Effect of surfactants on anti-\textit{Escherichia coli} ability of MgO nanoparticles

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Abstract. MgO nanoparticles (MgO NPs) were obtained by a simple hydrothermal-calcination method. The influence of three different surfactants on the surface morphology, particle size and antibacterial performance of MgO NPs was investigated. The SEM results indicated that compared with using benzoic acid and citric acid, the MgO sample has more regular flake morphology and well dispersion, moreover, its particle size has more small and uniform when EDTA-2Na was used as the surfactant. The colony-forming unit count and optical density (OD) measurement results suggested that MgO NPs prepared using EDTA-2Na had the best antibacterial activity against \textit{Escherichia coli} (\textit{E. coli}), which might be originated from the small particle size and low aggregation. Our study demonstrated that using proper surfactant was a key way during the synthesis process to control the size and morphology of MgO NPs and enhance its antibacterial efficiency, and this way was expected to be applied to other inorganic antibacterial agents.

1. Introduction

MgO nanomaterials as the inorganic antibacterial agents have attracted extensive attention because of non-toxicity, high stability and cheap price, etc. For the first time in 1995, Sawal et al. found that MgO could cause the death of \textit{E. coli} [1]. Subsequently, a number of works about the antibacterial mechanism of MgO have been reported. On the one hand, rich defects (such as oxygen vacancies) on MgO surface could effectively improve the antibacterial activity [2-4]. Many studies have shown that the content of surface defects has increased with a decrease in particle size, which was advantageous to induce the formation of ROS and kill bacteria [5-7]. On the other hand, the bacteria-MgO contact mechanism has also been confirmed [8, 9]. The relatively small particle size of MgO with good dispersion was liable to contact and penetrate the bacterial membrane, leading to the inactivation of bacteria [10].

Based on these mechanisms, the particle size of MgO as a crucial role has been reported to significantly improve its antibacterial performance. For example, Cui et al. found that Cu doping was a key strategy to diminish the size of MgO and enhance its antibacterial ability due to the increase of surface defects [5]. Huang et al. proposed that controllable particle size of MgO could be achieved via regulation of different precipitators. The result indicated that MgO with a small particle size exhibited high antibacterial activity [6]. Sundrarajan et al. reported that 30-130 nm of MgO NPs were prepared by a wet chemical method under different calcination temperatures. The results further confirmed that small size was beneficial for the enhancement of antibacterial property [7]. However, little has been reported about the effect of different surfactants on particle size, morphology and antibacterial property of MgO NPs.
Herein, MgO NPs were prepared by a facile hydrothermal-calcination method using three different surfactants (EDTA-2Na, benzoic acid and citric acid). The morphologies and particle sizes of MgO samples were characterized by a scanning electron microscope (SEM). The effect of different surfactants on the antibacterial property of MgO NPs was also investigated by treating *E. coli* (ATCC 25922).

2. Experimental section

![Flowchart](image)

Fig. 1. The preparation process of MgO nanoparticles.

In this work, all chemical reagents were obtained from Tianjin Guangfu Fine Chemical Research Institute (China). The precursors of MgO NPs were prepared as described elsewhere [11]. As shown in Fig. 1, the precursors were synthesized with MgCl₂·6H₂O, NH₃·H₂O and different surfactants as raw materials by hydrothermal method and then MgO samples were obtained by calcining the precursors in air at 450 °C for 4 h. The MgO samples prepared using the EDTA-2Na, benzoic acid and citric acid were named as MgO-E, MgO-B and MgO-C, respectively. The surface morphologies and sizes of MgO samples were observed by SEM at an accelerating voltage of 5 kV (Hitachi S-4800, Japan).

![Flowchart](image)

Fig. 2. The antibacterial process.
To evaluate the antibacterial ability of MgO samples against *E. coli*, the colony-forming unit counts method was used [12] and the detailed experiment procedures were illustrated in Fig. 2. The antibacterial ratio was calculated according to the following equation (1):

\[
\text{Antibacterial ratio (\%) = (A-B)/A \times 100\%}
\]  

(1)

where A and B are the surviving number of bacterial colonies corresponding to the negative control and the MgO sample group, respectively. Besides, the *E. coli* growth curve was also determined by using the optical density (OD) method. The OD measurements were achieved by a spectrophotometer (Shimadzu UV-2600, Japan) at 600 nm [13, 14]. And all biological reagents were obtained from Beijing Aooboxing Biotechnology Co., Ltd. (China).

