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Antibacterial Characteristics of MgO-ZnO Solid Solution with Different Chemical Compositions

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MgO-ZnO solid solutions with different chemical compositions were prepared by heating at 1200℃ for 5h in air. By XRD, a single phase of MgO was observed above the molar ratio (MgO/ZnO) of 1.86, of which the lattice constant increased linearly. Diffraction peaks corresponding to ZnO when the ratio was 1.22 were detected in addition to diffraction peaks of MgO. No change in lattice constant of MgO was recognized at the ratio of 1.22. Antibacterial characteristics towards *Staphylococcus aureus* and *Esherichia coli* were examined in four solid solutions by colony count method. In the results, antibacterial activity of MgO-ZnO solid solution was found to enhance with decreasing the molar ratio in the range from 9.00 to 1.86. At the ratio of 1.22, however, the antibacterial activity reduced. That is, antibacterial activity of the MgO-ZnO solid solution was found to be dependent on the chemical composition.
Key words: MgO-ZnO solid solution, chemical composition, lattice constant, antibacterial characteristic

1. INTRODUCTION

Magnesium oxide (MgO) and zinc oxide (ZnO) have been known as oxide ceramics which show strong antibacterial activity without the presence of light [1-3]. Sawai et al. [4] and Yamamoto et al. [5] have reported that occurrence of the antibacterial activity on MgO and ZnO was assumed to be due to the generation of active oxygen, such as O_2^- and H_2O_2. That is, chemical species contributing to antibacterial activity of MgO is different from that of ZnO.

In these powders, some effective factors on the occurrence of antibacterial activity have been anticipated, i.e., pH value, particle size, specific surface area and lattice constant [6-8]. In MgO-ZnO solid solution, however, it is not yet clear what change in antibacterial activity is expected to be dependent on the chemical composition.

According to a phase diagram of MgO-ZnO system [10], periclase phase (MgO) can be formed above the molar ratio (MgO/ZnO) of 1.86. Below the ratio of 1.86, zincite phase (ZnO) appears in addition to MgO.

Yamamoto et al. [9] has reported that the increase of lattice constant in ZnO resulted in the enhancement of the antibacterial activity. Applying this report to MgO, the lattice constant of MgO may affect antibacterial activity, which is possible to control the doping amount of ZnO in MgO-ZnO solid solution.

In the present work, antibacterial activity of MgO-ZnO solid solution with different chemical compositions was evaluated, with emphasis on effect of the lattice constant.

2. EXPERIMENTAL

2.1 Powder characterization

Sample code, molar ratio (MgO/ZnO) and chemical composition of MgO-ZnO solid solution are summarized in Table I. The number in sample code represents molar fraction of ZnO; For example, MZ10 represents 90mol% MgO-10mol%ZnO powder sample. MgO powder (Ube Chemical IND, Ltd. Purity; 99.9%) and ZnO powder (Kanto Chemical, Co., Purity; 99.9%) were used as starting materials. The mixtures of MgO and ZnO were heated at 1200℃ for 5h in air to prepare powder samples of MgO-ZnO solid solution. As reference materials, MgO and ZnO were prepared at same heat-treatment. The formation of MgO-ZnO solid solution was confirmed by X-ray diffraction measurement (XRD; Rigaku, RAD- C SYSTEM). Specific surface area of the powder samples was determined by measuring the adsorption isotherms of N_2 at -196℃ (BET; BELJAPAN, INC. BELSORP-mini).

In order to examine the pH values when the powder samples were suspended in water, the powder samples were dispersed into distilled water at the powder concentration of 0.63 g dm^{-3}. After keeping the dispersed solution at 30℃ for 1h, the pH value of the solution was measured.

