Effects of pH on the Crystallinity and Chemical Properties of Gallium Oxyhydroxide

BACKGROUND RESEARCH

Gallium oxide (Ga_2O_3) and its precursor, gallium oxyhydroxide (GaOOH), are wide bandgap semiconductors known for their optical and physical properties. Nano and microparticles of these versatile inorganic materials are used in gas sensors, drug carriers, UV detectors, and optoelectronic devices (Luo et al., 2007). β-Ga_2O_3(Figure 1b) is the most common crystalline form of Ga_2O_3 (Shi et al., 2021). GaOOH (Figure 1a) is a precursor to the synthesis of Ga_2O_3. GaOOH crystals exhibit a variety of morphologies and shares similar semiconducting properties with Ga_2O_3. The morphologies of GaOOH particles are influenced by the chemical composition of the growth precursors and synthesis methods employed.

Synthesis techniques such as chemical vapor deposition, sol-gel, and pulsed laser deposition, can produce Ga_2O_3 and GaOOH, but the hydrothermal method stands out for its cost-effectiveness and precise control over time, temperature, precursor concentrations, and pH (Zhang et al., 2005). The hydrothermal method involves using a high-pressure environment to dissolve and crystallize materials in an aqueous solution. It utilizes an autoclave for nucleation. Subsequent cleaning and drying steps remove extraneous matter and excess liquids.

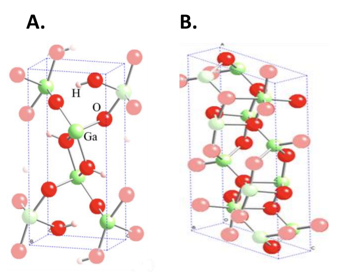

Figure 1. **Crystal structure of a) orthorhombic structure of GaOOH b) monoclinic structure of β-Ga_2O_3.** Source of image (Ichimuraz, 2023)

Previous studies on zinc oxide synthesized using hydrothermal methods have revealed that pH impacts the dimensions and optical properties of zinc oxide microparticles (MPs) (Torkamani et al., 2022). This study uniquely focuses on GaOOH since it has not been studied in terms of antibacterial properties. The project investigates the influence of pH on GaOOH MPs chemical, crystalline, and optoelectronic properties including morphology and defect density and their relationship to antibacterial properties.

In response to the increasing threat of bacterial antibiotic resistance, understanding the role of growth pH in the antibacterial properties of nano and microscale particles becomes crucial. While Ga_2O_3 particles ae known for their antibacterial efficacy, there is limited research available on the antimicrobial properties of GaOOH. The project's purpose is to synthesize GaOOH MPs with high crystallinity and uniformity by manipulating pH values during growth. Subsequently, the antibacterial activity of crystals formed at different pH values will be tested against both Gram-positive and Gram-negative bacteria to discern any correlation with pH-dependent morphology.

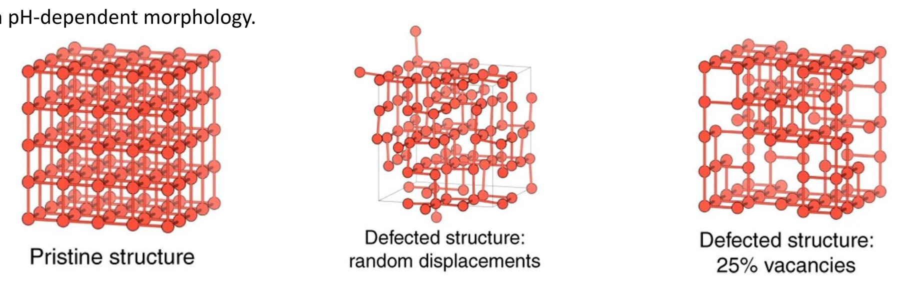

Figure 2. **Images of Pristine surface and different severity cases of crystal structures.** Source of image (Ziletti et al., 2018)

QUESTION & HYPOTHESIS

QUESTIONS

1. How does pH influence the chemical structure and crystallinity of gallium oxyhydroxide MPs?
2. Does the chemical structure and crystallinity of gallium oxyhydroxide MPs influence their antibacterial action?

