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Preparation of highly dispersed MgO and its bactericidal properties

L. Huang, D.-Q. Li, D.G. Evans, and X. Duan[a]

Key Laboratory of Science and Technology of Controllable Chemical Reactions, Ministry of Education, Beijing University of Chemical Technology, Beijing 100029, P.R. China

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Abstract. Samples of layered double hydroxides (LDHs) were prepared by a method involving separate nucleation and aging steps recently developed in our laboratory, using varying $[Mg^{2+}]/[Al^{3+}]$ ratios and different aging conditions. The samples were characterized by X-ray diffraction (XRD), FT-IR spectroscopy and laser granulometry. The results showed that LDHs with different particle sizes could be obtained by controlling the reaction temperature and degree of supersaturation. Calcination of these materials affords mixtures of highly dispersed MgO and mixed metal oxides. Bactericidal experiments against *Bacillus subtilis var. niger* and *Staphylococcus aureus* were carried out using materials formed by calcination of the LDHs at 500 °C. The mechanism of bactericidal activity was also investigated. It is known that MgO is very readily hydrated and that reaction with dissolved oxygen affords superoxide anions O_2^-, which attack the secondary amide structure of proteins leading to destruction of the bacteria. The bactericidal activity of the MgO increases with specific surface area because this leads to an increased number of surface hydroxyl groups and higher concentrations of O_2^- in solution. The bactericidal ability of MgO therefore increases with decreasing particle size.

PACS. 81.07.-b Nanoscale materials and structures: fabrication and characterization

1 Introduction

Layered double hydroxides (LDHs) are a well-known type of anionic clay and have extensive anion exchange properties. Calcination of LDHs affords mixed metal oxides having high surface area and degree of dispersion which have been widely used in catalysis [1,2]. There have been relatively few studies of the use of these materials for sterilization however. Recently it has been found that the superoxide ion O_2^- produced by MgO in aqueous suspension can kill bacteria very effectively and thus shows high sterilization activity [3]. It is therefore of interest to investigate the bactericidal activity of related materials produced by calcination of LDHs.

LDH samples with Mg^{2+}/Al^{3+} ratios of 2, 3 and 4 having different particle sizes were prepared by methods involving separate nucleation and aging steps and their calcination products used to treat *Bacillus subtilis var. niger* and *Staphylococcus aureus*. The effect of particle size on sterilization activity and the relationship between the degree of dispersion of MgO in the calcined materials and sterilization performance were investigated.

[a] e-mail: duanx@mail.buct.edu.cn

2 Experimental

2.1 Materials

$Mg(NO_3)_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$, NaOH and Na_2CO_3 were all A.R. grade reagents. *Bacillus subtilis var. niger* (spore, ATCC9372) and *Staphylococcus aureus* (bacterium, ATCC6538) were obtained from the Chinese Center for Disease Control and Prevention in the form of slice packages prepared according to international standards, with a bacteria or spore number of 10^6 cfu/piece (cfu = colony forming units).

2.2 Preparation of calcined LDHs with different particle sizes

Samples of calcined LDHs are described by the notation n-X, where n represents the $[Mg^{2+}]/[Al^{3+}]$ ratio in the precursor solution and X represents the synthesis method (A, B, C or D) as described below

Method A: aqueous mixtures of $Mg(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ with $[Mg^{2+}]/[Al^{3+}]$ ratios of 2, 3 or 4 were prepared. Mixed alkali solutions containing NaOH and Na_2CO_3 were also prepared. The solutions were mixed rapidly following our published procedure involving separate nucleation and aging steps [4–6] such that

$[OH^-]/([Mg^{2+}]+[Al^{3+}]) = 1.6$ and $[CO_3^{2-}]/[Al^{3+}] = 2.0$. The resulting suspensions were aged at 60 °C for 6 h and dried at 70 °C for 24 h. Samples 2-A, 3-A and 4-A were obtained by calcining the resulting LDHs for 4 h at 500 °C.

Method B: samples of LDHs were also obtained by a modification of method A in which the degree of supersaturation during the aging process was varied [7]. Samples 2-B, 3-B and 4-B were obtained by calcining the resulting LDHs for 4 h at 500 °C.

Method C: calcined samples 2-C, 3-C and 4-C were obtained following the procedure for method A except that the mixtures were aged at 100 °C for 6 h.

