

Structure and Antibacterial Property of ZnO-B₂O₃-P₂O₅ Glasses

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ABSTRACT

The glass structure and antibacterial properties of (65-x)ZnO-xB₂O₃-35P₂O₅ glasses were investigated. Zinc borophosphate glasses were prepared using a conventional melt-quenching technique at 1000°C. Glass transition temperature and CTE were studied and the structure of zinc borophosphate glasses was evaluated by FTIR. The Zn²⁺ state increase with increasing ZnO content was investigated by XPS and a single sharp Zn 2P_{3/2} peak was confirmed, showing that Zn 2P_{3/2} exists as Zn²⁺. In order to evaluate the antimicrobial activity, *Escherichia coli* (*E. coli*) was used following the Japanese Industrial Standard JIS Z 2801; the *E. coli* death rate was found to increase with increasing Zn²⁺ content of glasses.

Key words : Zinc borophosphate glass, Antibacterial property

1. Introduction

Commercial glasses, which are used mainly in kitchens and laboratories, require a low coefficient of thermal expansion, good thermal shock resistance, high chemical durability and electrical resistivity, etc. Generally, borosilicate glass satisfies these requirements; there are several commercial borosilicate glasses such as Pyrex[®] and Duran[®]. However, the melting points of borosilicate glasses are very high, exceeding 1500°C, resulting in high energy consumption during production. In order to solve this problem, phosphate and borate glasses have been extensively studied. Phosphate glasses, which have a high coefficient of thermal expansion, low melting and softening temperatures, and high electrical conductivity, and which respond to specific types of light, have gained considerable attention over the last two to four years in various fields.¹⁾ However, the poor chemical durability of phosphate glasses makes them unsuitable for many commercial applications; to overcome the poor durability, various studies on the dissolution behavior of phosphate glasses have been performed.²⁻⁴⁾ Takebe *et al.* studied the compositional dependence of chemical durability in meta-phosphate glasses, and reported that the chemical durability of ZnO-P₂O₅ glasses of water was improved by addition of ZnO.³⁾ In the case of zinc borophosphate glass, the first investigation of the glass-forming regions in the ZnO-B₂O₃-P₂O₅ system was reported by Ushakov *et al.*,⁵⁾ who reported two glasses-forming regions: one adjacent to the ZnO-P₂O₅ system and the other placed between Zn

metaborate and ZnO-3ZnO·P₂O₅ composition. L. Koufelka *et al.* verified the data on glass formation in the ZnO-B₂O₃-P₂O₅ system and characterized the obtained homogeneous glasses using physico-chemical and spectral methods.⁶⁾

ZnO is interesting not only for its wide variety of physical and chemical properties but also for its antibacterial activity. The antimicrobial properties of ZnO, without toxicity and environmental effects, have been widely investigated. And, ZnO is an effective and stable photocatalyst in the waste treatment and renewable energy fields. It is also known to have antibacterial properties.⁷⁻¹¹⁾ Therefore, stable phosphate glasses have attracted significant interest as functional materials. Research into effective photocatalyst powders that can be obtained using a conventional coating or chemical vapor deposition method has attracted attention recently in studies of photocatalytic and antibacterial materials. However, because attempts to assess the photocatalytic and antibacterial functions require the separation of the powder and the solution, such studies are difficult at general laboratory scale. To overcome this problem, it is necessary to fix the material on a substrate.

In this paper, the antibacterial properties and glass structure of borophosphate containing ZnO were studied. The structural changes in zinc borophosphate glass with added B₂O₃ and the changes in the thermal properties were confirmed by Thermo Mechanical Analyzer (TMA) and Fourier Transform Infrared spectroscopy (FTIR) analysis. Structural analysis was carried out to investigate the effects of B₂O₃ addition on the chemical durability and CTE changes. In addition, the effect of the Zn²⁺ content on the antibacterial properties was investigated through X-Ray Photoelectron Spectroscopy (XPS); an antibacterial test with *Escherichia coli* (*E. coli*), following the Japanese Industrial

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Standard JIS Z 2801, showed the relationship between *E. coli* death rate and Zn²⁺ content in the glasses.

