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# Antibacterial activity and in vitro cytotoxicity studies of Ag-doped CaO nanoparticles

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Abstract

CaO nanoparticles can be used in different fields, such as medical and food, some studies suggest that they have poor antimicrobial properties. In this work, CaO nanoparticles doped with different concentrations of Ag were synthesized by the sol-gel method, to improve their antimicrobial activity. Cytotoxicity was also determined by MTT assay. The results of this study showed that it was possible to increase the antimicrobial activity of the CaO nanoparticles by doping them with different concentrations of Ag. According to a cytotoxicity study, a percentage of cell viability above 70 % was obtained, thus the nanoparticles of CaO doped with Ag are candidates to be used in medical and food applications.

Keywords

Nanoparticles, CaO, Ag-doped, Antimicrobial activity, Cytotoxicity

## 1. Introduction

Nanoparticles have been studied extensively since they have shown antimicrobial activity. Silver nanoparticles are recognized due to their high antimicrobial activity against different microorganisms, but also its potential toxicity, cell oxidative and inflammatory response, has been reported, so its use has been severely questioned [1]. In contrast, calcium oxide nanoparticles have received attention

for their properties such as lower toxicity, high basicity, and histocompatibility [2] and their potential applications in several fields: antimicrobial agent, absorbent, catalyst, and drug delivery agent [3]. Different works have shown that doping with different metal cations may enhance the antimicrobial properties of metallic oxides [4] [5].

In the present work, Ag-doped CaO nanoparticles were prepared by a sol-gel method. Antibacterial activity of Ag-doped CaO nanoparticles was evaluated against two pathogenic bacteria, *E. coli* and *S. aureus*. Cytotoxicity analysis was carried out in human fibroblasts cell using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

## 2. Experimental

### 2.1 Preparation of Ag-doped CaO nanoparticles and characterization

Ag-doped CaO nanoparticles were prepared via a sol-gel method with briefly modifications [6]. Firstly, (1-x) mol $Ca(NO_3)_2 \cdot 4H_2O$ and x mol $AgNO_3$ were dissolved in 20 mL deionized water. Then a solution containing 2 mol $C_6H_8O_7 \cdot H_2O$ was added into the above solution under continuous stirring. The mixture was kept in a water bath at 80 °C until a wet gel was formed. Secondly, the wet gel was heated up to 150 °C in an oven to obtain a dry gel. Finally, the dry gel was calcined at 700 °C in air atmosphere to get Ag-doped CaO nanoparticles. The obtained samples were designated as pure CaO, 0.1% Ag-doped CaO and 0.5% Ag-doped CaO respectively. The samples were analyzed by X-ray diffraction (XRD) (PANalytical Empyrean), X-ray photoelectron spectroscopic (XPS) (Thermo Scientific K-Alpha), and scanning electron microscope (SEM) (JEOL JMS 70000F).

### 2.2 Antibacterial activity

Growth kinetics were performed, both for *S. aureus* and *E. coli*, by placing test tubes with 4 ml of brain heart infusion medium for each microorganism, adding different amounts of samples (3, 6, and 9 µg) and controls, in triplicate. The tubes were inoculated with 100µL of infectious inoculum, corresponding to tube 4 on the McFarland scale, and incubated at 37 ° C [7].

### 2.3 Cytotoxicity evaluation

All samples and controls (Ag-doped CaO and cells without material) were seeded with fibroblast line 3T3.1.5 x 104 cells during 24 and 72 h, in Dulbecco′s modified eagle′s medium (D-MEM) supplemented with fetal bovine serum (FBS) and penicillin-streptomycin. The viability was determined

using MTT assay [8].

## 3. Results and discussion

### 3.1 Characterization of Ag-doped CaO nanoparticles

Figure 1a shows the XRD patterns for CaO nanoparticle samples with the different Ag doping percentages. All samples exhibited the characteristic peaks corresponding to the pure cubic phase of CaO (JCDPS No. 77-2376). It is also possible to observe that the intensity of the diffraction peaks of the CaO phase increases as the Ag content does, indicating that doping has a positive effect on the CaO crystallization and growth gain [5].

