SONICATION-ASSISTED PREPARATION OF CaO NANOPARTICLES FOR ANTIBACTERIAL AGENTS

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The effect of calcination conditions on the size and killing activity of CaO nanoparticles towards *L. plantarum* was studied in this paper. The results showed that CaO nanoparticles with a diameter of 20 nm could be obtained under the investigated conditions. The lethal effect of CaO nanoparticles after incubation of 6 or 24 h increased with increasing calcination time. Using CaO-SA, CaO-SB, and CaO-SC after a 24-h exposure, 2.25, 3.37, and 5.97 log *L. plantarum* were killed, respectively, at a concentration of 100 ppm. The current results show that the use of CaO nanoparticles as antibacterial agents has significant potential in food-relevant industries.

Keywords: nano CaO; preparation; antibacterial activity.

INTRODUCTION

CaO nanoparticles can be used as bactericides, adsorbents, and in particular as destructive adsorbents for toxic chemical agents.[1,2] Few studies on the preparation of CaO nanoparticles have been reported. At present, methods for the preparation of CaO include thermal decomposition,[3,4] as well as sol–gel[5] and microwave processes.[6] Microscale CaO particles can be easily obtained by decomposition of limestone (mineral $CaCO_3$). Normally, $CaCO_3$ can be decomposed at high calcination temperatures of greater than 900 °C.[7] In our previous study, CaO nanoparticles obtained from the precursor $Ca(OH)_2$ using the thermal decomposition method were reported.[8] The sol–gel method is cost prohibitive on account of the high cost of the reagents and laboriousness of the process. The microwave-assisted route is another method for the synthesis of nanometal oxides, and has previously been used for preparing CaO nanoparticles. Roy and Bhattacharya investigated the preparation of CaO nanoparticles using the microwave irradiation method, with $Ca(NO_3)_2$ and NaOH as starting materials.[6] Their results showed that microwave irradiation is a simple and efficient method for the production of CaO nanoparticles with a regular shape, small size, narrow size distribution, and high purity. Recently, sonication has been applied in the synthesis of novel nanomaterials in aqueous solutions.[9,10] The chemical effect of ultrasound originates from the formation of ultrasonic cavitation, with the growth and collapse of microbubbles in the liquid phase generating very high temperatures and pressures followed by rapid cooling. These extreme conditions have been exploited for the preparation of nanoparticulate metal oxides.[11,12]

L. plantarum, a gram-positive bacterium, has a motionless shape and can convert lactose and other simple sugars into lactic acid. Usually, *L. plantarum* has complex nutritional requirements and can grow at temperatures ranging from 15 to 45 °C. Moreover, this type of bacterium is known to be very tolerant to acidity. In 1990, McDonald et al. showed that the growth of *L. plantarum* could be stopped when the internal pH reached 4.6–4.8 independently from pH 5.4–5.7 of the culture medium.[13] This feature allows *L. plantarum* to grow easily in juice drinks.[14] In fact, it may metabolize citric and malic acids present in fruit juices and fruit drinks, reducing the acidity of the product.

In recent years, inorganic agents have been used increasingly for control of microorganisms in various applications. The key advantages of inorganic agents are improved safety and stability compared with organic antimicrobial agents. Basic metal oxides such as MgO and ZnO, have been shown to exhibit antibacterial activity, where the particle size of the oxides appears to have an impact.[15,16] Thus, in this work, the preparation of calcium oxide nanoparticles used as antibacterial agents by a sonication method was studied in detailed. All results obtained form the basis for further application in food-relevant fields. Until now, no relevant references on this topic have been reported.

EXPERIMENTAL

Materials

Calcium tetrahydrate ($Ca(NO_3)_2.4H_2O$) (Mallinkrodt Baker Inc, ACS), sodium hydroxide (BMD Chemicals Inc, ACS), and ethylene glycol (99%) were purchased from BDH Inc; *L. plantarum* maintained in glycerol 20% solution at −80 °C was grown in Difco *Lactobacillus* media (MRS-agar and broth) at 35 °C for 24 h. Other reagents were obtained from local supplies.