3. Results and discussion

![Fig. 3. The SEM images of MgO samples.](image)

According to the SEM image in Fig. 3, it was obvious that when benzoic acid was added as the surfactant, the flake-like MgO samples whose particle sizes were in the range of 400-500 nm were prepared. Compared to MgO, the SEM image of MgO clearly showed that small-sized MgO samples were uniformly dispersed flake-like particles with a diameter of about 200-300 nm. Interestingly, the morphology of MgOC was irregular with the complex needle structure when citric acid was used as the surfactant. Meanwhile, there was the existence of agglomeration for MgOC samples. Thus, EDTA-2Na as the surfactant, the particle size could be controlled successfully, which would effectively keep MgO NPs from aggregation.

| Samples | Morphology | Size (nm) | Antibacterial ratio (%) |
|---------|------------|-----------|------------------------|
| MgOE    | flake-like | 200-300   | 72.7                   |
| MgOB    | flake-like | 400-500   | 32.1                   |
| MgOC    | needle-like| aggregation| 62.2                   |
Fig. 4. Surviving cells of *E. coli* (diluted 10^5 times, 10^8 CFU/mL) on LB solid medium: (a) negative control, (b) MgOE, (c) MgOB, (d) MgOC at 100 μg/mL concentration and (e) the antibacterial ratio.

As shown in Fig. 4, there were a large number of *E. coli* colonies on LB solid medium of negative control without treatment, from which could be concluded that the bacteria had the normal growth activity. The *E. coli* colonies on the other solid mediums of MgOE, MgOB and MgOC were less than that of negative control. The results indicated that MgOE, MgOB and MgOC had a certain antibacterial activity against *E. coli* and showed that the drop in the antibacterial performance was in the following order: MgOE > MgOC > MgOB (in Table 1). Meanwhile, the growth curves of *E. coli* were measured. In Fig. 5, MgOE was the most efficient at inhibiting the *E. coli* growth, closely followed by MgOC, while MgOB was the least antibacterial efficient, which was in good agreement with the results of colony-forming unit counts. These results further demonstrated that compared with MgOB and MgOC, the MgOE prepared with EDTA-2Na ranked best in the antibacterial activity, which resulted from the small particle size and low aggregation.
Fig. 5. The growth curves of the *E. coli* exposed to 300 μg/mL different MgO samples in the LB liquid medium.

As the particle size of MgO particles decreased, abundant surface defects were formed on surface, which led to the production of active oxygen [2]. These active materials have the strong inhibiting effect on bacterial activity based on free radical oxidative damage, improving the antibacterial efficiency [15-19]. On the other hand, the small-scale MgO_E were more likely to contact with bacterial, which resulted in more effective destruction of the membrane of bacteria, leading to the death of bacteria [20-24]. Therefore, the MgO_E samples had a high antibacterial performance in comparison with MgO_B and MgO_C, which might be attributed to the small size. However, there might be a very complex interaction between MgO and *E. coli*. The relationship between the application of surfactants and the exact antibacterial mechanism of MgO NPs needs further investigation and perfection.

4. Conclusions
In summary, MgO NPs were successfully synthesized by a hydrothermal-calcination method using three different surfactants. Among which, the more regular flake-like morphology and small size of MgO_E without large agglomerations were well controlled by using EDTA-2Na as the surfactant. The antibacterial results indicated that MgO NPs prepared with EDTA-2Na had the highest antibacterial ratio (72.7%) in comparison with other samples prepared using benzoic acid (32.1%) and citric acid (62.2%). The reason for the high activity of MgO NPs was closely related to their small particle size and high dispersion. This simple preparation method by using an appropriate surfactant to enhance the antibacterial ability of MgO nanoparticles might promote the application in designing inorganic antibacterial materials in the nearest future.

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Conflict of Interest
The authors declare no conflict of interest.

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