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RESEARCH ARTICLE

Mushroom-Derived Chitosan–Copper Oxide Nanoparticle Hydrogel: A Multifunctional Biomaterial for Biomedical and Environmental Applications

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Abstract: Pleurotus species are widely grownup all over the world and are considered as the essential mushrooms for the trade. Approximately 25% of all grown mushrooms worldwide are produced only from Pleurotus mushrooms. Pleurotus ostreatus, also known as the oyster mushroom, is a widespread edible mushroom cultivated worldwide. The main objective of the study was to aimed at the fabrication and evaluation of a chitosan-based hydrogel infused with copper oxide nanoparticles (CuONPs), focusing on its potential as a biocompatible and multifunctional biomaterial, we develop a novel hydrogel incorporating mushroom-derived chitosan and CuONPs, evaluating its physicochemical, biological, and antimicrobial performance. The resulting composite hydrogel demonstrates significant potential for advanced biomedical applications, including wound healing, infection control, and drug delivery, as well as environmental uses such as water purification. This sustainable and high-performance material represents a promising step toward next-generation solutions in both healthcare and environmental remediation.

Keywords: Pleurotus ostreatus; Mushroom chitosan; Copper oxide nanoparticles; Composite hydrogel; Sustainable materials.

INTRODUCTION

As demand for sustainable and biocompatible materials increases, researchers are exploring cutting-edge alternatives to standard biopolymers. Chitosan, a naturally derived polysaccharide, has long been sourced from crustacean shells, but recent advances have introduced mushroom-derived chitosan as a superior, allergen-free, and eco-friendly alternative [1]. Fungal chitosan retains the beneficial properties of its crustacean counterpart—including biocompatibility, biodegradability, antimicrobial activity, and film-forming ability—while offering additional advantages such as enhanced antioxidant potential due to the presence of fungal polysaccharides like β-glucans [2]. Furthermore, its slightly lower degree of deacetylation influences solubility and charge density, potentially improving its bioactive performance in biomedical applications.

Copper oxide nanoparticles (CuONPs) have emerged as a versatile nanomaterial due to their exceptional physicochemical properties, including high surface area-to-volume ratio, catalytic efficiency, and potent antimicrobial activity [3]. Their ability to generate reactive oxygen species (ROS) makes them promising candidates for antibacterial, antifungal, and anticancer therapies, as well as environmental applications such as water purification and pollutant degradation [4].

Hydrogels, three-dimensional hydrophilic polymer networks, are extensively used in biomedical and environmental applications due to their high-water retention, biocompatibility properties [5]. Their porous structure facilitates controlled drug release, wound healing, and tissue regeneration, making them ideal for advanced wound dressings, drug delivery systems, and tissue engineering scaffolds [6]. The integration of chitosan with CuO NPs into a hydrogel matrix can synergistically enhance mechanical strength, antimicrobial activity, and bioactive functionality, offering a multifunctional platform for biomedical and environmental field [7].

Mushrooms are widely valued for their distinctive flavours and potent nutritional characteristics, which includes a wide range of dietary supplements. Thus, Oysters have been identified as a high source of vitamins, carbohydrates, proteins, dietary fibre, minerals, amino acids, and protein contents [8], making them an excellent vegetarian meat substitute with a low calorific value [9]. Additionally, studies have demonstrated the immune stimulating properties of mushrooms and anticancerous activity, as well as antioxidant, antidiabetic, and antitumor properties [10].

Pleurotus ostreatus (Basidiomycota) is a member of the Pleurotaceae family which is native to China but has now spread around the world. *P. ostreatus* contains substances with strong pharmacological properties, as it contains easily digested vitamins, proteins, and mineral

salts, making it an excellent nutritional and medicinal product. Mineral salts of copper, phosphorus, magnesium, potassium, zinc, calcium, iron, and selenium have been found in high concentrations in P. ostreatus mycelium *P. ostreatus* protein has a high concentration of exogenous amino acids, which are not produced by the human system and must be obtained in large quantities from diet. These exogenous amino acids include phenylalanine, lysine, and leucine [11].

In this study, we fabricate a novel composite hydrogel using mushroom-derived chitosan and CuONPs and evaluate its physicochemical, biological, and antimicrobial properties. By leveraging the unique advantages of fungal chitosan and the multifunctionality of CuONPs, this composite hydrogel holds significant potential in wound healing, infection control, drug delivery, and environmental remediation.

MATERIALS AND METHODS

Extraction of Chitosan from mushroom

Fresh *Pleurotus ostreatus* mushrooms was collected from Tamil Nadu Agricultural University, Coimbatore. The mushrooms were thoroughly cleaned to remove surface impurities and then dried by air-drying to preserve structural integrity. The dried mushrooms were ground into a fine powder using a mechanical grinder to increase surface area for subsequent chemical treatments.

