UDC 549.67:543.544-414.7]:666.856

Yuriy Sukhatskiy, Martyn Sozanskyi, Roman Mnykh, Zenovii Znak

SONOCHEMICAL SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF Cu_2O NANOPARTICLES/CLINOPTILOLITE COMPOSITE

Lviv Polytechnic National University, Lviv, Ukraine

The Cu_2O nanoparticles/clinoptilolite composite was synthesized using a sonochemical method (ultrasound frequency of 20 kHz; ultrasound power density of 200 W/L) with an aqueous solution of copper sulfate as a precursor and hydrazine as a reducing agent for Cu^{2+} ions in the clinoptilolite framework, forming Cu_2O nanoparticles. X-ray diffraction confirmed the presence of the cuprite (Cu_2O) phase with a cubic structure in the synthesized composite. The average crystallite diameter of Cu_2O, calculated using the Debye-Scherrer equation, was approximately 13 nm. Energy-dispersive X-ray spectroscopy was used to determine the Cu content in the composite. SEM imaging confirmed the uniform distribution of Cu_2O nanoparticles (weighted average size of 61 nm) across the clinoptilolite framework. The antibacterial activity of the Cu_2O nanoparticles/clinoptilolite composite was evaluated using the direct addition method in a nutrient medium. Complete bacterial elimination (100%) was observed for both Gram-positive *Staphylococcus aureus subsp. aureus* ATCC 25923 and Gram-negative *Escherichia coli* ATCC 25922 at a composite concentration of 3 g/L in suspension.

Keywords: Cu_2O nanoparticles, clinoptilolite, composite, sonochemical synthesis, direct addition method, antibacterial activity.

DOI: 10.32434/0321-4095-2025-160-3-65-70

Introduction

Zeolites are microporous crystalline aluminosilicates, also known as «molecular sieves» [1]. The tetrahedra $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$, linked together by oxygen atoms, are the building units for an aluminosilicate zeolite framework [2]. Zeolites are divided into two major groups: natural and synthetic zeolites. Natural zeolites are primarily formed from volcanic and sedimentary rocks (e.g., clinoptilolite, mordenite, and chabazite) [3], while synthetic zeolites are produced by heating China clay, soda ash, feldspar, and other sources [4].

The controllable acidity and pore size, stability, high efficiency, and selectivity of zeolites and composite materials based on them in organic transformations [5] make them applicable in catalysis for the production of industrially important hydrocarbons and oxygenates (e.g., light olefins, aromatic compounds, liquid fuels, methyl and ethyl alcohols, acetic acid from non-petrochemical feedstock), for hydrocarbon separation, desalination and dehydration, for host–guest assembly [6–8], as well as for the adsorptive removal of dyes, antibacterial agents, and heavy metal ions [9]. Zeolites in the sodium/potassium form and exchanged with transition metals, acting as Lewis acids/bases, perform the functions of universal catalysts for multicomponent reactions [10].

To create materials with antimicrobial (antibacterial, antifungal, and antiviral) properties, zeolites are modified with Ag^+, Zn^{2+}, Cu^{2+} ions, as well as metallic Ag^0 or Ag nanoparticles/clusters. The mechanism of antimicrobial action of functionalized zeolites is associated with direct contact damage to membranes, followed by penetration into the cell. This leads to leakage of cell contents and its death. Additionally, the interaction of the antimicrobial agent

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with the respiratory enzyme system results in the generation of reactive oxygen species (the occurrence of the so-called «oxidative stress»), which causes cellular damage to nucleic acids and proteins [11].

Cuprous oxide (Cu_2O) is a p-type semiconductor material with a theoretical bandgap of ~2.2 eV, which shows a light-emitting coherence character [12]. It is known that the good electrical and optical properties of Cu_2O are mainly controlled by intrinsic defects (e.g., by Cu and/or O vacancies) [13]. Due to these properties, Cu_2O has many practical applications: in optoelectronics (particularly in solar cells), photocatalysis, in nano-magnetic devices, as a material for the production of thin-film transistors, electrodes in lithium-ion batteries, in glucose and gas sensors, biosensors, and biomedicine (as antibacterial, antifungal, and antiviral agents).

The aim of the work was to investigate the antibacterial activity of the Cu_2O nanoparticles/clinoptilolite composite synthesized by the sonochemical method against Gram-positive (*Staphylococcus aureus* subsp. *aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922) bacteria.