Preparation of CaO nanoparticles

$Ca(NO_3)_2.4H_2O$ (11.8 g) was dissolved in ethylene glycol (25 mL) and then NaOH (12.5 mL; 2.10 g) was added into the mixture under sonication. After 10 min of sonication, the solution obtained was left to stand for 5 h. Subsequently, the precipitate was removed by filtration, washed with water, and dried at −50 °C. Finally, CaO nanoparticles of different sizes were obtained through calcination. The sample obtained under conditions of 530 °C, 5 h, 3 °C/min under vacuum is denoted CaO-SA. Similarly, samples obtained under calcination conditions of 530 °C, 10 h, 3 °C/min, under vacuum, and 530 °C, 15 h, 3 °C/min, under vacuum are denoted as CaO-SB and CaO-SC, respectively.

Antibacterial effects of CaO nanoparticles

Before CaO nanoparticles were used for antibacterial experiments, they were activated for approximately 2 h at 180 °C. The

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activated powder samples were sterilized at 121 °C for 15 min, and then suspended in peptone water (0.1%) to reach a concentration of 100 ppm.

The antibacterial effect of CaO nanoparticles was evaluated on *L. plantarum*. An overnight bacterial pre-culture was centrifuged, rinsed twice with sterile peptone water (0.1%), and then re-suspended to obtain a concentration approximately 1×10^8 CFU/mL. The suspension was added into 50-mL flasks containing CaO nanoparticles (20 mL; 100 ppm). Approximately 1×10^6 CFU/mL were submitted to the stress and incubated at 24 °C with agitation (250 rpm). Samples (1.0 mL) were taken at the indicated time (6 and 24 h), diluted 10-fold in distilled water, and then grown on MRS agar plate incubated at 35 °C for 48 h. The enumeration was performed in CFU/mL.

Characteristics analysis of CaO nanoparticles

Characteristic measurements of CaO nanoparticles have been carried out previously by us.[8,15–18] Thermal gravimetric analysis (TGA) measurements were carried out using Netzsch STA 409 Apparatus. A helium flow of 40 cm^3 min^{-1} and a heating rate of 10 K min^{-1} were used; the size and particle size distribution was recorded in ethanol using submicron particle sizer (NICOMP 370, USA). A Rigaku Geiferflex X-ray diffractometer with Ni-filtered Cu Ka radiation (40 kV; 30 mA) was used to determine the crystallinity and phase of the samples. X-ray diffraction (XRD) patterns were recorded in the range of 20°–70° with a scan speed of 2 °/min. Transmission electron microscopy (TEM) photomicrographs were obtained using the Philips 201 transmission electron microscope operated at 80 kV. The deposit was scraped away from the support and then transferred to the Fromvar 1595 E (Merck) membrane-coated Cu grid (mesh 400);

RESULTS AND DISCUSSION

Preparation of CaO nanoparticles

TGA of the intermediate

TGA was adopted to study the decomposition characterization of the intermediates. According to TGA (Figure 1), two major weight-loss peaks were identified; 350 to 500 °C and from 500 to 700 °C. The peak at 400 °C was assigned to the decomposition of Ca(OH)$_2$ to CaO + H$_2$O.
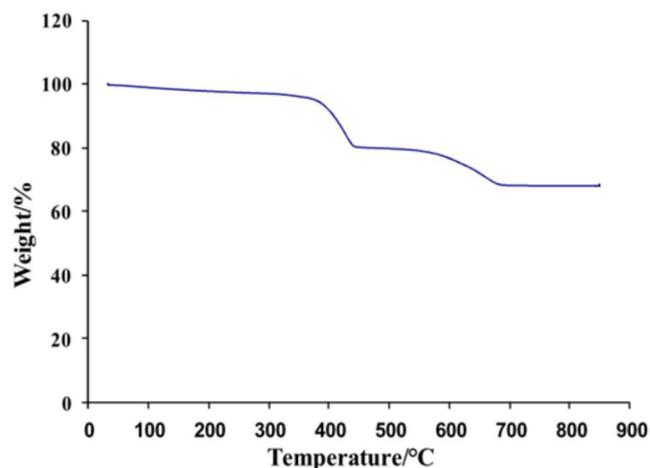
Figure 1. *TGA of the intermediate. Conditions: hold for 1 min at 50 °C; heat from 50 °C to 1000 °C at a rate of 10 °C/min; hold for 1 h at 1000 °C*

This result was consistent as that previously reported by Olga et al.[2] The peak from 500 to 700 °C was due to the removal of

chemisorbed water. Zhu et al. (2011) reported similar findings in which the starting and completed decomposition temperatures of nano CaCO$_3$ were 594.4 and 721.1 °C, respectively.[19] CaO nanoparticles could be obtained through calcination at 500 °C in this study.