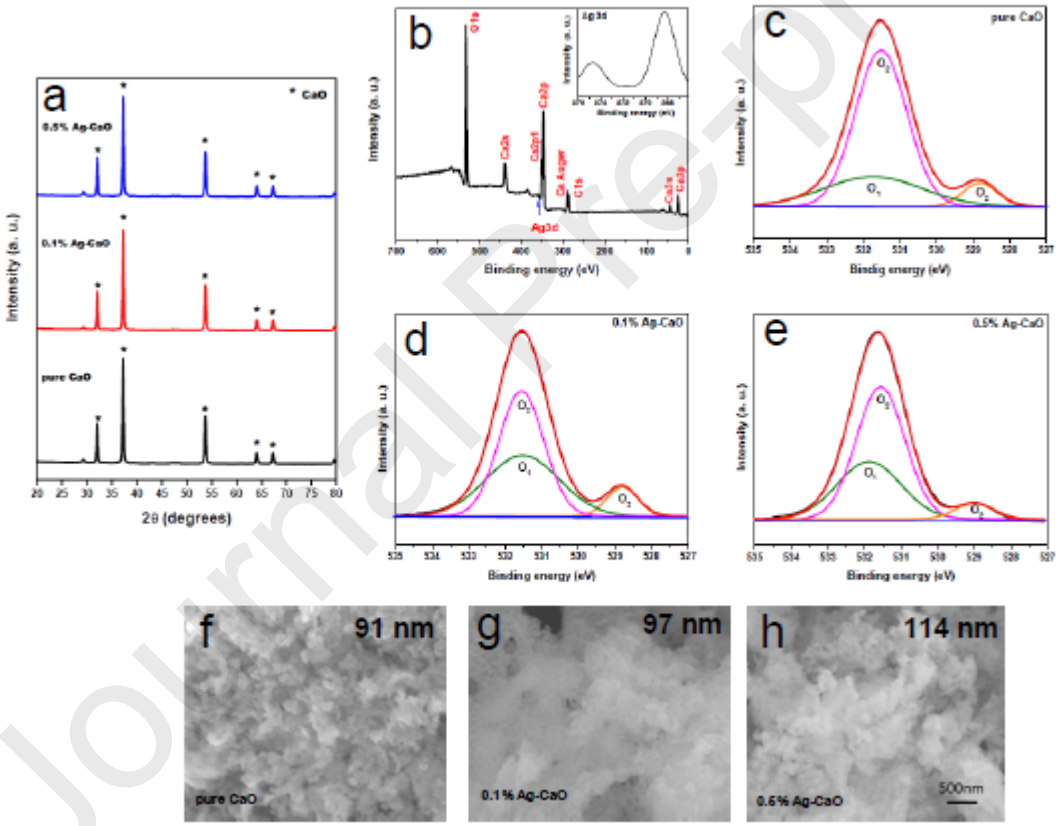


**Figure 1.** XRD patterns of pure CaO and Ag-doped CaO (a), XPS survey spectrum of 0.1% Ag-doped CaO (b), high resolution O1s XPS spectra of the samples (c, d, e) and SEM images (f, g , h).

The typical XPS survey in Fig. 1b confirms the signals from Ca, Ag y O. Fig. 3c-e shows the O1s high-resolution XPS spectra of pure CaO and doped CaO. The O1s peaks of each sample are composed of three subpeaks. The peak about 531.3 eV is attributed to the chemisorbed oxygen ($O_1$), and the peaks centered at 529 and 531.5 eV are associated with the lattice oxygen ($O_2$) of CaO. The molar percentage of $O_1$ in O species varies with de silver content, the percentage for pure CaO, 0.1% Ag-CaO, and 0.5%Ag-CaO is 25%, 41% y 34%, respectively. The above suggests that Ag doping promotes the amount of oxygen absorbed and the generations of the oxygen vacancies [6]. The SEM images show an irregular spherical shape. The particle size increases as the percentage of Ag doping increases.

3.2 Antibacterial activity of Ag-doped CaO nanoparticles

Antimicrobial efficiency was evaluated by monitoring microbial growth; Figure 2 shows the behavior of microbial growth in CFU / ml as a function of time. It is possible to appreciate that the growth curves show the four characteristic growth phases (lag, exponential growth, stationary, and death) [9].