Experimental

The two-stage synthesis of the Cu_2O nanoparticles/clinoptilolite composite included the following:

1) adding an aqueous solution of copper sulfate with a concentration of 10 wt.% $CuSO_4$ to a suspension of clinoptilolite (from the Sokyrnytsia deposit, Zakarpattia region, Ukraine) under synchronous action of an ultrasonic (US) cavitation field to this heterogeneous system for 10 min. The clinoptilolite content in the suspension was 200 g/L of distilled water, and the particle size of the clinoptilolite fraction was 1.0–1.5 mm. A Bandelin Sonopuls HD 2200.2 ultrasonic homogenizer was used to generate US with a frequency of 20 kHz. The power density of the cavitation treatment applied to the reaction medium was 200 W/L. In order to achieve complete ion exchange of cations in the clinoptilolite framework with Cu^{2+} ions (the source of which was the precursor), the formed heterogeneous system was stirred (frequency of 300 rpm) at room temperature for 2 h.

2) adding hydrazine (in a 2-fold molar excess) to the system to reduce Cu^{2+} ions in the clinoptilolite to Cu_2O nanoparticles. Before adding hydrazine, the pH of the system was adjusted to ~7.5 using an aqueous ammonia solution. The reaction system, which acquired a reddish-brown color, was stirred with a frequency of 300 rpm for 2 h and left for 12 h. Within 12 h, sedimentation of the synthesized composite particles occurred. After that, the liquid phase was decanted, and the composite was washed with distilled water and dried at 60^0C until a constant mass was achieved.

X-ray diffraction (XRD) experiments to determine the phase composition of the product were performed using an AERIS Research (Malvern PANalytical) diffractometer with CuKα radiation (λ=1.5406 Å). The XRD data were acquired in the 2θ range of 10 to 105^0. The average crystallite size was calculated using the Debye-Scherrer equation [13]. The elemental composition of the synthesized composite was analysed using energy-dispersive X-ray spectroscopy (EDX) method on an INCA Energy 350 (Oxford Instruments). SEM observations to study the morphology of the obtained material were carried out using a ZEISS EVO 40XVP microscope. The weighted average size (diameter) of Cu_2O nanoparticles was estimated using specialized software product (Carl Zeiss Vision AxioVision Viewer 4.8).

The antibacterial activity of the Cu_2O nanoparticles/clinoptilolite composite against Gram-positive (*Staphylococcus aureus* subsp. *aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922) bacteria was determined by the direct composite addition method into the nutrient medium for microorganisms growth. For this purpose, a nutrient medium (meat-peptone agar) was added to a suspension with different concentrations of the composite (1, 2, 3, 4, and 5 g/L, respectively), prepared using sterile distilled water. The obtained samples were placed in an autoclave for 20 min (temperature of 121^0C and pressure of 1.2 atm) and poured into sterile Petri dishes. Once the medium solidified, a bacterial suspension (accordingly, *Staphylococcus aureus* subsp. *aureus* ATCC 25923 or *Escherichia coli* ATCC 25922) in physiological saline, with turbidity equivalent to the 0.5 McFarland standard (approximately 10^8 cells), was applied to the surface. Then the samples were kept in a thermostat for 2 days at 30^0C. The presence of antibacterial action was indicated by a reduced number of microorganisms (or absence of their growth) on the Petri dishes. Petri dishes containing nutrient medium without applying the composite suspension were used as controls, and they were similarly sowed with the respective bacterial strain.

Results and discussion

The XRD patterns of clinoptilolite with a cubic structure (Ref Code 01-087-1619) [14] and the Cu_2O nanoparticles/clinoptilolite composite prepared by the sonochemical method are shown in Fig. 1. A decrease in the intensity of clinoptilolite peaks in the range of 2θ=10^0–28^0 (Fig. 1b) was observed compared to the intensity of the clinoptilolite peaks in Fig. 1a.

Additionally, new peaks appeared in the range of $2\theta=30^0-93^0$, corresponding to the crystalline planes (110), (111), (200), (220), (311), (222), and (400) of cuprite (Cu_2O) with a cubic structure (JCPDS No. 05-0667) [15]. The average diameter of the Cu_2O crystallite, calculated using the Debye-Scherrer equation, was approximately 13 nm. The absence of peaks corresponding to other phases indicated the high purity of the Cu_2O crystals.

The EDX spectrum of the synthesized material (Fig. 2), in addition to peaks corresponding to elements such as Si, Al, and O, also contained peaks for Cu at 1.0 and 8.05 keV. It was determined that the Cu content in the composite was 56.71 wt.%.

