO powder slurries, there were no changes in MIC with an increase in heating temperature. On the other hand, in the case of the ZnO powder slurry, the MIC increased with increasing heating temperature, which showed a decrease in antibacterial activity. And, the MIC of the ZnO powders heated at 1023 and 1273 K against *E. coli* was higher than 100 mg/ml. Based on the results of BHI broth, the MgO and CaO powder slurries

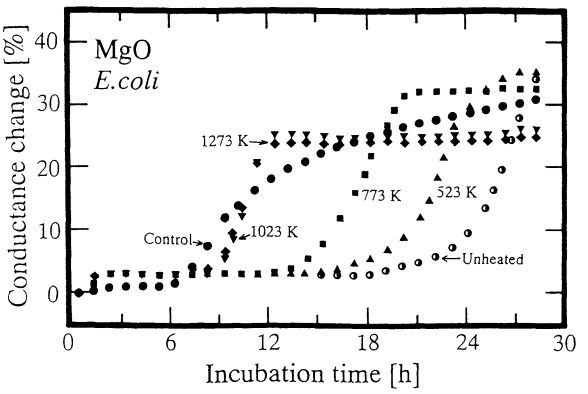


Fig. 7 Variation of DT values of *E. coli* by MgO powder heated at different temperature

exhibited a bactericidal action and the ZnO powder slurry exhibited a bacteriostatic action at higher than their MIC.

Figure 6 shows the results of *S. aureus*. The MIC of the MgO powder heated at 1273 K increased. The MIC of the CaO powder slurry against *S. aureus* did not change like that against *E. coli*. Both the powder slurries exhibited a bactericidal action against *S. aureus* over the MIC. For the ZnO powder slurry, a similar tendency was obtained in the case of *E. coli*. The MIC of the ZnO powder slurry against *S. aureus* markedly increased with an increase in heating temperature. From the results of BHI broth incubation, the ZnO powder heated at less than 773 K acted in a bacteriostatic manner up to 50 mg/ml and acted in a bactericidal manner over 50 mg/ml. Also, the ZnO powders heated at higher than 1023 K exhibited a bacteriostatic action but not a bactericidal action at 100 mg/ml.

Figure 7 displays the conductance curves showing the antibacterial activity of the MgO powders heated at different temperature against *E. coli*. The slurry concentrations of the MgO powder were 12.5 mg/ml each. As shown in Fig. 6, addition of the MgO powders caused delays of the DT values of *E. coli*. Also, an increase in heating temperature shortened the delays of the DT values even the MIC did not change as shown in Figs. 4 and 5. A similar result was obtained in the case of the CaO powders.

3. Discussion

3.1 Antibacterial activity and particle size

Though for the CaO powders, the effect of particle size could not be examined, increases in particle size of the MgO and ZnO decreased their antibacterial activity. These ceramics are porous powders. Since their pore size ranges from nanometers to tens of nanometers, the bacteria are unable to penetrate the pores. In the previous study (Sawai *et al.*, 1995a), it was suggested that the antibacterial activity of ceramic powders originated from the powder surface or very near to the surface. Therefore, the decrease in the antibacterial activity with increasing particle size is probably attributable to the decrease in powder surface area available for contacting with bacteria.

The influence of particle size depended on the

Table 3 Shape or test bacteria

Bacteria		Shape [μm]
Gram-negative	*E. coli*	1.1~1.5 × 2.0~6.0
	S. typhimurium	0.4~0.6 × 2.0~3.0
Gram-positive	*S. aureus*	0.8~1.0
	B. subtilis	0.3~2.2 × 1.2~7.0

(Kasai *et al.*, 1977; Mizushima *et al.*, 1992)

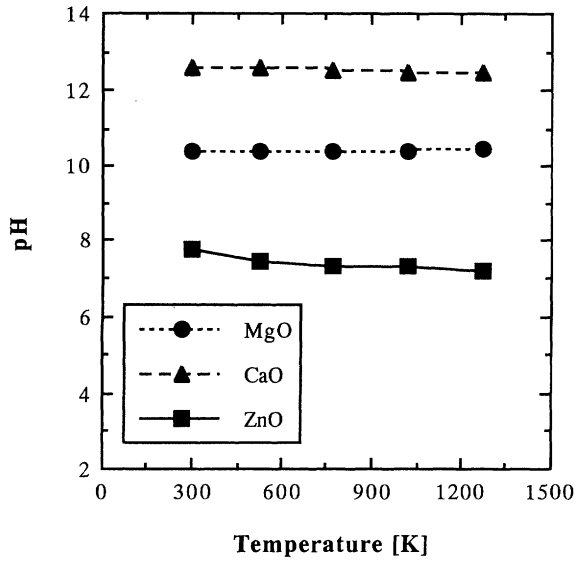
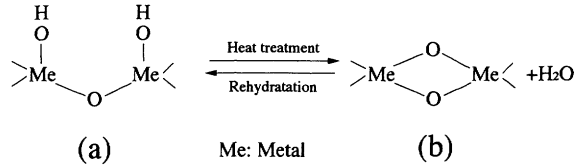


