NOTE

Transparent porous $\text{La}_2\text{Mo}_2\text{O}_9$ thin film preparation and antibacterial and antiviral activities

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Thin $\text{La}_2\text{Mo}_2\text{O}_9$ (LMO) films were prepared using wet processing with acetylacetonates as starting materials. After acetylacetonates of La and Mo were dissolved into a mixture of 2-methoxyethanol and acetylacetone, the obtained precursor solution was coated onto Pyrex glass plates by spin coating. Transparent porous LMO thin film was obtained after vacuum ultraviolet light illumination and firing at 500 °C for 1 h in ambient air. The film exhibited antibacterial and antiviral activities that were almost equivalent to those of LMO powder against *Escherichia coli*, *Staphylococcus aureus*, bacteriophage Q β , and bacteriophage Φ 6.

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Today, coronavirus (SARS-CoV-2) has spread among humans worldwide. Its infectivity and lethality pose major threats to human life. Suppression and prevention of the spread of viral infections of this kind are becoming important issues, increasingly necessitating the use of antiviral materials.

Recently, studies of inorganic antiviral materials are increasing gradually because they have low probability of causing development of resistance by viruses. Moreover, they can affect various viruses under widely various temperatures. Various inorganic antiviral materials exist: metals (e.g. Ag and Cu^{1–3}), photocatalysts (e.g. TiO₂^{4,5}), and others (e.g. ZnO, alkaline earth oxides, and polyacids^{6–9}). Protein inactivation, reactive oxygen species generation, and pH change are proposed as mechanisms of antiviral activity. Nevertheless, these materials present some shortcomings such as discoloration and activity degraded by oxidation, high cost, and restricted usage environments (requiring light illumination or alkalization). New inorganic antiviral materials must be developed to overcome these shortcomings.

Very recently, we prepared $\text{La}_2\text{Mo}_2\text{O}_9$ (hereinafter, LMO) using a polymerizable complex method. We demonstrated that this material exhibits antibacterial activity against both gram-negative (*Escherichia coli*, *E. coli*) and gram-positive (*Staphylococcus aureus*, *S. aureus*) bacteria, and antiviral activity against non-envelope type (bacterio-

phage Q β , hereinafter denoted as Q β) and envelope type (bacteriophage Φ 6, hereinafter denoted as Φ 6) viruses, simultaneously with slight cytotoxicity.^{10,11} Nutritional inhibition (La) and pH decrease (Mo) are anticipated as useful mechanisms with antibacterial activity. Polyacid adsorption at specific active sites of viruses is proposed as a mechanism of antiviral activity for this material. During usage, the color of this material is white, with little discoloration; light illumination is not necessary. The raw materials are not as expensive as Ag. Therefore, the material is attracting attention as one that can overcome shortcomings of inorganic antiviral materials that have been used to date. In fact, LMO has been studied as an ion-conductive material. Thin films of LMO have been prepared using sol-gel method,¹² sputtering,¹³ and pulsed laser deposition.^{14,15} Nevertheless, no report to date has described transparent film processing or antibacterial or antiviral activities of prepared LMO films.

Wet coating is beneficial for small-volume production of great varieties and for application to items having complicated shapes. A porous structure is expected to be ideal for films with high antibacterial and antiviral activities because of large reaction fields. Given this background, we have conducted preparation of a transparent porous LMO film by wet processing. Then the prepared film antibacterial and antiviral activities were evaluated.

All starting chemicals used for this study were reagent-grade. At the beginning of this investigation, we conducted preliminary experiments to prepare LMO films using the reported sol-gel method. Nevertheless, we were unable to

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obtain the film that we had expected because of the poor wettability of the precursor solution on the glass substrate and because of the carbonization of organic compounds during firing. Therefore, we changed our strategy and used acetylacetonates as starting materials. We performed solubility tests with various organic solvents on both lanthanum(III) acetylacetonate hydrate ($\text{La}(\text{C}_5\text{H}_7\text{O}_2)_3 \cdot x\text{H}_2\text{O}$, $\text{La}(\text{acac})_3$, Sigma-Aldrich Corp., MI, U.S.A.) and bis(acetylacetonato)dioxomolybdenum(VI) ($\text{MoO}_2(\text{C}_5\text{H}_7\text{O}_2)_2$, $\text{MoO}_2(\text{acac})_2$; Fujifilm Wako Pure Chemical Corp., Tokyo Japan). These chemicals were found to dissolve into a mixture of 2-methoxyethanol ($\text{C}_3\text{H}_8\text{O}_2$, MtxE; Fujifilm Wako) and acetylacetone ($\text{C}_5\text{H}_8\text{O}_2$, Ac; Fujifilm Wako) ($\text{MtxE}/\text{Ac} = 5\text{--}20$), although $\text{La}(\text{acac})_3$ does not dissolve into either pure solvent. Stabilization of the precursor solution, including La by the MtxE-Ac mixture, has been reported also for another system.¹⁶⁾ After detailed investigation of solid concentration and solution stability, we optimized the final molar ratio of the starting materials as $\text{MtxE}:\text{Ac}:\text{La}(\text{acac})_3:\text{MoO}_2(\text{acac})_2 = 420:35:1:1$ for the precursor solution of the wet coating. We confirmed that the pot life of the mixture solution for the film processing was at least one week at room temperature.