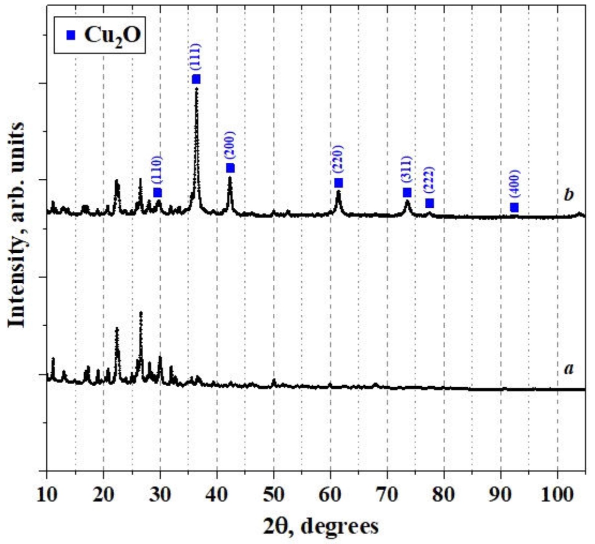


Fig. 1. The XRD patterns of (a) clinoptilolite and (b) the Cu_2O nanoparticles/clinoptilolite composite prepared by the sonochemical method

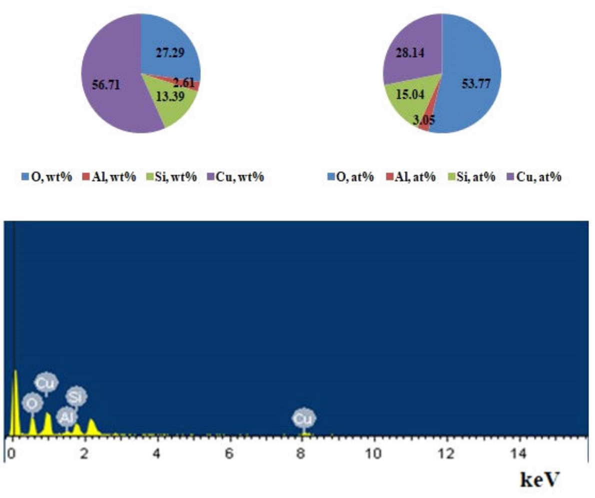


Fig. 2. EDX spectrum of the Cu_2O nanoparticles/clinoptilolite composite prepared by the sonochemical method

The SEM image of the obtained composite (Fig. 3a) confirmed the uniform distribution of Cu_2O nanoparticles across the surface of the clinoptilolite framework. The weighted average size of Cu_2O nanoparticles, determined taking into account the particle size distribution (Fig. 3b), obtained using specialized software (Carl Zeiss Vision AxioVision Viewer 4.8), was 60.5 nm.

Two primary mechanisms of the antibacterial action of nanoparticles (including Cu_2O nanoparticles) are distinguished [11]. The first mechanism involves the release of positively charged cuprous ions (Cu^+), which, due to electrostatic interaction forces, neutralize the negative charges of bacterial cell walls and plasma membranes. This leads to the leakage of bacterial cell contents and, consequently, their death. The second mechanism involves the Cu_2O nanoparticles-induced generation of excess reactive oxygen species (primarily free radicals), which cause oxidative destruction of proteins, lipids, and DNA (the so-called «oxidative stress»).

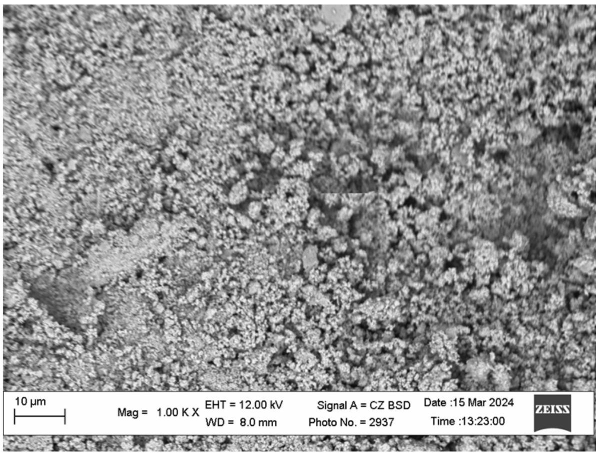
Based on studies of the antibacterial activity of the Cu_2O nanoparticles/clinoptilolite composite by the method of its direct addition into the nutrient medium, it was found that 100% bacterial death (both Gram-positive *Staphylococcus aureus subsp. aureus* ATCC 25923 and Gram-negative *Escherichia coli* ATCC 25922) was achieved at a composite concentration of 3 g/L in the suspension (Tables 1 and 2).

Therefore, the Cu_2O nanoparticles/clinoptilolite composite synthesized by the sonochemical method is an effective antibacterial agent.