Fig. 8 Effect of heat treatment on pH value of ceramic powder slurry

species of bacteria. *E. coli* and *S. typhimurium* are gram-negative bacteria and rod-shaped *S. aureus* and *B. subtilis* are both gram-positive bacteria, but the former is of spherical-shape, and the later is of rod-shape (**Table 3**). Gram-positive and negative bacteria differ from each other in structure of cell wall. The MIC of the ceramic powders against rod-shape bacteria markedly changed. On the other hand, the variation of MIC against spherical-shape bacteria, i.e., *S. aureus*, was small. The difference is probably due to the shape of the bacteria, but not the character of the bacteria, such as the structure of cell wall. It is not clear how these bacteria behave in the ceramic powder slurry. Usually, *S. aureus* form grape-like irregular clusters. The cluster is considerable larger than the rod-shape bacteria used in this study. Also, some *S. aureus* which exist in a cluster would not directly contact with the ceramic powders. Therefore, it was considered that the influence of the variation of particle size on *S.aureus* was relatively smaller than those on other bacteria.

3.2 Decrease in antibacterial activity by heating

Hydroxyl groups and water molecules adsorbed on ceramic surfaces are removed by heat treatment (Morimoto *et al.*, 1968). Most physisorbed water molecules are removed at about 523 to 573 K. The removal of the surface hydroxyl groups required heat treatment at higher temperature (Seiyama, 1978). In this case, it is considered that the structure of the ceramic surface changes as shown in the following equation.



(1)

When the powder with the structure (b) in Eq.(1) is immersed in water, rehydration readily occurs. However, the structure (b) is stabilized with an increase in heating temperature, and the stabilized surfaces have a tendency not to rehydrate (Kiyono, 1991; Koishi and Tsunoda, 1982). The pH values of the ZnO powder slurries slightly decreased with an increase in heating temperature (**Fig. 8**).

It is well known that the surface hydroxyl groups on metallic oxides play an important role as an acid or a base in adsorption and catalytic action. The antibacterial activity of the powders heated over 523 K decreased. Therefore, it was considered that the hydroxyl groups and water molecules on the surface of the ceramic powders play an important role, even in the inhibition of bacterial growth by the ceramic powder slurries.

The decrease in antibacterial activity of the CaO or MgO powders with an increase in heating temperature was much smaller than that of the ZnO powder. The contribution of ionic bond to the bond of the CaO or MgO is about 80 or 73 %, respectively, and CaO and MgO are relatively soluble among ceramics (Koishi and Tsunoda, 1982). There was a slight variation of antibacterial activity between the CaO and have powders because alkaline earth metallic oxides such as CaO and MgO have a high hydrophilicity and are extremely easy to hydrate and rehydrate. The MgO and CaO powder slurries prepared with physiological saline are saturated, and no variation of pH values of the slurries by heating was observed (Fig. 8).

On the other hand, the contribution of ionic bond to the bond of ZnO is about 63 % and smaller than those of CaO and MgO. When the slurries were prepared, the ZnO powders heated over 1023 K did not disperse and soon sedimented in physiological saline. Rehydration originates from the interaction between ceramic powder and water, and mainly depends on electrostatic interaction and the formation of hydrogen bonds. The increase in interaction between the powder and water enhances dispersibility of the powder. Therefore, it was considered that the ZnO powders heated at high temperature readily sedimented because the interaction decreased with the stabilization of the ZnO powder surfaces.

It was reported that the presence of O_2^- on the surfaces of MgO, CaO, and ZnO powders was observed (Iizuka and Tanabe, 1975; Tanabe *et al.*, 1978). Active oxygen such as O_2^- and H_2O_2 exhibited a powerful oxidative ability. The active oxygen generation of these powders may take part in growth inhibition by these powder slur-

ries. Further work is in progress to detect active oxygen generating from these powders and to examine the contribution of active oxygen to antibacterial activity.

Concluding Remarks

In this study, it was found that particle size and heating temperature of ceramic powders (MgO, CaO, and ZnO) affected their antibacterial activity. The following conclusions were obtained.

1) The antibacterial activity of these ceramic powder slurries decreased with increasing particle size. However, the variation of the antibacterial activity against *S. aureus* was smaller than those against other rod-shaped bacteria. It was considered that this difference depended on the shape of the bacteria, but not the character of the bacteria, such as structure of the cell wall.

2) The antibacterial activity of these ceramic powder slurries decreased with an increase in heating temperature of the powders. The decrease in the activity of the ZnO powder slurry was larger than those of the MgO and CaO powder slurries. It was suggested that these decreases in antibacterial activity were due to stabilization of the surface of the powder heated at high temperature.

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