Effect of calcination conditions on the size of CaO nanoparticles

To investigate the effect of calcination temperature and calcination time on particles size, five calcination temperatures (450, 480, 500, 530, and 550 °C) and six calcinations times (1, 5, 8, 12, 15, and 20 h) were selected. The results are showed in Figures 2 and 3.
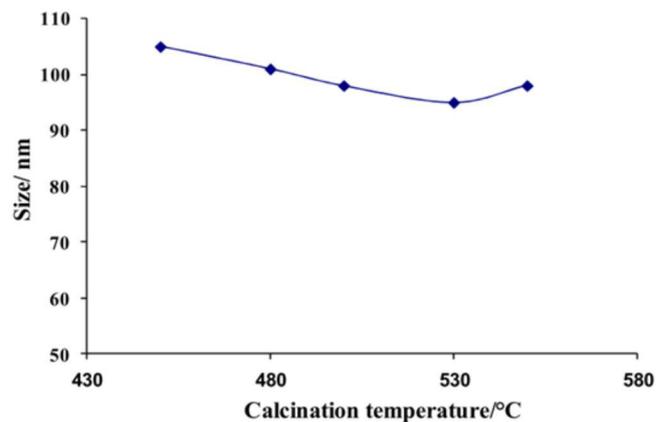
Figure 2. *Effect of calcination temperature on size. Conditions: heating rate, 3 °C/min; calcination time, 15 h*
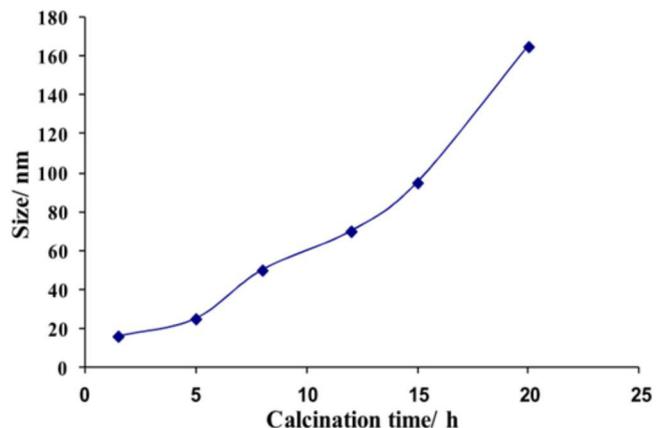
Figure 3. *Effect of calcination time on size. Conditions: calcination temperature, 530 °C; heating rate of calcination, 3 °C/min*

When the calcination temperature reached above 530 °C, the size of the CaO nanoparticles grew significantly. Below calcination temperatures of 530 °C, the decrease in CaO nanoparticle size with increasing calcination temperature might be explained by incomplete decomposition of the intermediates. Prolonging the calcination time at a fixed calcination temperature led to a rapid increase in nanoparticles size. Although the CaO nanoparticles increased as consecutive calcination time increased, the growth of these nanoparticles was not unlimited. In fact, the sintering of CaO is a common phenomenon that occurs simultaneously with calcination.[15,16,18,20] Borgwardt (1989) studied that the sintering rate of CaO in an N$_2$ atmosphere at temperatures between 700 and 1100 °C and found that the surface area and porosity of the sorbent sharply decreased as calcination temperature increased.[21] In our case, when the intermediates were decomposed at high temperatures, small CaO nanoparticles formed, after which the contact nanoparticles formed necks and began to grow, thus resulting

in an increase in the average size.