Method D: LDHs were prepared using non-equilibrium aging conditions [8] and subsequently calcined for 4 h at 500 °C to afford samples 2-D, 3-D and 4-D.

3 Bactericidal tests

Samples of calcined LDHs (0.50 g) prepared by different methods were contacted with *Bacillus subtilis var. niger* and *Staphylococcus aureus* at 37 °C for 24 h. The number of living spores or living bacteria was counted after follow up cultivation for 48 h, and the bactericidal efficiency was calculated.

4 Analysis and characterization

Powder XRD patterns were recorded on a Shimadzu XRD-6000 X-ray powder diffractometer (Cu Kα radiation, $\lambda = 0.15406$ nm) between 3° and 90°. The scan speed was 5°/min. The particle size distribution was measured using a Malvern MS-2000 laser particle size analyzer. FT-IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer (as KBr discs, with a weight ratio of sample to KBr of 1:100).

5 Results and discussion

5.1 Preparation of calcined LDHs with different particle sizes

LDH samples were prepared by four methods A-D involving separate nucleation and aging steps with different aging conditions, using precursor solutions with $[Mg^{2+}]/[Al^{3+}]$ ratios equal to 2, 3 and 4. The XRD patterns of the LDHs prepared with $[Mg^{2+}]/[Al^{3+}] = 2$ are shown in Figure 1; the peak intensities and values of FWHM (Full Width at Half Maximum) were found to vary with the synthesis method.

Temperature is a key factor affecting crystal growth [9]. LDH samples 2-A and 2-C were prepared with aging temperatures of 60 and 100 °C respectively As shown in Table 1, the crystallite size in both *a*- and *c*-directions, as calculated using the Scherrer equation [10], increases with aging temperature. Supersaturation is another important factor in crystal growth. Crystal growth increases with increasing supersaturation. Adding water decreases the degree of supersaturation, resulting in smaller crystallites. Accordingly sample 2-B, which was prepared by adding water to the aging mixture, has a small particle size as shown in Table 1. Using method D involving non-equilibrium aging conditions, LDHs with relatively large crystallite size can be prepared (sample 2-D in Tab. 1).

Samples of LDHs prepared by the above methods were calcined at 500 °C. As shown in Figure 2, the XRD patterns of the resulting materials correspond to those of poorly crystalline MgO.

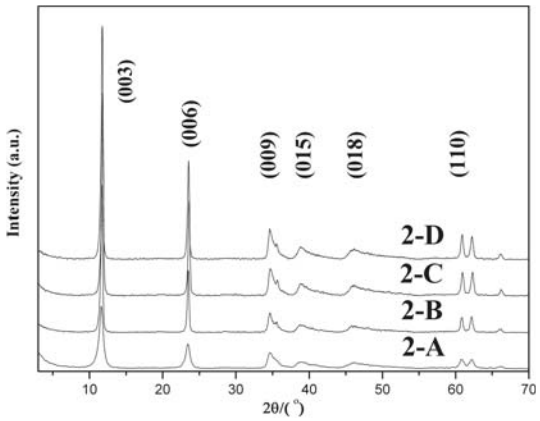

Fig. 1. XRD patterns for LDH samples.

Table 1. Indexing of XRD patterns for the LDHs.

Sample	2-A	2-B	2-C	2-D
d_{003}/nm	0.7627	0.7574	0.7510	0.7547
d_{110}/nm	0.1522	0.1522	0.1520	0.1521
$W_{1/2}$ for [003]/°	0.6143	0.2901	0.2840	0.2669
$W_{1/2}$ for [110]/°	0.5500	0.3462	0.2914	0.3020
Crystallite size in the *a*-direction/nm	16.58	26.33	31.30	30.20
Crystallite size in the *c*-direction/nm	12.87	27.25	27.84	29.62

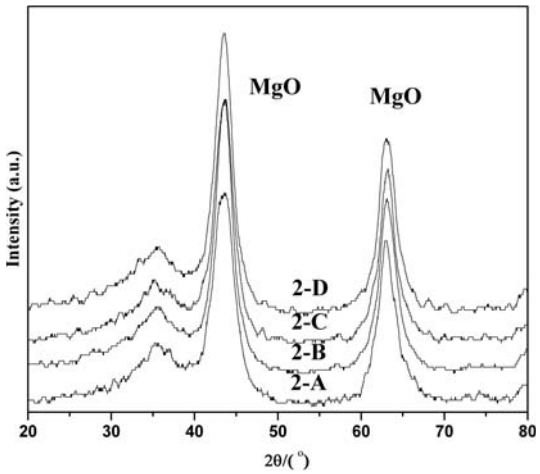

Fig. 2. XRD patterns for calcined LDH samples.