HYPOTHESES

1. Modulating the pH during the growth of gallium oxyhydroxide MPs to a more acidic environment can create microscale gallium oxyhydroxide particles with high-quality and uniform surfaces.
2. Gallium oxyhydroxide MPs with more defect sites will have greater bacterial inhibition. This is supported by previous studies on zinc oxide, which have shown that defects act as interaction sites for bacteria.

VARIABLES

In the hydrothermal synthesis experiment, the independent variable was the pH the GaOOH microparticles (MPs) were created. The dependent variables were the average length of the microparticles, second Ga-OH bending band position, first Ga-OH bending band position, and optoelectrical properties (relative intensity of defect emission compared to bandgap emission).

In the antimicrobial experiment, the independent variable was the pH during the formation GaOOH particles, and the dependent variable was cell growth measured through optical density.

MATERIALS

The following were used to make gallium oxyhydroxide: 1.2789 grams of Gallium Nitrate Salt, 50 mL of Deionized water, 30 mL of Ammonium Hydroxide, hot plate, stirring rod, plastic pipettes, steel autoclave, Teflon container, AI stabletemp forced air driving oven, centrifuge, and sonicator.

The following were used for Photoluminescence (PL): Kimmon IK He CD Helium Cadmium Laser, Horiba Jobin Yvon T 64,000 triple Raman spectrometer, and Janis CCS150 cryostat.

The following were used for SEM/EDX: JEOL FE-SEM, aluminum scanning electron microscopy (SEM) mounts, and carbon tape.

Fourier Transform Infrared Spectroscopy (FTR): ATR-FTIR spectrometer.

The following were used for antibacterial studies: Dimethyl sulfoxide, 1X Phosphate Buffered Saline, pipette, pipette tips, Spectrophotometer (Carolina model #64-3303), Mini Centrifuge (Costar), *Escherichia coli* (ATCC 8739) Supplier, VWR International, LLC cat 89504-606, and *Staphylococcus aureus* (ATCC 25923) - Supplier, VWR International, LLC cat 89502-706.

The following were used for analyzing data: ImageJ and Microcal Origin.

WORKS CITED

1. Bae, Hyun, et al. "High-aspect ratio β-Ga_2O_3 nanorods via hydrothermal synthesis." *Nanomaterials*, vol. 8, no. 8, 2018, p. 594, https://doi.org/10.3390/nano8080594.
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5. Liu, Guocong, et al. "Preparation and photoluminescence properties of EU-doped Ga_2O_3 nanorods." *Materials Chemistry and Physics*, vol. 110, no. 2–3, 2008, pp. 206–211, https://doi.org/10.1016/j.matchemphys.2008.02.012.
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10. Ziletti, Angelo, et al. "Insightful classification of Crystal Structures Using Deep Learning." *Nature Communications*, vol. 9, no. 1, 2018, https://doi.org/10.1038/s41467-018-05169-6.

All photos, images, and graphics were created by the researcher, except for Figures 1, 2, and 4 whose citations are provided in the Works Cited.