The effect of the calcination heating rate on the nanoparticles was also studied. The results are presented in Figure 4.
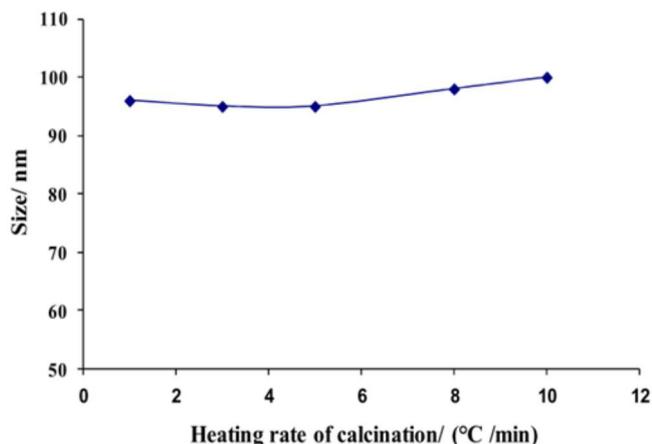
Figure 4. *Effect of heating rate of calcination on size. Conditions: calcination temperature, 530 °C, calcination time, 15 h*

As shown in Figure 4, the calcination heating rate had little effect on particle size. With increasing calcination heating rate, the size barely increased (i.e., from 95 to 98 nm). Therefore, calcination heating rate is not a key parameter for the preparation of CaO nanoparticles. Usually, calcination heating rate is related to the collapse of the intermediate rate. When the heating rate of calcination is very fast, aggregation of CaO nanoparticles happens more easily, particularly at high calcination temperatures.[15,16,18] Therefore, a calcination heating rate of 3 °C/min was selected for the preparation of CaO nanoparticles.

Through controlling calcination parameters, CaO nanoparticles with different sizes could be obtained. The size distribution of CaO-SC is presented in Figure 5.

Two peaks can be observed in Figure 5. One small peak was around between 15 nm and 20 nm, and another between 130 nm and 180 nm. This size distribution appears closely consistent with TEM (Figure 6) and its XRD result (Figure 7). From the XRD pattern in
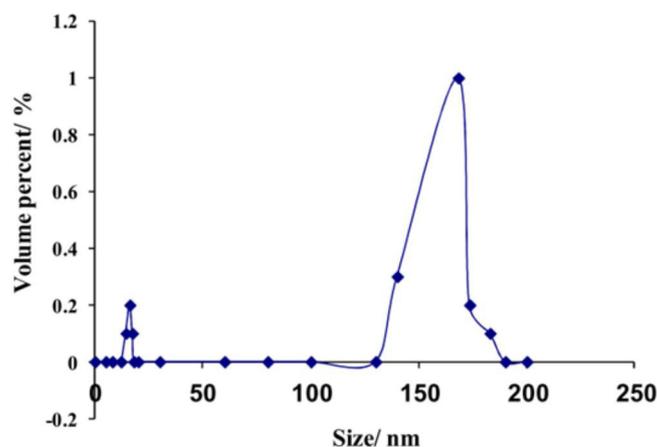
Figure 5. *Size distribution of CaO nanoparticles. Conditions: CaO-SC*