Deproteinization

4 g of lyophilized mushroom powder was treated with 1 M sodium hydroxide (NaOH) at a ratio of 1:30 (w/v). Mixture was heated to 80°C and stirred continuously for 2 h to facilitate protein removal. The alkali-insoluble residue (AIR) was separated by centrifugation at 10,000 × g for 10 min, followed by repeated washing with deionized water until a neutral pH was achieved. The residue was then freeze-dried and stored for further processing [12].

Chitin Isolation

The AIR obtained from deproteinization was subjected to acidic hydrolysis using 2% acetic acid (CH_3COOH) at a solid-to-liquid ratio of 1:100 (w/v). The mixture was refluxed at 95°C for 6 h to solubilize glucans and other acid-soluble components, leaving behind purified chitin. The suspension was centrifuged (10,000 × g, 10 min), and the chitin-rich pellet was washed multiple times with deionized water until neutral pH was attained. The purified chitin was then lyophilized. The degree of deacetylation (DDA) was determined using Fourier-transform infrared spectroscopy (FTIR) or potentiometric titration [13].

Synthesis of Copper Oxide Nanoparticles (CuONPs)

0.1 M copper sulfate ($CuSO_4 \cdot 5H_2O$) was dissolved in distilled water, followed by dropwise addition of 0.2 M sodium hydroxide (NaOH) under vigorous stirring. The resulting precipitate was centrifuged (8,000 × g, 15 min), washed with ethanol and water, and dried at 60°C. The obtained CuONPs were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), and dynamic light scattering (DLS) to confirm crystallinity, morphology, and particle size distribution [14].

Confirmatory Test for Chitosan

Solubility Test

Take a small amount of the dried chitosan powder and add it to 1% acetic acid If it dissolves completely, it confirms chitosan presence. Chitin is insoluble, while chitosan is soluble in weak acid [15].

Ninhydrin Test

Dissolve a small amount of chitosan in 1% acetic acid. Add a few drops of ninhydrin reagent. Heat the mixture at 100°C for 5–10 minutes. Purple or blue colour indicates the presence of free amine groups.

Potentiometric Titration

Dissolve chitosan in 0.1 M HCl and titrate with NaOH. A distinct inflexion point indicates the presence of free amino groups. Confirms the degree of deacetylation (DD%), which differentiates chitosan (high DD) from chitin [16].

Fabrication of Chitosan-CuO Composite Hydrogel

The hydrogel was prepared by dissolving mushroom-derived chitosan (2% w/v) in 1% acetic acid under stirring. A predetermined amount of CuONPs (0.5–2% w/w relative to chitosan) was dispersed in the chitosan solution via ultrasonication (30 min, 40 kHz). To induce crosslinking, 0.5% (w/v) glutaraldehyde was added, and the mixture was cast into molds and allowed to gel at room temperature for 24 h [17]. The resulting hydrogels were washed with distilled water to remove unreacted crosslinkers and lyophilized for further characterization [18].

Characterization Studies

X-Ray Diffraction (XRD)

Dry the hydrogel completely to remove moisture and grind into fine powder load into sample holder. Set appropriate parameters (typically Cu Kα radiation, 40kV, 30mA), select scan range of (5-80° 2θ) and step size (0.02°). Run the scanner and collect the diffraction patterns [19].

Scanning Electron Microscope (SEM)

Ensure the hydrogel sample is completely dry to prevent outgassing under vacuum. Critical point drying or freeze-drying may be used for hydrogels to maintain structure. Mount the prepared sample on an SEM stub using conductive tape or adhesive. Vacuum Chamber: Place the stub inside the SEM chamber and evacuate to high vacuum (typically ~10^{-5} to 10^{-6} Torr). Adjust (typically 5–20 kV) based on sample composition and desired resolution. Set an appropriate distance (usually 5–15

mm) between the sample and the detector for optimal focus [20].

Physical Property Test - Swelling Test

Take a known amount of dried hydrogel sample and immerse it in phosphate-buffered saline (PBS, pH 7.4) at room temperature. At specific time intervals, remove the hydrogel, blot gently to remove surface water, and weigh it, formula given as

Swelling (%) = (Wt−W0 / W0) ×100

where Wt - swollen weight, W0 - initial dry weight. This method is commonly used to assess the water absorption capacity of hydrogels, providing insights into their potential applicability in biomedical fields [21].