Hydration resistance of the powder samples was

Table I Sample code and chemical composition of powder sample used in this study

Sample code	Molar ratio (MgO/ZnO)	Chemical composition
MZ10	9.00	$Mg_{0.90}Zn_{0.10}O$
MZ25	3.00	$Mg_{0.75}Zn_{0.25}O$
MZ35	1.86	$Mg_{0.65}Zn_{0.35}O$
MZ45	1.22	$Mg_{0.65}Zn_{0.35}O$ + $Zn_{0.45}Mg_{0.55}O$

tested at the powder concentration of 0.63 g dm^{-3}. $Mg(OH)_2$ is easily formed by hydration of MgO, which can be detected by XRD. The hydration rate (α) was calculated by Eq.(1).

$$\alpha\ [\%] = \{[Mg(OH)_2]_{(101)} / ([Mg(OH)_2]_{(101)} + [MgO]_{(200)})\} \times 100 \quad \cdots\cdots (1)$$

where subscripts, (101) and (200), are Miller indices. Square brackets, $[Mg(OH)_2]$ and [MgO], express the diffraction peak area of each component detected by XRD.

2.2 Antibacterial test

Escherichia coli 745 (hereafter, *E. coli*) as a gram-negative bacterium and *Staphylococcus aureus 9779* (hereafter, *S. aureus*) as a gram-positive bacterium were used as test bacteria. *E. coli* and *S. aureus* were cultured at 36℃ for 48h in a LB medium on a reciprocal shaker, which contains 0.5% yeast extract (Becton, Dickinson and Co.), 1% bactopeptone(Becton, Dickinson and Co.) and 1% sodium chloride(WAKO PURE CHEMICAL IND., LTD, purity; 99.9%)(NaCl). The medium was rinsed four times in sterile water, and the bacterial culture was suspended in sterile water at a final concentration of 10^7 CFU dm^{-3} (CFU; Colony Forming Unit). Subsequently, the solution of bacterial suspension was added into sterile water containing powder samples with concentration in the range from 0.08 to 1.25 g dm^{-3} and then kept at 36℃ for different times on a reciprocal shaker. After sampling the bacterial suspension of 1×10^{-4} dm^3, the bacterial suspension was spread on nutrient agar (NA, Eiken Chemical, Co.) for *E. coli* and pearl-core plate count agar (PPCA, Eiken Chemical, Co.) for *S. aureus,* and cultured at 36 ℃ for 48 h without the presence of light. The colonies formed with bacterial growth were counted. By calculating the ratio (N/N_0) between the viable bacterial counts (N (CFU dm^{-3})) at specified time and the initial counts (N_0 (CFU dm^{-3})) of bacteria, antibacterial activity was evaluated.

3. RESULTS AND DISCUSSIONS

3.1 Powder characterization

Figure 1 shows XRD patterns of the powder samples obtained at 1200℃. A single phase of MgO with cubic structure was formed in three powder samples of MZ10, MZ25 and MZ35, indicating the formation of MgO-ZnO solid solution. In MZ45, however, the diffraction peaks corresponding to ZnO with hexagonal type were detected in addition to diffraction peaks of MgO. This result seems to be due to excess ZnO in the formation of the solid solution. Diffraction peaks corresponding to MgO shifted to low-angle side with increasing the doping amount of ZnO. The value of lattice constant in MgO is summarized in Table II , together with the value of formed ZnO, a_0 and c_0. The lattice constant of MgO on the powder samples was larger than that on original MgO. And the value increased with increasing the doping amount of ZnO, which reached 0.4238 nm at the molar ratio of 1.86.