DATA

SEM Images

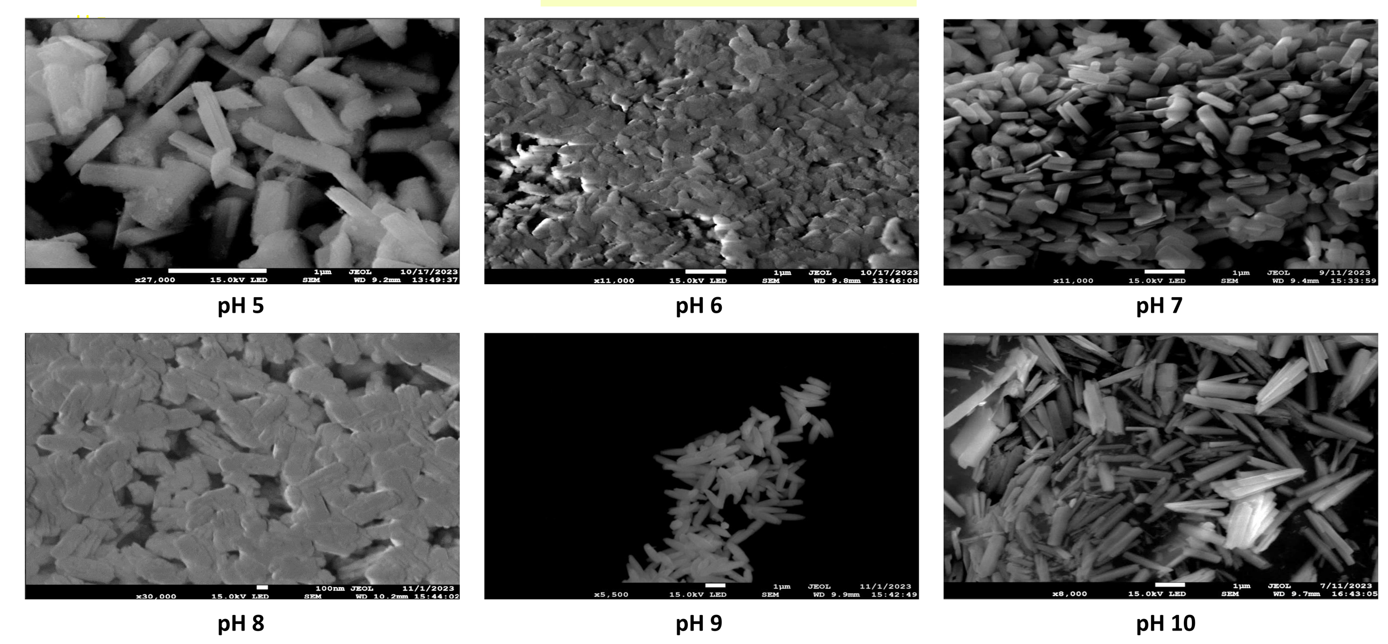


Figure 6. **SEM Images showing effect of pH on GaOOH MPs morphologies.**

SEM Analysis

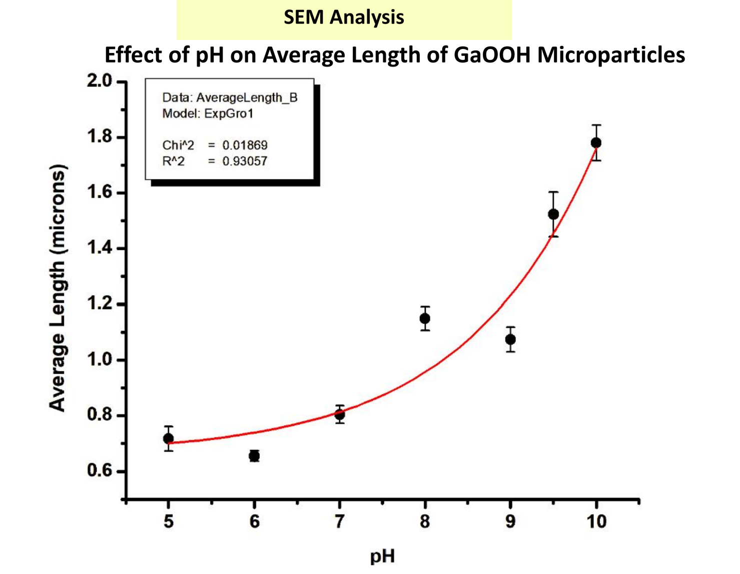


Figure 7. ImageJ software was used to measure the average length of particles (a sample size of 20 was used). This was plotted against pH, and exponential regression was performed.

