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Iraqi Journal of Applied Physics
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Synthesis of Calcium Oxide Nanoparticles and Their Synergism Applications

In this study, the calcium oxide nanoparticles (CaO NPs) were synthesized through the calcination of chicken eggshells at 900°C and their subsequent synergistic combination with naringin have been explored. A CaO NPs/naringin composite was synthesized by combining colloidal CaO NPs in a solution of naringin. Confirmation of the cubic crystalline nature and polydisperse size distribution of the particles was done through X-ray diffraction and transmission electron microscopy (TEM) analysis. The nanoparticles were subjected to an ultrasonic probe, resulting in particles with a mean of 9 nm, along with their synergistic effects, which increased to 19 nm. Antibacterial activity was examined by the agar diffusion assay, with a highest inhibition zone of about 18 ± 0.67 mm for the CaO NPs/naringin mix, and appreciably heightened activity when compared to monotherapies. Statistical analysis upheld the reproducibility of the results ($P \leq 0.001$). The results highlight the promise of CaO NPs as powerful nanocarriers for naringin that significantly enhance its antibacterial activity against resistant microbial strains.

Keywords: Calcium oxide; Nanoparticles; Eggshell waste; Antibacterial synergism
Received: 6 February 2025; **Revised:** 20 April 2025; **Accepted:** 27 April 2025

1. Introduction

Nanomaterials are characterized by at least one external dimension at the nanoscale (~1 nm) and are synthesized via top-down or bottom-up fabrication methods. Their unique physicochemical properties make them particularly valuable in the pharmaceutical industry, among other applications. Particle size, shape, surface morphology, crystallinity, and solubility are critical factors that significantly affect their functional behavior and, consequently, their efficacy in biomedical and industrial settings [1]. Metal oxide nanoparticles (MONPs) constitute a renaissance material class that has disruptive potential in biomedical, environmental, and industrial applications. Their distinctive physicochemical features—high surface-to-volume ratios, accessible band gaps, and facile functionalization—enable them to be engineered to provide bespoke solutions for multiconfronting technological and scientific challenges [2]. Due to their unique properties, MONPs have gained considerable interest among researchers, as they have been found to be effective in UV filtering, energy storage [3], gas sensing devices [4], pollution control [5], medicine, diagnostics, and imaging applications [6].

Among various metallic oxide nanoparticles, CaO NPs are of particular interest due to their biocompatibility, cost-effectiveness, and extensive applications in biomedical, environmental, and industrial fields. Physicochemical properties of CaO NPs greatly depend on the synthesis pathways [7,8]. For instance, Mirghiasi et al. [8] and Razali et al. prepared CaO NPs by thermal decomposition of $Ca(OH)_2$ and $CaCO_3$, respectively, while El-Dafrawy et al. employed a sol-gel process using $CaCl_2$ as a precursor [9].

During the recent few years, environmentally friendly synthesis strategies have attracted lots of attention towards the fabrication of nanoparticles. Ramli et al. demonstrated biosynthesis of CaO NPs with red dragon fruit peel extract (Hylocereus polyrhizus) as a green reducing agent [10]. Another cost-effective and sustainable method is the utilization of eggshells—a cheap source of $CaCO_3$, which is thermally reduced to CaO NPs by a low-cost combustion process [11]. Besides minimizing waste, this process also adheres to the concepts of circular economy by transforming agricultural and household waste products into valuable nanomaterials for medical and environmental uses [12].

Green synthesis methods enhance their sustainability without compromising performance. Biomedically, they exhibit antimicrobial effects via reactive oxygen species (ROS) generation and membrane disruption while serving as drug carriers in phototherapies [13,14]. Environmentally, they adsorb heavy metals with high efficiency [15], and convert CO_2 to syngas. Industrially, they are used to catalyze biodiesel production, wastewater treatment, and steel processing, with additional utility in gas sensing and optoelectronics due to their porous structure [16].

The present study has two prime objectives: First, thermal combustion synthesis of calcium oxide nanoparticles using eggshells and extensive structural, morphological, and topological characterization; and second, investigation of synergistic antimicrobial activity of CaO NPs and naringenin against drug-resistant *Staphylococcus* strains. The proposed combination therapy seeks to create three significant therapeutic advantages: reduction in antimicrobial concentration, enhancement of bactericidal activity, and compound stability. These studies will provide baseline data for the development of CaO NPs-based antimicrobial products with potential clinical significance.