In comparison on the ionic radius between Zn^{2+} ion (74pm) and Mg^{2+} ion (65pm), the reason that the lattice

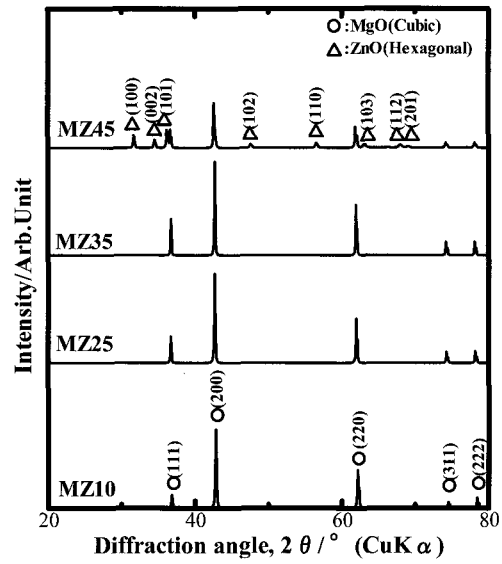

Fig. 1 XRD patterns of MgO-ZnO solid solutions with different chemical compositions.

Table II Lattice constant of the powder samples used in this work.

Sample code	Lattice constant of MgO / nm	Lattice constant of ZnO / nm	
		Along a_0	Along c_0
Original MgO	0.4218	—	—
MZ10	0.4220	—	—
MZ25	0.4234	—	—
MZ35	0.4238	—	—
MZ45	0.4238	0.3259	0.5192
Original ZnO	—	0.3254	0.5208

constant of MgO increased with increasing the doping amount of ZnO was presumed to be due to the replacement of Mg^{2+} ion with larger Zn^{2+} ion.

With emphasis on lattice constant of ZnO in MZ45, the a_0 value in hexagonal structure slightly increased, compared with original ZnO. The c_0 value in MZ45 was smaller than that in original ZnO, indicating the replacement of Zn^{2+} ion with smaller Mg^{2+} ion in hexagonal structure. Therefore, it is assumed that two solid solutions of $Mg_{0.65}Zn_{0.35}O$ and $Zn_{0.45}Mg_{0.55}O$ are formed at the molar ratio of 1.22. This result agreed with the phase diagram of the MgO-ZnO system [10]. At the molar ratio of 1.22, there was no change in lattice constant of MgO, i.e., identical value for $Mg_{0.65}Zn_{0.35}O$ (MZ35).

By specific surface area measurement, it was found that specific surface area of powder samples was approximately identical value of 6 m^2 g^{-1}.

The hydration rate and the pH value of original MgO and solid solution slurries at powder concentration of 0.63 g dm^{-3} are summarized in TableIII. The powder samples after hydration test were measured by XRD. The diffraction peaks corresponding to $Mg(OH)_2$ with

hexagonal type were detected in the powder samples obtained after hydration tests for original MgO and MZ10. In powder samples, MZ25, MZ35 and MZ45, no peaks of $Mg(OH)_2$ were detected, i.e., no hydration reaction of MgO with water. The hydration rate of original MgO and MZ10 was 84 and 59%, respectively. The powder samples, MZ25, MZ35 and MZ45, showed high hydration resistance, i.e., no hydration reaction. From the results, it is found that the hydration reaction is inhibited with increasing the doping amount of ZnO.

Small et al. [11] reported that the tolerance of *E. coli* in alkaline media reduced above pH 9.8; that is, antibacterial activity enhanced with increasing pH value. The pH value in aqueous solution containing powder samples was measured, because aqueous solution containing MgO shows alkalinity, due to the formation of $Mg(OH)_2$ with water. The pH values decreased with increasing the doping amount of ZnO. This was consistent with results of hydration test.

TableIII pH value of original MgO and solid solution slurries at powder concentration of 0.63g dm^{-3}.