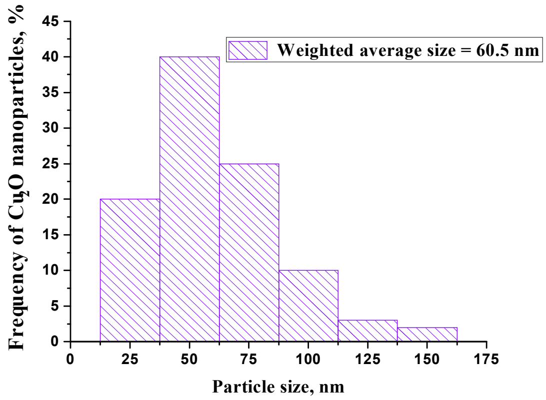
Conclusions

For the sonochemical synthesis (US frequency of 20 kHz and US power density of 200 W/L) of the Cu_2O nanoparticles/clinoptilolite composite, an aqueous solution of copper sulfate (precursor) and hydrazine (reductant of Cu^2z ions in the clinoptilolite framework to Cu_2O nanoparticles) were used.

A decrease in the intensity of clinoptilolite peaks on the XRD patterns of the synthesized composite was observed compared to the clinoptilolite diffractogram, along with the appearance of new peaks in the range of $2=30^0-93^0$, corresponding to the crystalline planes (110), (111), (200), (220), (311), (222), and (400) of cuprite (Cu_2O) with a cubic structure. The average diameter of the Cu_2O crystallite, calculated by the Debye-Scherrer equation, was approximately 13 nm. The SEM image of the synthesized composite indicated the uniform distribution of Cu_2O nanoparticles, with a weighted average size of 61 nm, across the surface of the clinoptilolite framework.



a



b

Fig. 3. SEM image of (a) the Cu_2O nanoparticles/clinoptilolite composite prepared by the sonochemical method, and (b) corresponding histogram of particle size distribution

Table 1

Antibacterial activity of Cu_2O nanoparticles/clinoptilolite composite against *Staphylococcus aureus* subsp. *aureus* ATCC 25923

Composite concentration in suspension, g/L	The average number of colony-forming units (CFUs)
0 (control)	cannot be determined (continuous growth)
1	cannot be determined (continuous growth)
2	100
3	0
4	0
5	0

Table 2

Antibacterial activity of Cu_2O nanoparticles/clinoptilolite composite against *Escherichia coli* ATCC 25922

Composite concentration in suspension, g/L	The average number of colony-forming units (CFUs)
0 (control)	cannot be determined (continuous growth)
1	cannot be determined (continuous growth)
2	50
3	0
4	0
5	0

The results of antibacterial activity studies confirmed the effectiveness of the synthesized product against Gram-positive (*Staphylococcus aureus* subsp. *aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922) microorganisms at a concentration of the Cu_2O nanoparticles/clinoptilolite composite in suspension ≥3 g/L.

Acknowledgments

The authors acknowledge the funding of the Ministry of Education and Science of Ukraine for the scientific research project of young scientists «Advanced oxidation processes, including nanocatalytic, based on cavitation technologies for purification of aqueous media from resistant N-substituted organic compounds» (state registration number 0122U000790).

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Received 03.02.2025

СОНОХІМІЧНИЙ СИНТЕЗ І АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ КОМПОЗИТУ НАНОЧАСТИНОК Cu_2O/КЛИНОПТИЛОЛІТ

Сухацький Ю., Созанський М., Мних Р., Знак З.

Композит наночастинки Cu_2O/клиноптилоліт було синтезовано сонохімічним методом (частота ультразвуку – 20 кГц; питома потужність ультразвуку – 200 Вт/дм^3) з використанням водного розчину мідного купоросу як прекурсора та гідразину для відновлення іонів Cu^{2+} у каркасі клиноптилоліту до наночастинок Cu_2O. Методом рентгенівської дифракції було підтверджено наявність фази куприту (Cu_2O) з кубічною структурою в одержаному композиті. За рівнянням Дебая-Шеррера було розраховано усереднений діаметр кристаліту Cu_2O, який становив приблизно 13 нм. Методом енергодисперсійної рентгенівської спектроскопії було визначено вміст Cu у композиті. На основі СЕМ-зображення композиту підтверджено рівномірність розподілу наночастинок Cu_2O із середньозваженим розміром 61 нм по поверхні каркаса клиноптилоліту. У результаті досліджень антибактеріальної активності композиту наночастинки Cu_2O/клиноптилоліт методом його прямого внесення у поживне середовище (м'ясо-пептонний агар) було встановлено, що загибелі 100% бактерій (як грам-позитивних *Staphylococcus aureus* subsp. *aureus* ATCC 25923, так і грам-негативних *Escherichia coli* ATCC 25922) досягали за концентрації композиту в суспензії 3 г/дм^3.

Ключові слова: наночастинки Cu_2O, клиноптилоліт, композит, сонохімічний синтез, метод прямого внесення, антибактеріальна активність.

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Yuriy Sukhatskiy *, Martyn Sozanskyi, Roman Mnykh, Zenovii Znak

Lviv Polytechnic National University, Lviv, Ukraine

*** e-mail: yurii.v.sukhatskyi@lpnu.ua**

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