PL Analysis

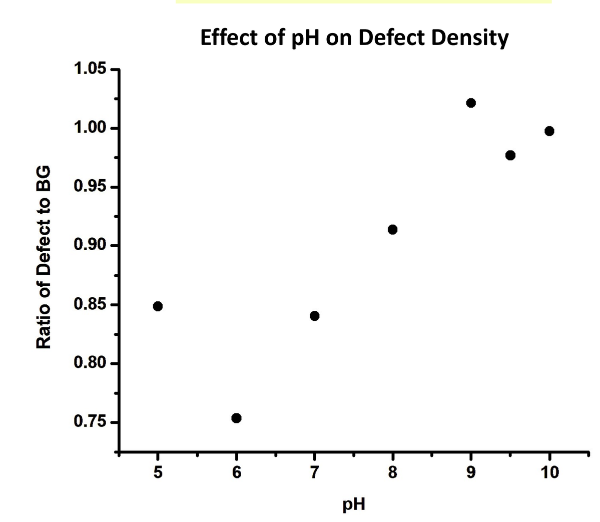


Figure 8. Photoluminescence spectra were plotted in Origin, and a ratio of the intensity of defect emission to band gap emission was taken. This was plotted against pH.

Table 1. **Statistical Analysis of Photoluminescence Spectroscopy Data**

Group 1	Group 2
pH 10	pH 5
pH 10	pH 7
pH 10	pH 8
pH 10	pH 6
pH 5	pH 8
pH 5	pH 9.5
pH 5	pH 6
pH 5	pH 9
pH 7	pH 8
pH 7	pH 9.5
pH 7	pH 6
pH 7	pH 9
pH 8	pH 6
pH 8	pH 9
pH 9.5	pH 6
pH 6	pH 9

One-way ANOVA with Tukey's multiple comparison test was used for analysis with p < .05. The pairs above are significantly different from each other.

ATR-FTIR Analysis

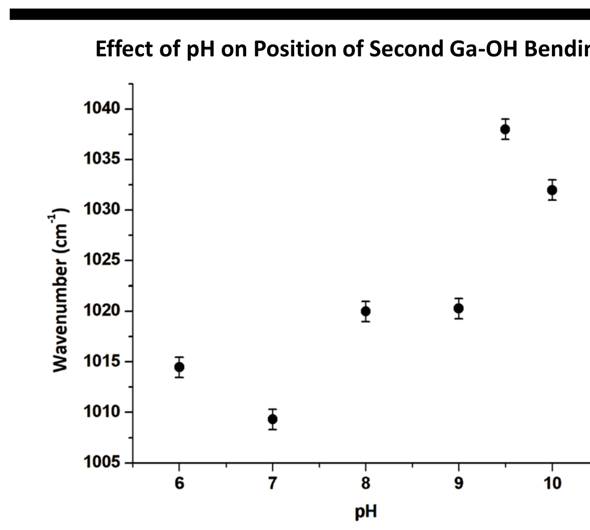


Figure 9. The FTIR transmittance spectra were plotted in Origin. The position of the second Bending Band (around 1027.1 cm^{-1}) was plotted against pH.

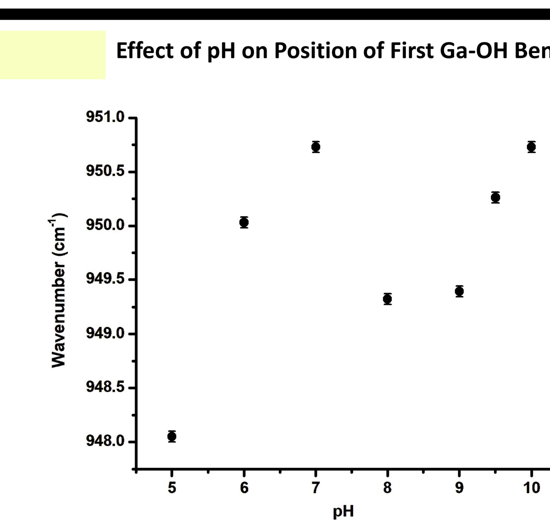


Figure 10: The FTIR transmittance spectra were plotted in Origin. The position of the first Bending Band (around 951.2 cm^{-1}) was plotted against pH.