Sample code	pH value / -	Hydration rate / %
Original MgO	11	84
MZ10	10	59
MZ25	8.9	0
MZ35	8.7	0
MZ45	8.4	0

3.2 Antibacterial activity of powder samples

In colony count method, the powder sample when the value of survival ratio changes with a steep decrease at the specified time can be understood to have stronger antibacterial activity. Fig. 3 (a) and (b) show change in survival ratio of *S. aureus* and *E. coli*, respectively, using four powder samples at a powder concentration of 0.63 g dm^{-3}. In the case of *S. aureus* (see Fig. 3 (a)), the survival ratio on powder samples showed the steep reduction in the molar ratio ranging from 9.00 to 1.86. It was found that antibacterial activity towards *S. aureus* enhanced with increasing the doping amount of ZnO. However, the survival ratio on MZ45 was larger than that on MZ35. This result indicated the reduction of the antibacterial activity. In *E. coli* (see Fig. 3 (b)), the reducing behavior of the survival ratio corresponded to *S. aureus*.

It has been known that the pH value above 10 inhibits bacterial growth [12]. The effect of pH on bacterial growth is generated above 10. In MZ10, the pH value was 10, which might affect antibacterial activity. The pH value decreased with increasing the doping amount of ZnO, which reached 8.4 at the molar ratio of 1.22. That is, the effect of pH value on antibacterial activity might be small in the molar ratio below 9.00. However, antibacterial activity of MZ35 was stronger than that of MZ25. This indicates that the pH value does not affect the enhancement of the antibacterial activity. Antibacterial activity of MgO occurs due to the generation of O_2^- from its surface [4]. The generated O_2^- was found to have stronger antibacterial effect than pH value [13]. Therefore, the chemical species contributing to occurrence of the antibacterial activity on solid solution should be O_2^- generated on its surface.

Yamamoto et al. [9] have studied the effect of lattice constant on antibacterial activity of ZnO, resulting in the enhancement of the antibacterial activity with the increase of the lattice constant along c axis. In present work, the lattice constant of MgO increased with decreasing the molar ratio. In the molar ratio ranging from 9.00 to 1.86, the enhancement of antibacterial activity on the solid solution corresponded to the increase in lattice constant of MgO. From the results, the increase in lattice constant of MgO is found to be effective factor affecting antibacterial action of MgO-ZnO solid solution.

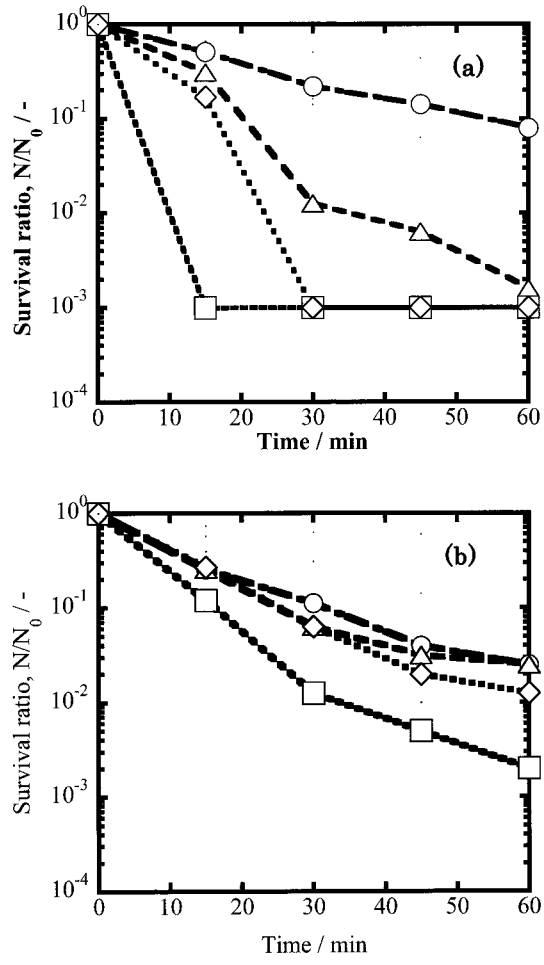

Fig. 3 Change in survival ratio of (a) *S. aureus* and (b) *E. coli* on solid solution slurries at powder concentration of 0.63g dm^{-3}. ○ ; MZ10, △;MZ25, □; MZ35, ◇; MZ45.