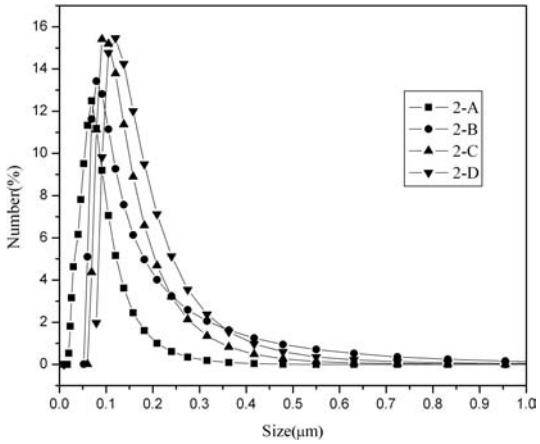

Fig. 3. Particle size distribution data for calcined LDHs.

Table 2. Sterilization ratios of calcined LDH samples.

Sample	Sterilization ratio of *S. aureus* /%	Sterilization ratio of *B. niger* /%
2-A	99.99	90.76
2-B	99.99	89.57
2-C	99.93	73.80
2-D	99.99	68.60
3-A	99.99	91.12
3-B	99.99	89.30
3-C	99.99	88.45
3-D	99.93	68.00
4-A	99.99	96.35
4-B	99.93	94.87
4-C	99.99	89.77
4-D	99.99	86.48

The laser particle size distributions for calcined LDHs prepared with $[Mg^{2+}]/[Al^{3+}] = 2$ are shown in Figure 3. It can be seen that the particle size of the calcined material is determined by that of the corresponding LDH precursor. Therefore, calcined samples with different particle sizes can be synthesized by tailoring the particle size of the precursor.

5.2 Effect of particle size of calcined LDH on bactericidal ability

Staphylococcus aureus is a ubiquitous bacterium in nature, which has a considerable impact on human health. *Bacillus subtilis var. niger* is a type of spore with a compact cell wall and has a strong resistance against chemical agents. The bactericidal activities of calcined LDHs having different particle sizes against *Staphylococcus aureus* and *Bacillus subtilis var. niger* were investigated. Table 2 lists the bactericidal results after contacting bacteria and spores with calcined LDHs for 24 h. As shown in Table 2, calcined LDHs with different particle sizes all exhibit excellent bactericidal activity against *Staphylococcus aureus*, which is relatively easily destroyed. The bactericidal efficiencies against *Staphylococcus aureus* are all above 99.9%. In contrast, the efficiency against *Bacillus subtilis var. niger* spores shows a marked decrease with increasing particle size as shown in Table 2, indicating that a high degree of dispersion of MgO is necessary for efficient bactericidal activity in this case.

The calcined LDH can be considered to consist of a mixture of MgO and Al_2O_3. It is known that the latter has no antibacterial activity [3]. Sawai et al. have shown that MgO is very readily hydrated and forms a layer of $Mg(OH)_2$ on the surface. Oxygen dissolved in the solution can generate superoxide anions O_2^- [11] by a single-electron reduction reaction, which are stable in a basic environment. The bactericidal ability of MgO increases with increasing surface area and degree of dispersion as a result of the higher number of surface hydroxyl ions and increasing concentration of O_2^- in solution. Proteins in the cell walls of bacteria and spores contain many peptide linkages. The superoxide anion will attack the carbonyl carbon atom in the peptide linkages eventually leading to destruction of the bacteria. The bactericidal activity of the calcined LDHs tends to increase with decreasing particle size.

6 Conclusions

(1) LDH samples were prepared by different methods with varying ratios of $[Mg^{2+}]/[Al^{3+}]$. Bactericidal experiments were carried out using LDHs calcined at 500 °C, which contain highly dispersed MgO.

(2) The bactericidal activity of highly dispersed MgO against *Staphylococcus aureus* is excellent, and its efficiency against *Bacillus subtilis var. niger* increases with decreasing particle size.

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