Antibacterial Activity

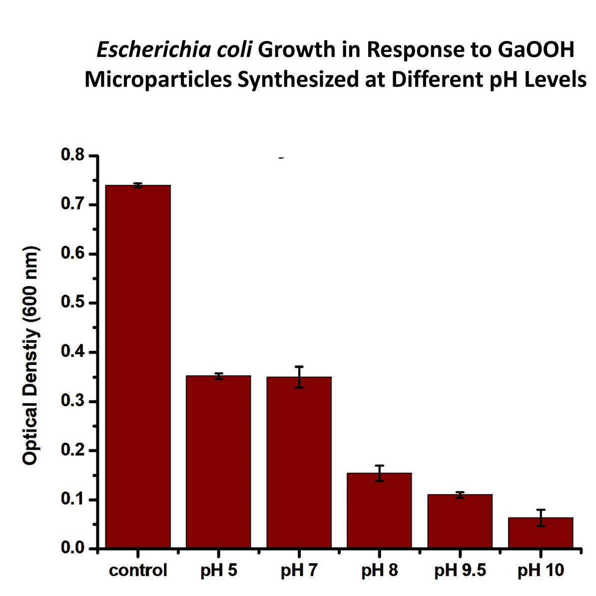


Figure 11. **Antibacterial Activity of GaOOH MPs**. Bacterial growth of Gram-negative bacteria was monitored by measuring the OD at 600 nm. Bacterial growth was compared with untreated bacterial control cultures for three trials.

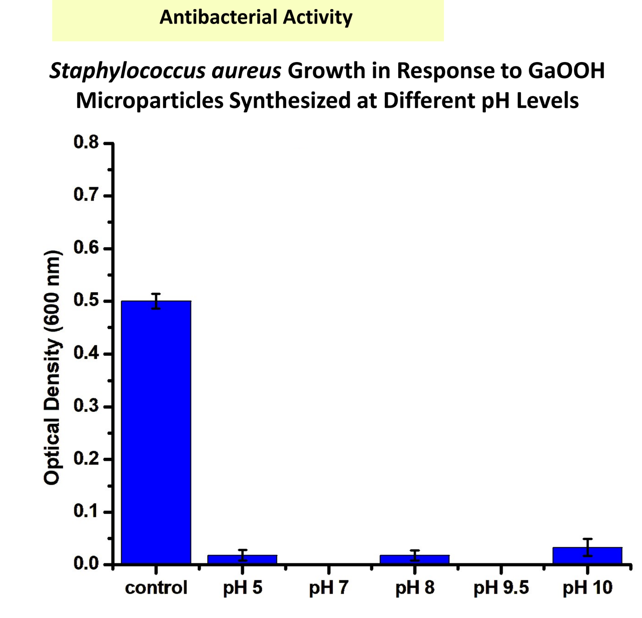


Figure 12. **Antibacterial Activity of GaOOH MPs**. Bacterial growth of Gram-positive bacteria was monitored by measuring the OD at 600 nm. Bacterial growth was compared with untreated bacterial control cultures for three trials.