Figure 7, all peaks were consistent with the peaks of standard CaO. XRD patterns showed broadening of the peaks, indicative of the ultra-fine nature of the crystalline material. The crystallite size calculated using Scherrer's formula was approximately 139 nm (Figure 7). No peaks from any other phases of CaO were observed.
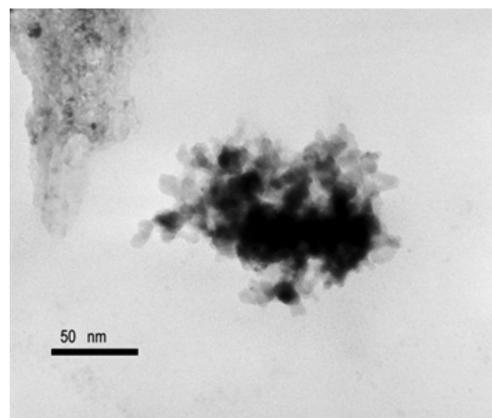
Figure 6. *TEM of CaO nanoparticles. Conditions: CaO-SC*
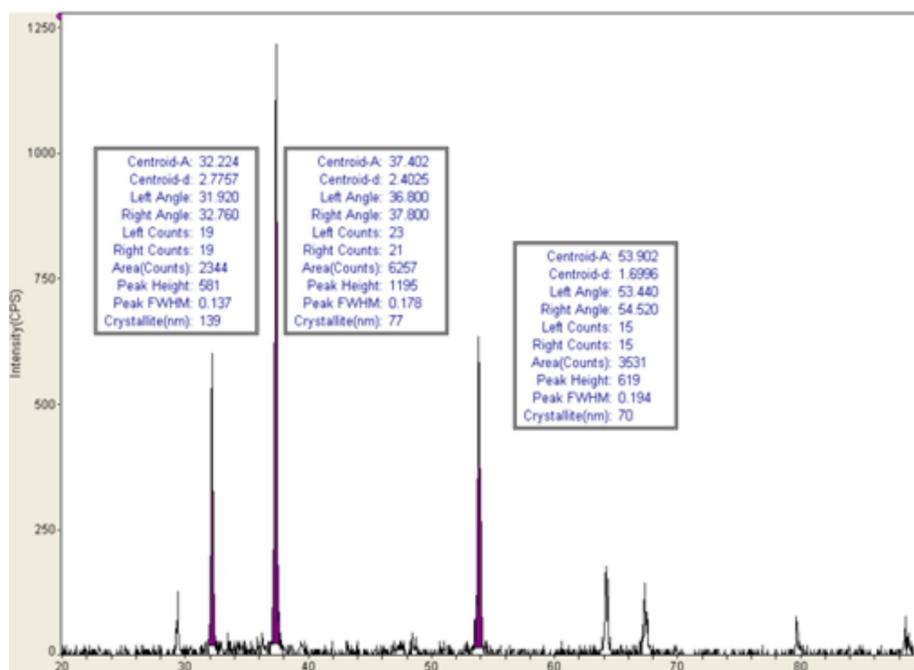
Figure 7. *XRD of CaO nanoparticles. Conditions: CaO-SC*

Antibacterial activity of CaO nanoparticles

The lethal effect of CaO nanoparticles is shown in Table 1. After 24-h exposure, the lethal effect of CaO nanoparticles increased with increasing calcination time; i.e., using CaO-SA, CaO-SB and CaO-SC, 2.25 log, 3.37 log and 5.97 log *L. plantarum* reductions were killed, respectively, at a concentration of 100 ppm. All CaO nanoparticles samples had little antibacterial activity after 6-h exposure. Through TGA (Figure 8), it was possible show that nanoparticles (CaO-SA) had undergone a weight loss 9% (Figure 8-A). Impurities or chemically absorbed water could conceal active spots of nanoparticles.[16,18] With increasing calcination time, antibacterial activity of CaO nanoparticles was also increased. When calcination time was increased to 15 and 10 h from TGA (Figure 8, -B, and -C), CaO nanoparticles had a higher antibacterial activity compared with CaO-SA. For the

antibacterial mechanism of CaO nanoparticles, it could be assumed that a concentration of O_2^- generated from the surface increased with decreasing particle size, because the number of CaO powder particles per unit volume of powder slurry increased with decreasing particle size. As per the abovementioned observations, the increase in antibacterial activity was assumed to be caused by the increase in O_2^- generated from the surface of CaO on reducing the sintering of the powder samples.[2,16,18]

Table 1. Effect of CaO nanoparticles on *L. Plantarum* viability

Samples	Log Reduction	
	6 h	24 h
CaO-SA, 100 ppm	0.08	2.25
CaO-SB, 100 ppm	0.12	3.37
CaO-SC, 100 ppm	0.18	5.97

CONCLUSIONS

CaO nanoparticles as antibacterial agents were prepared by a sonication method. Nanoparticles with the smallest size of 20 nm could be obtained, which showed good antibacterial activity. At a concentration of 100 ppm, CaO-SC could kill the quantity equivalent to *L. plantarum* 5.97 log reductions. These results suggest that CaO nanoparticles from the sonication method have a potential application in food-relevant fields.

Figure 8. TGA of CaO nanoparticles. (A) CaO-SA; (B) CaO-SB; (C) CaO-SC

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