2. Materials and Methods

Naringenin (purity 97 %) was purchased from MACKLIN (China). Bacterial culture media such as Mueller-Hinton agar and Mannitol salt agar were obtained from Himedia (India).

Chicken eggshells were collected, cleaned well, washed three times with deionized water to remove impurities, and then ground into a fine powder using a blender. The resulting powder was roasted for one hour at 900 °C, the powder was cooled, and then the CaO powder was crushed in deionized water using an ultrasonic probe for 15 minutes [17,18]. CaO powder of 0.035 g was added to 10 ml of deionized water and 0.27 g of Naringenin was prepared in 10 ml of methanol (99%)[19]. Then 1 ml of CaO NPs (3.5 mg/ml) was mixed with 1 ml of Naringenin solution (27 mg/ml) under stirring conditions at 30 °C. This resulted in the formation of a yellow substance. Then serial dilutions of all the above-mentioned materials were made from their stock solutions.

CaO NPs were characterized by determine of their structural, morphological, and topological properties, using the X-ray diffraction analysis (XRD), the scanning electron microscope (SEM), and the transmission electron microscope (TEM) techniques respectively. XRD spectrum recorded for 2θ =10° to 100° with scanning speed of 0.011° for 1.54060 Å.

The antibacterial activity of CaO NPs, Naringenin, and CaO NPs/Naringenin were investigated against *S. aureus* (MDR). After culture of the organisms on Mueller-Hunten agar, different concentrations of calcium oxide nanoparticles, naringin (NAR), and CaO NPs/NAR were added to the designated wells in the agar. The cultured plates were incubated for 24 hours at 37 °C. Then, the diameter of the inhibition zone was measured and recorded for three replicates.

3. Results and Discussion

XRD pattern of the CaO NPs showed crystalline multiphases with cubic structure according to Card No ICDD 00-037-1497. XRD peaks of the CaO NPs located at 32.3°, 37.4°, 54.0°, 64.2°, 67.5°, 79.8°, 88.5°, and 91.4° correspond to the directivity of (111), (200), (220), (311), (222), (400), (331), and (420), respectively, the dominant phase was (200) with crystalline size about 55 nm. Figure (1) illustrates the XDR pattern of the prepared CaO NPs.

Transmission electron microscopy (TEM) was employed to enhance the accuracy of our examination, as shown in Fig. (2). TEM image of the synthesized CaO NPs revealed a particle size distribution ranging from 2 to 23 nm, with an average size estimated at 9 nm. This observation highlights the presence of quantum dots within the prepared nanoparticles and the previously mentioned nanosheets, as demonstrated in Fig. (2). The addition of naringenin enhanced the nanoparticles' agglomeration, leading to an increase in the average particle size observed during microscopic examination—the average particle size measured approximately 19 nm, as illustrated in Fig. (2c).

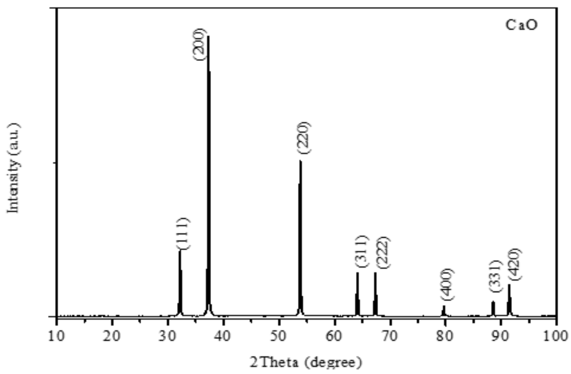

Fig. (1) XRD pattern of the prepared CaO nanoparticles

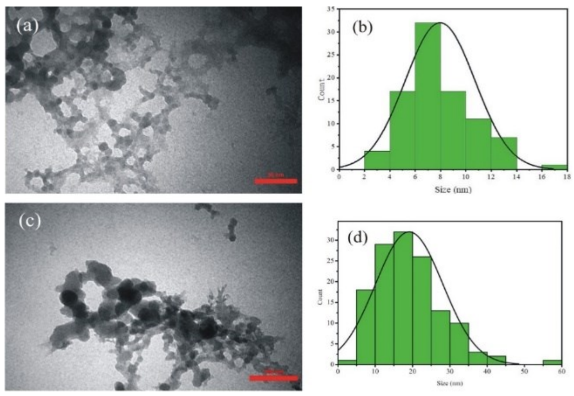

Fig. (2) (a) TEM image of the prepared CaO NPs (b) Size distribution of the prepared CaO NPs, (c) TEM image of the prepared CaO NPs/NAR, and (d) Size distribution of the prepared CaO NPs/NAR