According to the analysis of variance performed ($\alpha<0.05$), it is possible to determine that the maximum inhibitory level was achieved with the sample treated with 9 mg of CaO at 0.5% Ag, evidencing from 10 h, the maximum level of microbial development, which when compared with the control sample shows a statistically significant decrease corresponding to 1 logarithmic cycle in the cell proliferation of *S. aureus*. This level of inhibitory efficiency against *S. aureus* is comparable to that which can be obtained for food preservation treatments, such as cooked ham, treated by high hydrostatic pressures and refrigeration [10].

In the case of growth kinetics for *E. coli*, the sample containing 9 µg of CaO doped at 0.1%Ag, delays the lag phase up to 4 h, which denotes the antimicrobial effect. The sample containing 9 mg CaO at 0.1% Ag has the maximum inhibitory effect, achieving a reduction of 9 logarithmic cycles with respect to the control. The levels of inhibition obtained are consistent with those of the preservation for mozzarella cheese coated with chitosan and polypropylene films, where inhibition levels of 0.49 logarithmic cycles for the development of *E. coli* were reached [11].

Antibacterial activity is attributed to damage caused to the cell wall of microorganisms due to the production of reactive species of oxygen (ROS). In the XPS analysis, it was observed that by increasing the doping percentage, the amount of absorbed oxygen increased, which causes oxidative stress in the cells of microorganisms, which translates into damage to the cell wall that can cause: leakage of intracellular contents, DNA damage that will prevent cell replication or the interruption of metabolic processes that ultimately lead to the death of the cell.

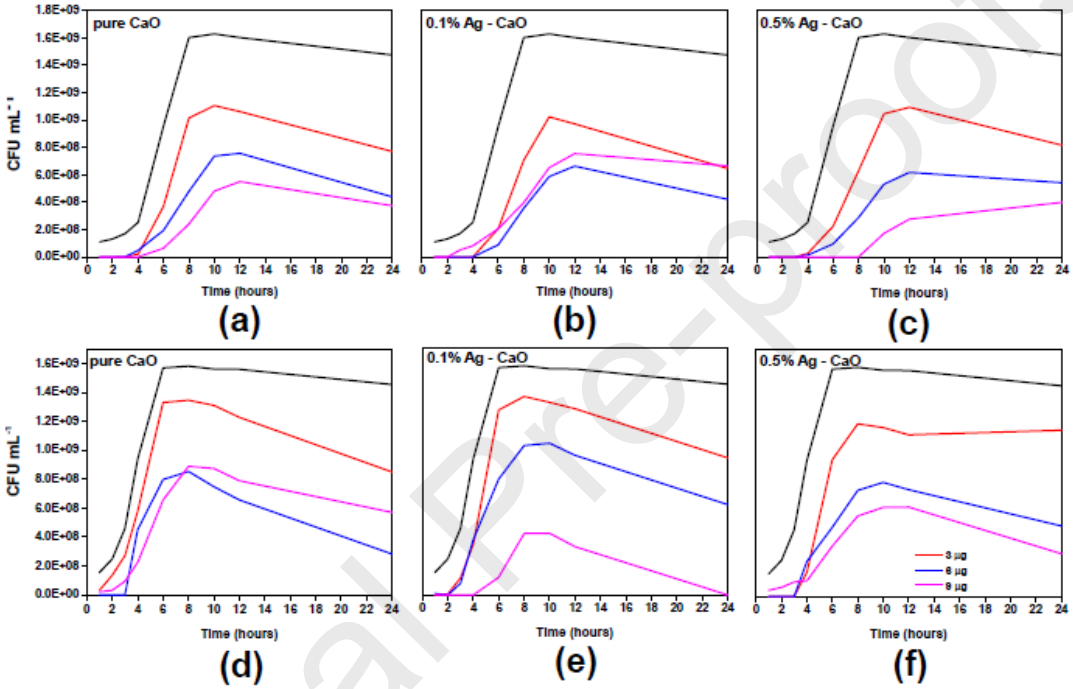


**Figure 2.** Microbial growth kinetics during 24 hours, *S. aureus* (a, b, c) and *E. colli* (d, e, f)

3.3 Cytotoxicity evaluation of Ag-doped CaO nanoparticles

Figure 3 shows the cell viability percentage. In these types of studies, treatments are considered non-toxic when cell viability is 70% or higher by ISO 10993-5: 2009, which shows that Ag-doped CaO nanoparticles are biocompatible at this range of concentrations. Viability percentages are above the 70% value, for all samples of Ag-doped CaO nanoparticles and at both incubation times.