Table 2. **Statistical Analysis of Antibacterial Activity of GaOOH MPs.**

Escherichia coli

Group 1	Group 2
Control	pH 10
Control	pH 5
Control	pH 7
Control	pH 8
Control	pH 9.5
pH 10	pH 5
pH 10	pH 7
pH 10	pH 8
pH 5	pH 8
pH 5	pH 9.5
pH 7	pH 8
pH 7	pH 9.5

Staphylococcus aureus

Group 1	Group 2
Control	pH 10
Control	pH 5
Control	pH 7
Control	pH 8
Control	pH 9.5
pH 10	pH 7
pH 10	pH 9.5

One-way ANOVA with Tukey's multiple comparison test was used for analysis with p < .05. The pairs above are significantly different from each other.

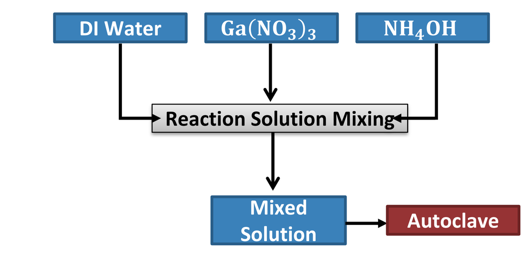
RESULTS

- **SEM Analysis:**
  - Lower pH created more uniform surfaces of GaOOH MPs, as revealed by SEM analysis (Figure 6).
- **pH Impact on Particle Characteristics:**
  - pH influenced the size, chemical structure, and crystallinity of GaOOH MPs.
  - Superlinear correlation observed between pH and average particle length (Figure 7).
- **Photoluminescence Analysis:**
  - Higher pH increased the relative intensity of defect emission to bandgap emission (Figure 8).
    - Indicates that higher pH samples have greater defect density, suggesting lower crystal quality.
- **Fourier Transform Infrared Spectroscopy (FTIR) Analysis:**
  - Trend observed with the bending band around 1027 cm^{-1} (Figure 9).
  - No clear trend found for the other band (Figure 10).
- **Bacterial Growth Inhibition:**
  - GaOOH MPs grown at higher pH inhibited growth of *Escherichia coli* more than lower pH samples (Figure 11).
  - No clear correlation was observed with *Staphylococcus aureus* at the 0.25 mg/mL, likely due to significant bacterial death (Figure 12).

PROCEDURES

Growth of Gallium Oxide Crystals

1. Measured 1.2789 grams of gallium nitrate salt using an electronic scale.
2. Dissolved the salt in 50 mL of DI water using a magnetic stirrer.
3. Heated the sample on a hot plate to 60°C.
4. Measured pH.
5. Added small amounts of ammonium hydroxide until a target pH was reached.
6. Heated and mixed the sample at 60°C for 2 hours.
7. Placed sample in oven at 140°C for 10 hours.



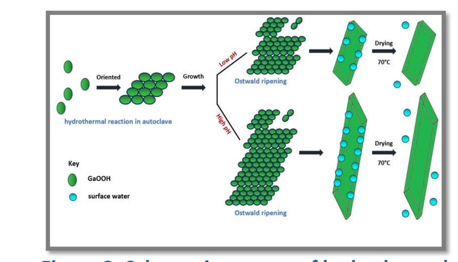


Figure 3: Schematic process of hydrothermal synthesis of GaOOH particles.

Cleaning and Drying of Gallium Oxide Crystals

Once the samples were done growing in the oven, they were cleaned using the following procedure.

1. Poured solution from Teflon container to two test tubes then centrifuged.
2. Removed excess liquid and replaced it with sample left from Teflon container.
3. Filled test tubes with deionized water (DI) water, centrifuged, and removed excess liquid.
4. Step 3 three was repeated three times.
5. Sonicated the test tubes for 5 minutes and then centrifuged.
6. Step 3 three was repeated three times.
7. Loaded samples from test tubes onto two glass plates.
8. Placed glass plates in oven at 70°C for 6 hours.

Analysis of Crystal Structure

1 The samples were probed at an operating voltage of 15 kV with a JEOL FE-SEM.
2. The SEM scans were uploaded into ImageJ, an image processing system developed by the National Institute of Health and the Laboratory for Optical and Communication Instrumentation.
3. Dimensions of the particles were taken using ImageJ software.