The biological characteristics of CaO NPs are largely determined by their surface, and the nanoparticles can be engineered to exhibit the desired biological characteristics [20]. When the nanomaterial has a small size, which gives it a high ability to interact with the smallest sizes, such as bacterial cells, it can reduce the thickness of the bacterial cell wall, or even cause the formation of small holes or fractures in the wall. Once the nanomaterial breaks or penetrates the cell wall, the second material can enter the bacterial cell more freely. Thus, CaO NPs facilitate the entry of naringenin into the bacteria, which leads to greater effectiveness than individual treatments. Figure (3) shows the zones of inhibition at serial dilutions of CaO NPs, NAR, and CaO NPs/NAR. CaO NPs/NAR

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showed inhibition at concentrations of 1/2, 1/4, and 1/8, while the maximum inhibition of CaO NPs and NAR was 1/2 and 1/4, respectively. ANOVA analysis confirmed that the effect of treatments was highly significant at the significance level of $P < 0.0001$.

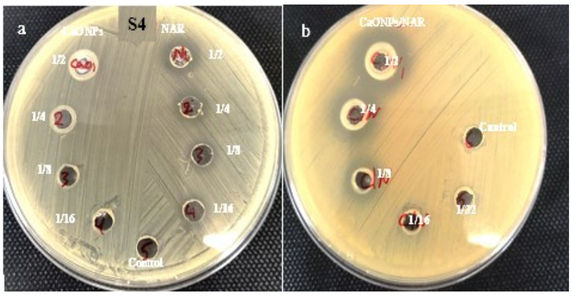

Fig. (3) Antibacterial activity on *S. aureus* (MDR) (a) with CaO NPs, NAR, and (b) with CaO NPs/NAR

The effect mean being 10.67 mm for CaO NPs and NAR and 18.67 mm for CaO NPs/NAR LSD results comparing the means of treatments showed that the difference between NAR, CaO NPs/NAR, CaO NPs, and CaO NPs/NAR was greater than LSD Table 1. Some nanoparticles cause the destruction of bacterial membranes through chemical or physical reactions that lead to a disruption in the membrane structure, and this disruption allows the cell contents to leak out and thus the bacteria to die [21]. In the synergistic formulation, the tetracycline silver nanoparticles interacted more strongly with *Salmonella spp.* cells and this may cause the release of more silver nanoparticles, thus creating a temporary high concentration of silver nanoparticles near the bacterial cell wall, which results in the inhibition of bacterial growth [22]. Synergistic treatment use of facilitates rapid access to the target [23,24]. The antibacterial strategy based on nanomaterials using photodynamic therapy (PDT) and photodynamic thermal therapy (PTT) works in multiple synergy, where raising the temperature reduces the cellular activity and increases the sensitivity of target cells to reactive oxygen species (ROS), thus facilitating their removal. The composite nanofilms with porous structure, nanoscale size, large surface area, and good penetration can effectively prevent the invasion of pathogenic bacteria [25,26].

4. Conclusion

Chicken eggshells are a green and natural precursor for the synthesis of CaO nanoparticles. Through thermal treatment and their subsequent ultrasonication, the nanoparticles derived from eggshells can be minimized to quantum dot-like dimensions with a polycrystalline character, as determined from XRD patterns and TEM investigations. Surprisingly, the incorporation of naringenin led to visible agglomeration of particles in the case of CaO NPs, which was responsible for the increase in average nanoparticle size. Moreover, the resultant CaO NPs/naringenin hybrid displayed significantly enhanced antibacterial activity against *Staphylococcus aureus* and also against multidrug-resistant strains. The synergistic system demonstrates the promising application of environmentally friendly, waste-derived nanomaterials as a possible solution to counter the increasing problem of antibiotic resistance.

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ISSN (print) 1813-2065, (online) 2309-1673

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ISSN (print) 1813-2065, (online) 2309-1673