Figure 4 (a) and (b) show change in survival ratio of *S. aureus* and *E. coli*, respectively, using original MgO and ZnO powders at a powder concentration of 0.63 g dm^{-3}. Survival ratio reduced with increasing incubation time, irrespective of the kind of bacteria. The reduction of the survival ratio on MgO was larger than that on ZnO, indicating that antibacterial activity of MgO was stronger than that of ZnO. As shown in Fig. 3(a) and (b), the reducing behavior of the survival ratio on MZ45 was gentler than that on MZ35, irrespective of the kind of

bacteria. That is, the antibacterial activity of MZ35 was stronger than that of MZ45.

Tamai et. al. [14] has reported that activated carbon containing MgO showed strong effect for inhibiting growth of bacteria, compared with activated carbon containing ZnO. By XRD, MZ45 showed two phases of MgO and ZnO. Therefore, the enhancement of antibacterial activity is anticipated to be dependent on the amount of MgO component. The amount of MgO component in MZ45 is smaller than that in MZ35, because MZ35 shows a single phase of MgO. The reason that antibacterial activity of MZ45 is weaker than that of MZ35 is presumed to be due to the decrease in amount of MgO component.

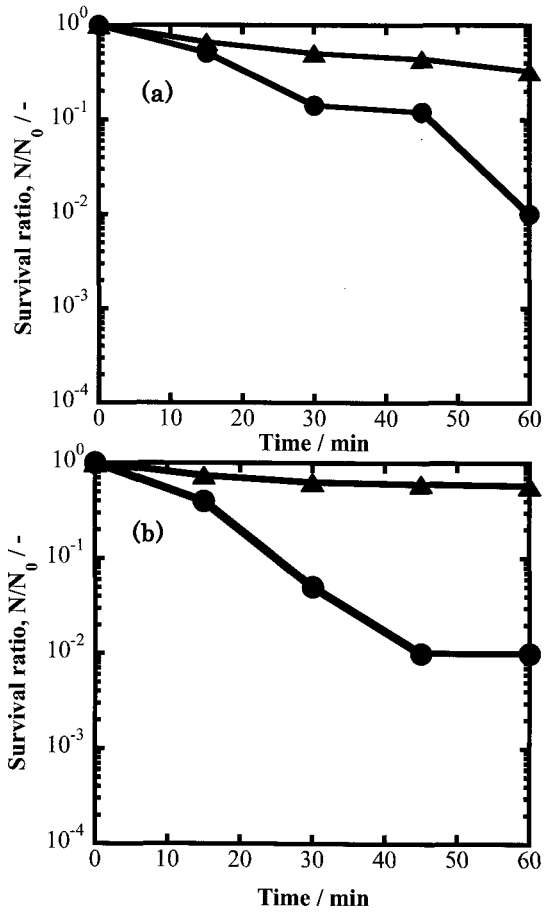

Fig. 4 Change in survival ratio of (a) *S. aureus* and (b) *E. coli* on original MgO and ZnO slurries at powder concentration of 0.63g dm^{-3}. ●; Original MgO, ▲;Original ZnO.

4. CONCLUSIONS

MgO-ZnO solid solution powders in molar ratio (MgO/ZnO) ranging from 9.00 to 1.22 were prepared by heating at 1200℃ for 5 h in air. By XRD, diffraction peaks corresponding to MgO with cubic structure were detected in the molar ratio above 1.86. At the molar ratio of 1.22, however, diffraction peaks of ZnO were observed in addition to diffraction peaks of MgO. In the range above the molar ratio of 1.86, the decrease of the ratio resulted in the increase in lattice constant of MgO, which showed the enhancement of antibacterial activity. At the molar ratio of 1.22, the reduction of antibacterial activity was found to be due to the formation of two phases, MgO and ZnO. Antibacterial activity was clarified to be dependent on the chemical composition of MgO-ZnO solid solution.

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