Analysis of Optoelectronic Properties using Photoluminescence

Samples were excited at a wavelength of 325 nm with a Kimmon IK HeCd CW laser and an optical train. The resulting signal from the laser was collected by a Horiba Jobin Yvon T64000 Triple Raman Spectrometer with a Synapse CCD.

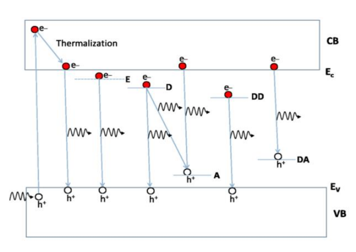


Figure 4. **Photoluminescence.** The electrons in the valence band are excited to energy states in the conduction band. In order to minimize energy, the electrons transmit back to the valence band and emit a photon (Davis, 2014).

Antibacterial Studies

GaOOH MPs at varying pH were dissolved in DMSO (5 mg/mL). *Staphylococcus aureus* and *Escherichia coli* were cultured in 5 mL nutrient broth for 24 hours. Then, 250 μL of GaOOH-DMSO solution (5 mg/mL) was added to 2.5 mL nutrient broth and 2.25 mL 1X PBS to achieve a final GaOOH concentration of 0.25 mg/mL. Tubes were inoculated with 25 μL of pre-cultured bacteria, mixed at 200 RPM, and incubated at 37°C for 17 hours. Growth was halted in an ice bath, followed by centrifugation at 10,000 RPM for 60 seconds to pellet GaOOH particles. A spectrophotometer recorded absorbance values at 600 nm after calibrating with a blank solution (2.5 mL media, 250 μL DMSO, and 2.5 mL 1X PBS). Absorbance values at 600 nm indicate bacterial growth, allowing assessment of GaOOH MPs' inhibitory effects on growth.

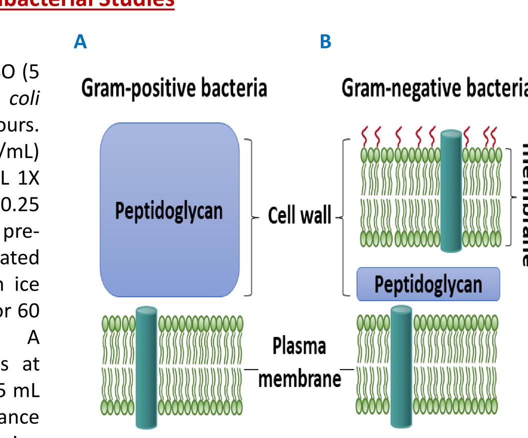


Figure 5. Gram-positive and Gram-negative bacteria differ in their cell walls.

CONCLUSION

SEM and ImageJ Analysis:
- The hydrothermal method produced GaOOH MPs with diverse morphologies.
- Lower pH values correlated with higher crystallinity and uniformity.
- Smooth surfaces and absence of clusters observed in lower pH samples.

Photoluminescence Analysis:
- Lower pH samples showed lower defect density.
- Higher pH samples increased defect density due to more rapid chemical reactions.

FTIR Analysis:
- No significant trend was found at bending band around 951.2 cm^{-1} but observed at bending band around 1027.1 cm^{-1}. Ga-OH bond changes with pH and morphology.

Overall hypothesis supported: Lowering pH enhances uniformity and crystallinity of GaOOH MPs.

Bacterial Studies:
- GaOOH inhibited the growth of *Staphylococcus aureus,* a Gram-positive bacterium, more than of *Escherichia coli,* a Gram-negative bacterium.
- GaOOH grown at higher pH more effected inhibited *Escherichia coli* growth.
- Similar morphology and effects on bacteria were observed for samples at pH 5 and 7.