After mixing the starting materials in due ratio, sonication was conducted. A clear solution was obtained. After the solution was coated onto Pyrex glass plates ($5\text{ cm} \times 5\text{ cm} \times 0.5\text{ mm}$) using spin coating at 1500 rpm for 5 s, it was dried at room temperature for approx. 10 s. This coating–drying cycle was repeated five times. Vacuum ultraviolet (VUV) light was illuminated onto the film for 10 min using a Xe excimer lamp (172 nm wavelength, UEM20-172; Ushio Inc., Tokyo, Japan). The LMO film was obtained after firing the glass plate at 500 °C for 1 h in ambient air atmosphere.

The film morphology was observed using scanning electron microscopy (SEM, JSM-7500F; JEOL Co., Tokyo, Japan). The crystalline phase of the powder was identified using X-ray diffraction (XRD, X'Pert-Pro-MRP D; Philips (PANalytical), Tokyo, Japan) with $\text{Cu K}\alpha$ radiation. Film thickness was measured using a contact probe profilometer (DEKTAK 6m; Bruker Corp., MA, U.S.A.). The amount and state of functional groups of organic compounds in the film were examined using the attenuated total reflectance mode of a Fourier transform infrared spectroscope (FT-IR, FT/IR-6100; Jasco Corp., Tokyo, Japan). Transmittance of the samples in the visible wavelength range was measured using a UV–Vis spectrophotometer (UV–vis, V-660; Jasco Corp.) with the same pristine glass plate used as a reference.

Figure 1 presents FT-IR spectra of the film before and after VUV illumination. Absorbances at 1517, 1535, and 1598 cm^{-1} , which were assigned to symmetric or asymmetric stretch modes of $\text{C}=\text{O}$ bonds of the acetylacetonates used as starting materials,^{17),18)} decreased after VUV treatment. The film thickness was also decreased by approx. 15 % by this treatment. These results indicate decomposition of some chelated acetylacetone in the precursor film. Because DTA/TG analysis revealed that crystal-

Fig. 1. FT-IR spectra of the prepared film before and after VUV illumination.

Fig. 2. XRD pattern of the prepared film.

Fig. 3. Transmittance and appearance of the prepared film.

lization of the dried precursor solution occurs at temperatures (570–600 °C) higher than the firing temperature of the film (500 °C), we inferred that the film crystallization is facilitated by removal of some organic compounds from the film through the VUV treatment. Similar trends were reported also for precursor films of TiO_2 prepared from a Ti-alkoxide solution.¹⁹⁾

Figure 2 displays the XRD pattern of the obtained film. Almost all peaks in the XRD pattern were identified as $\text{La}_2\text{Mo}_2\text{O}_9$ (card No. 28-0509), suggesting almost single phase of LMO. **Figure 3** depicts the appearance and transmittance of the film against light in the visible wavelength range. The film possessed high transmittance: the value was more than 88 %. **Figure 4** presents top-view and

Fig. 4. SEM images of the prepared film: (a) top-view (b) and cross-section.

Fig. 5. Results of antibacterial and antiviral activity experiments using film adhesion against (a) *Escherichia coli*, (b) *Staphylococcus aureus*, (c) bacteriophage Q β , and (d) bacteriophage Φ 6.

cross-section SEM images of the film. The porous film included 50–300 nm pores. The microstructure was apparently of an intermediate stage of sintering for LMO particles after removal of organic compounds during firing. The film thickness was almost 200 nm. It became almost half that of the precursor film after firing at 500 °C for 1 h. The film adhered onto the glass plate with a certain degree of strength: it did not peel easily during rubbing with fingers.

We evaluated antibacterial and antiviral activities of the film using procedures described for our earlier studies.^{10),11),20)} The test was conducted using the entire film on a 5-cm-square glass substrate. A pristine glass plate or a PP film of the same size was used for control data. Before the evaluation, disinfection treatment was conducted at 80 °C for 15 min for all samples. The antibacterial test and the antiviral test were performed respectively using the film adhesion methods described in JIS R 1702 (ISO 27447) and JIS R 1706 (ISO 18061), with minor modifications. For this study, we used *E. coli* (NBRC 3972) and

S. aureus (NBRC 12732) to evaluate antibacterial activity. For antiviral activity measurements, we used two bacteriophages, a virus whose host is a bacterium: Q β (NBRC 20012) and Φ 6 (NBRC 105899).

The activity test results are displayed in Fig. 5. We also present results for LMO powders reported by Matsumoto et al.¹⁰⁾ The y-axis in the figure shows the logarithm of the number of colonies for viable bacteria or that of plaques for virus. Values of both the *E. coli* and *S. aureus* dropped below the detection limit in 2–4 h. The titer of Q β also reached the detection limit during 6 h. That of Φ 6 decreased by two orders in 6 h. Activity against Φ 6 was found to be inferior to that against Q β . The reason for the tailing trend of Φ 6 against time for LMO remains unclear at this stage. We extended the measurement time to 24 h and confirmed that the number (*N*) of Φ 6 continued to decrease gradually even at 24 h. Very recent studies revealed that antiviral activity against Φ 6 can be improved by substituting Ce for La in LMO.^{11),20)} We can infer that Ce

is more effective for Φ6 than La is; however, finding can only be characterized as a characteristic of Φ6 at this time. Investigation of this reason remains as a subject to be addressed in future work. However, all these results and trends correspond to those of LMO powder in work reported by Matsumoto et al., suggesting that prepared transparent porous LMO films possess antibacterial and antiviral activities that are almost equivalent to those of LMO powder.

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