2. Experimental Procedure

ZnO (Junsei, CP, Japan), B₂O₃ (Junsei, CP, Japan), and NH₄H₂PO₄ (Junsei, EP, Japan) were used as the starting materials. As shown in Table 1, five glasses with high ZnO content were fabricated. For each composition, 30 g of powder was placed in an alumina crucible and melted in an electric furnace at 1000°C for 1 h. After melting, the sample was poured into a stainless-steel mold and quenched. The quenched specimens were annealed for 1 h at near their glass transition temperature (T_g : 480°C) and cooled slowly. Vitrification of the manufactured glass was confirmed by X-Ray Diffraction (XRD, Rigaku Corporation, Cu, 30 kV, 15 mA) analysis. The glass transition temperature and coefficient of thermal expansion were measured by Thermo Mechanical Analyzer (Q-400 TMA, T.A. Co, USA). FTIR spectroscopy (PerkinElmer, Spectrum GX) with KBr method used to investigate the structure of each glass composition. The investigated sample was dried in a dryer at 100°C and tested after powder milling (particle size of 44 μm or less). KBr powder was mixed at a ratio of 1 : 1200. XPS was performed to determine the Zn state and the structure of the glass. XPS spectra were measured using an ESCALAB XPS and Theta Probe XPS system under monochromatic Al K α ($h\nu = 1486.6$ eV) radiation. An analytical area 400 μm in diameter was used and the results were calibrated using a reference C 1s line (284.6 eV).

In order to analyze the antibacterial properties of the glasses, a Japanese Industrial Standard (JIS) Z 2801 standard test was conducted.¹² This test measures that the visible number of bacteria or fungi on a test piece after 1.0×10^7 cells are cultured for 24 h at a relative humidity of > 90% at 35°C. The test was conducted 20 times for reproducibility.

The number of viable bacteria was calculated by the following equation.

$$N = C \times D \times V \quad (1)$$

where N is the number of viable cells of bacteria, C is the average number of colonies in the two petri dishes adopted, D is the dilution ratio, and V is the volume (ml) of SCDLP broth used for washing out the bacteria.

Antibacterial activity was calculated by the following equation.

Table 1. (65- x)ZnO- x B₂O₃-35P₂O₅ Glasses Composition (mol%)

Sample No.	ZnO	B ₂ O ₃	P ₂ O ₅
ZBP 1	65	0	35
ZBP 2	55	10	35
ZBP 3	45	20	35
ZBP 4	35	30	35
ZBP 5	25	40	35

$$R = \log (B/A) \quad (2)$$

where R is the value of the antimicrobial activity, A is the average of the number of viable cells of bacteria after 24 h inoculation of the treated test piece, and B is the average of the number of viable cells of bacteria on the untreated test piece after 24 h. Conversion of antimicrobial activity values can be classified as follows: i) $\sim 1.99 = -$, ii) $2.00 \sim 2.99 = 99.0\%$, iii) $3.00 \sim 3.99 = 99.9\%$, iv) $4.00 \sim = 99.99\%$.

3. Results and Discussion

3.1. Structure analysis

Figure 1 shows the XRD patterns of the (65- x)ZnO- x B₂O₃-35P₂O₅ glasses. Patterns are only the characteristic halo pattern of amorphous material at a low diffraction angle (near $2\theta - 20^\circ - 30^\circ$); this clear ups up the idea that there is no crystal effect in this study.

Table 2 and Fig. 2 show the glass transition temperature, T_g , and the coefficient of thermal expansion, C.T.E., in the vitrification zone. The experimental results show that the glass transition temperature increases and the coefficient of thermal expansion decreases with increasing B₂O₃ content. Brow *et al.* reported that there are forms of BO₃ with three-coordinated boron atoms and BO₄ with four-coordinated boron atoms in borate glasses; their mutual participation in the structure of borophosphate glasses was investigated by B NMR analysis.¹³ Therefore, the incorporation of BO₄ units in the chain-like phosphate glass structure results in

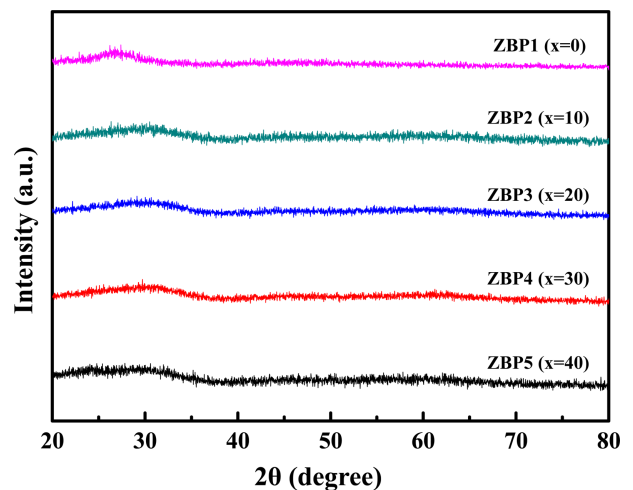


Fig. 1. XRD Patterns of (65- x)ZnO- x B₂O₃-35P₂O₅ glasses.