For the 24 h incubation time, the viability levels are of the order of 130% with a minimum of 100%, which indicates that Ag-doped CaO does not exhibit cytotoxicity during this incubation time. At 72 h incubation time, maximum viability percentages of 130% are reached with a minimum of 90%, which

indicates that at this incubation time there is a decrease in the viability percentage, without the samples accomplishing the cytotoxicity limits [12]. It can be stated that Ag-doped CaO nanoparticles used in this research are non-cytotoxic, additionally they promote cell growth, which is in accordance with a study of Ag nanocomposites that present viability levels greater than 100%, which indicates it promotes cell growth.[13].

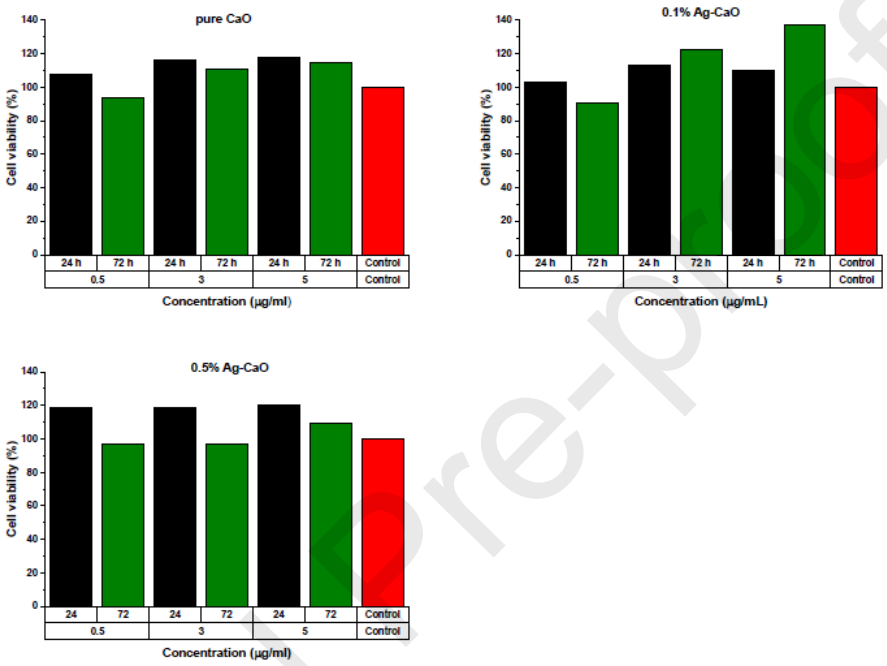


**Figure 3.** Cellular viability analysis of pure CaO and Ag-doped CaO nanoparticles.

## 4. Conclusions

Nanoparticles of pure CaO and Ag-doped CaO were obtained by the sol-gel method according to the XRD and XPS analyses. The XPS analyses reveal the influence of silver doping promotes the generation of oxygen vacancies and the formation of ROS that increase de antimicrobial properties. Regarding pure CaO nanoparticles antimicrobial properties were presented for *E. colli* and *S. aureus*. Ag-doped CaO nanoparticles exhibit more strong antibacterial activity than pure CaO, and 0.1% Ag-CaO can reduce 9 logarithmic cycles for E. *colli*.

Cytotoxicity tests showed that in general, pure CaO and Ag-doped CaO nanoparticles; does not show cytotoxic effects on fibroblast cells at 24 and 72 hours of exposure. The effect presented is the promotion of cell growth, being this greater at 24 h.

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Highlights

Ag-doped CaO nanoparticles were synthesized by the sol-gel method.

The obtained nanoparticles have shown good antimicrobial activity.

Ag-doped CaO nanoparticles exhibited cell growth promotion as well as non-cytotoxic effects.

**C.M. López-Badillo:** Conceptualization, Resources, Project administration; M. **Hernández-González:** Writing - Review & Editing, Resources; **F. Hernández-Centeno:** Formal analysis, Validation; **I. Olivas-Armendáriz:** Resources, Methodology; **C. A. Rodríguez-González:** Resources, Supervision; **E. M. Muzquiz-Ramos:** Supervision , **Jorge López-Cuevas:** Resources, Data curation, **H. Y. López-De la Peña:** Conceptualization, Methodology, Writing - Original Draft, Funding acquisition, Investigation.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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