This study shows a correlation between pH and the morphology of GaOOH MPs. Morphologies with a higher defect density had a more potent inhibitory effect on the growth of *Escherichia coli*. Furthermore, GaOOH MPs may have more effectively inhibited the growth of *Staphylococcus aureus,* a Gram-positive bacterium, in comparison to *Escherichia coli,* which is Gram-negative. This difference could stem from the structural differences in their cell walls. Gram-negative bacteria have an outer membrane containing molecules that restrict the entry of external substances. On the other hand, Gram-positive bacteria lack this outer membrane and have a thicker peptidoglycan layer, making their cell walls more susceptible to damage by substance like GaOOH.

Future Studies

This project analyzed only the effect of pH on the properties of gallium oxyhydroxide. However, many more parameters, such as time, temperature, and concentrations of the precursors, could affect the morphology of GaOOH MPs. Combined with the results from this experiment, a sample with very high crystallinity can be created. Further testing on bacteria with varying morphologies can be done to better understand the impact of morphology on antibacterial processes.

RELEVANCE & APPLICATION

Antibiotic resistance is a major health concern in treating bacterial infections worldwide. This resistance limits treatment options for many bacterial infections. GaOOH, which is inexpensive, bio-safe, and widely accessible, presents a promising alternative to antibiotics. Studying the effects of different pH levels on the morphology of GaOOH particles (Figure 7), and subsequently, the impact of these morphologies on bacterial growth, can provide insights into the mechanism behind GaOOH's inhibition of bacterial growth.

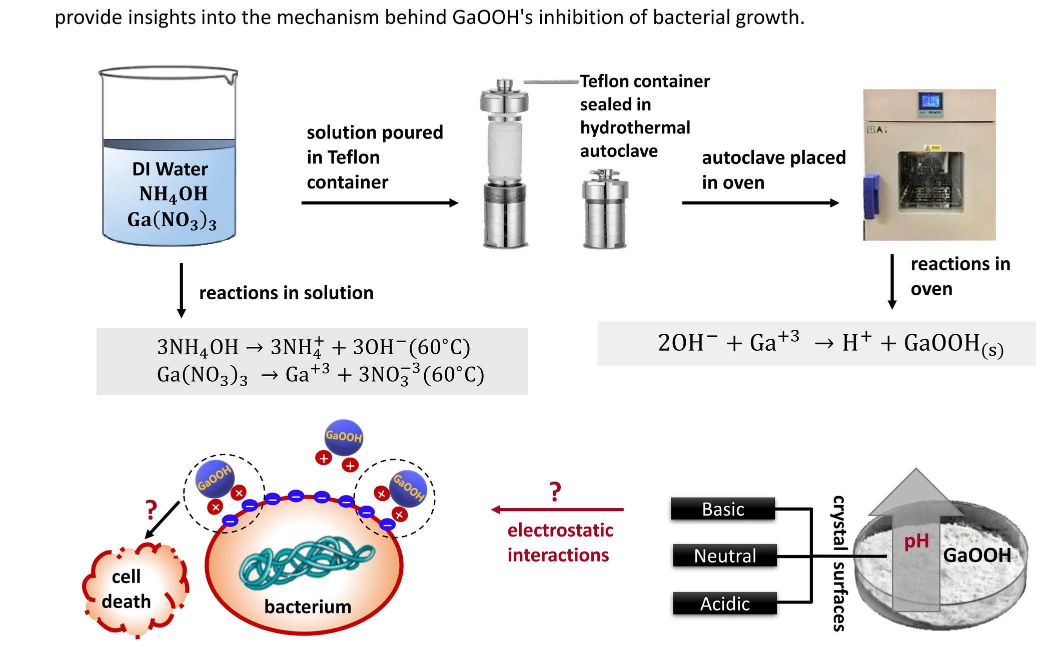


Figure 13. **Synthesis of GaOOH microparticles, and the proposed mechanism by which antibacterial effects are induced.**