Table 2. Thermal Properties of (65- x)ZnO- x B₂O₃-35P₂O₅ Glasses

Sample No.	T_g (°C)	CTE ($\times 10^{-7}/^\circ\text{C}^{-1}$)
ZBP 1	421	87.86
ZBP 2	438	73.81
ZBP 3	443	79.54
ZBP 4	460	75.04
ZBP 5	471	60.31

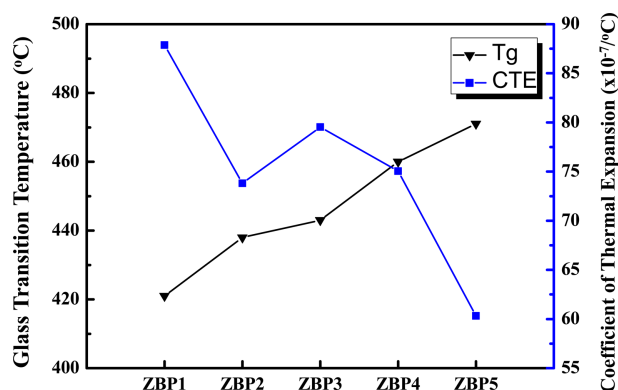


Fig. 2. Thermal properties of (65-x)ZnO-xB₂O₃-35P₂O₅ glasses.

the enhancement of dimensionality of the structural network from one-dimensional to three-dimensional. Such a cross-linking of the structural network gives glasses with increased *T_g*; the thermal expansion coefficient generally decreases with increasing content of B₂O₃. In the case of ZBP3 (20 mol% B₂O₃), the coefficient of thermal expansion increases because of boron anomalies in which BO₃ and BO₄ units are mixed. When the B₂O₃ content exceeds 20 mol%, the bridging oxygens are exhausted and B₂O₃ tends to occur in the form of planar BO₃ units.⁶

Figure 3 shows the FTIR spectra of the (65-x)ZnO-xB₂O₃-35P₂O₅ glasses. Phosphate glasses are composed of network forming, corner-sharing PO₄ tetrahedra, in which three corners are shared and a double bond forms between the phosphorus atoms and one of the four oxygen neighbors. The band in the 1200 - 1250 cm⁻¹ range is ascribed to asymmetric stretching vibration *v_{as}*(PO₂) of nonbridging oxygen and another band in the 1000 - 1080 cm⁻¹ range is assigned to the symmetric stretching vibration *v_s*(PO₂) of nonbridging oxygen in the phosphate chain. The band in the 900 - 970 cm⁻¹ range belongs to asymmetric vibrations *v_{as}*(P-O-P) of bridging oxygen and the weaker band at 700 - 800 cm⁻¹ is assigned to symmetric stretching vibration *v_s*(P-O-P) of bridging oxygen in the phosphate chains. The broad band at

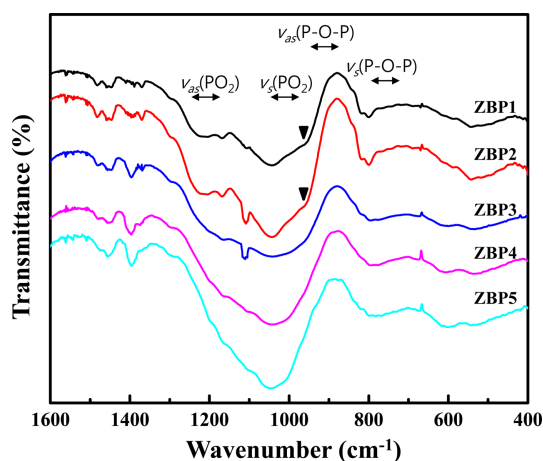


Fig. 3. FTIR Spectra of (65-x)ZnO-xB₂O₃-35P₂O₅ glasses.

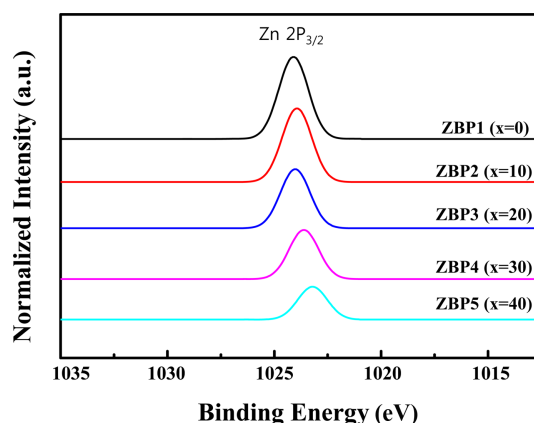


Fig. 4. XPS Spectra of (65-x)ZnO-xB₂O₃-35P₂O₅ glasses.

500 cm⁻¹ belongs to the bending vibrations of basic structural units of phosphate glasses.¹⁴ The vibration at 1000 - 1200 cm⁻¹ is the result of vibration of the B₂O₇²⁻ ionic group, which indicates that planar BO₃ units are changing into tetrahedral BO₄. With increasing B₂O₃ content, the high-frequency bands corresponding to stretching vibrations become broader and less distinct and overlap each other.⁶ The results for samples ZBP4 and ZBP5 indicate that the rapid increase in the content of planar BO₃ units weakens the glass structure because the bridging oxygens are exhausted when B₂O₃ > 20 mol%. The peak at 970 cm⁻¹, which is shown in the high ZnO content samples, results from a combined effect of Zn-O stretching vibration, [ZnO₆] characteristic absorption, and P-O-P asymmetric vibration.¹⁵

The ZnO ionic state was investigated by XPS (Fig. 4). The peak movement (1023.3 eV to 1024.2 eV) with increasing ZnO showed the same results as in a previous study; the single peak of Zn 2P_{3/2} show that ZnO exists only in the Zn²⁺ state.¹⁶ The peak intensity can be confirmed for which Zn²⁺ increases with increasing ZnO content.

3.2. Antibacterial property

Figure 5 and Table 3 show the rate of decrease of *E. coli* obtained using the JIS Z 2801 standard test. The *E. coli*

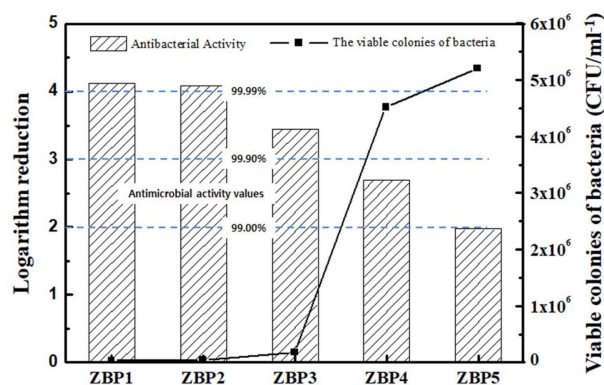


Fig. 5. Antibacterial activity and viable colonies of bacteria after 24 h contact on (65-x)ZnO-xB₂O₃-35P₂O₅ glasses.

Table 3. Antibacterial Activity of (65-x)ZnO-xB₂O₃-35P₂O₅ Glasses against *E. coli*

Sample No.	The viable colonies of bacteria before Test (CFU/ml ⁻¹)	The viable colonies of bacteria after 24 h contact (CFU/ml ⁻¹)	Antibacterial activity	Conversion of antibacterial activity
Blank	1.0×10^7	5.06×10^8	No effect	No effect
ZBP 1	1.0×10^7	3.87×10^4	4.12	99.99 %
ZBP 2	1.0×10^7	4.24×10^4	4.08	99.99 %
ZBP 3	1.0×10^7	1.81×10^5	3.45	99.90 %
ZBP 4	1.0×10^7	4.52×10^6	2.69	99.00 %
ZBP 5	1.0×10^7	5.21×10^6	1.97	-

death rate increases with increasing ZnO content of the glass and with ZBP 1 and ZBP 2; the death rate is 99.99% or more, which is superior to those of the other three samples. Padmavathy *et al.*¹⁷⁾ reported that ZnO can be activated with visible and/or ultraviolet light, generating reactive oxygen species (ROS); H₂O₂ penetrates the membrane, causing oxidative stress and the death of the cell. Xie *et al.*¹⁸⁾ suggested that ZnO has a direct effect on the membrane, modifying its permeability, which increases the ZnO concentration inside the cell, where it induces oxidative stress. Those studies state that Zn²⁺ directly induces a significant increment in intracellular reactive oxygen species (ROS) due to the Zn²⁺ interacting with the thiol-groups of the enzymes in charge of the breathing cycle of the bacteria; the result in this study also suggests that the antibacterial mechanism of ZnO is likely the oxidative stress in the *E. coli*.

4. Conclusions

The glass structure and antibacterial properties of ZnO-B₂O₃-P₂O₅ glasses were studied. B₂O₃ in the cross-linking of phosphate glass structure brought an increase in the glass value of T_g and CTE decreased with increasing B₂O₃ amount. The single peak of Zn 2P_{3/2}, obtained by XPS, showed the ZnO state: Zn²⁺ increases with increasing ZnO content. The viable colonies of bacteria decreased with increasing ZnO; ZnO-B₂O₃-P₂O₅ glass with high ZnO concentration in this work showed over 99.99% antimicrobial activity values.

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