***In vitro* Degradation Test**

To study enzymatic degradation, a known weight of the dried hydrogel sample is immersed in phosphate-buffered saline (PBS, pH 7.4) containing 1 mg/mL lysozyme, which simulates *in vivo* enzymatic conditions. The samples are incubated at 37 °C under mild shaking (50–60 rpm). At predetermined time intervals (e.g., days 1, 3, 7, 14, and 21), the hydrogels are taken out, rinsed gently with distilled water to remove surface enzyme, dried to constant weight, and reweighed, formula given as

Degradation (%) = (Wt−W0 / W0) ×100

where W0 is the initial dry weight and Wt is the weight after degradation at each time point. Lysozyme specifically degrades chitosan by cleaving its β-(1→4)-linkages, enabling assessment of hydrogel stability in biologically relevant enzymatic environments [22,23].

Antimicrobial Testing

The antibacterial efficacy of *Pleurotus ostreatus* - derived CuONPs was tested against *Escherichia coli* and *Staphylococcus aureus* using the agar diffusion assay. Mueller-Hinton Agar (MHA) plates were prepared with 20 mL of medium and inoculated uniformly with bacterial suspensions using sterile swabs. Filter paper discs impregnated with CuONPs (test), ampicillin (positive control), and distilled water (negative control) were aseptically placed on the agar surface. After 24-hour incubation at 37°C, the zones of inhibition were measured, with larger diameters indicating greater antibacterial activity.

RESULTS AND DISCUSSION

Extraction of Chitosan

Deproteinization of 4 g *Pleurotus ostreatus* produced 2.6 g of alkali-insoluble residue (AIR). Chitin isolation from AIR yielded 1.8 g of purified chitin. Deacetylation yielded as 1.4g. Each step showed effective removal of unwanted components. Similarly, chitosan derived from *P. ostreatus* waste exhibited a molecular weight of approximately 47 kDa and a DD between 79% and 84%, demonstrating significant prebiotic potential by promoting the growth of beneficial Lactobacillus strains and inhibiting pathogenic bacteria [24].

Confirmatory Test for Chitosan

Solubility Test

The dried chitosan powder dissolved completely in 1% acetic acid, confirming successful deacetylation. This solubility behaviour distinguishes chitosan from chitin, which remains insoluble under the same conditions.

Ninhydrin Test

The chitosan solution developed a purple coloration upon heating with ninhydrin, indicating the presence of free amine groups. This confirms successful deacetylation of chitin to chitosan.

Potentiometric Titration (ACID-BASE TITRATON)

To confirm the presence of free amino groups and determine the degree of deacetylation (DD%), 50 mg of chitosan was dissolved in 25 mL of 0.1 M HCl. The solution was titrated with 0.1 M NaOH while recording pH. A distinct inflection point was observed at 4.3 mL of NaOH, indicating the neutralization of $–NH_3^+$ groups and confirming successful chitin deacetylation, it was given in Figure 1.

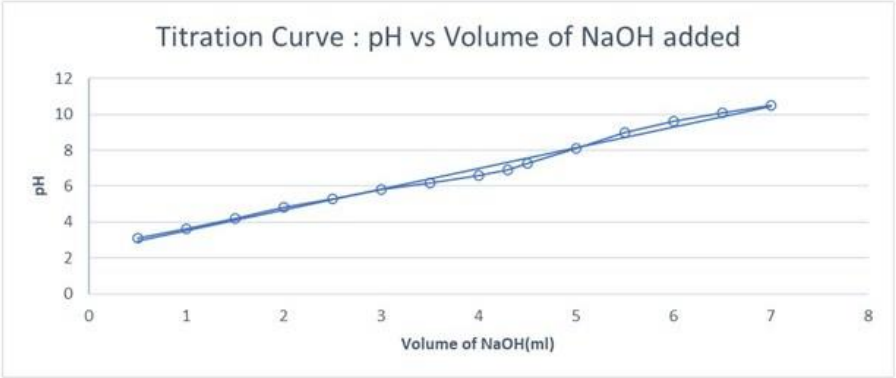

Figure 1: Acid- Base Titration Curve pH Vs NaOH

Synthesis of Copper Oxide Nanoparticles

A visible colour changes from blue to dark brown / black was observed within 2 hours of incubation, indicating the successful formation of copper oxide nanoparticles (CuONPs). After 24 hours, centrifuge at 5000rpm till a dark precipitate appears. Nanoparticles were thoroughly washed and dried at 80°C. The final yield of CuONPs was approximately 8–10 mg, confirming effective green synthesis using *Pleurotus ostreatus* extract, work has been highlighted the antioxidant and anticancer properties of CuONPs synthesized using *P. ostreatus* extracts, indicating their potential applications in biomedical fields [25].

Characterization Studies

Scanning Electron Microscope (SEM)

The provided SEM (Scanning Electron Microscope) Figure 2 (a, b, c and d) reveal the microstructural morphology of the sample at varying magnifications of 250x, 1000x, 2500x and 5,000x. The particles appear to be agglomerated with irregular shapes, displaying both fine and coarse granular structures.

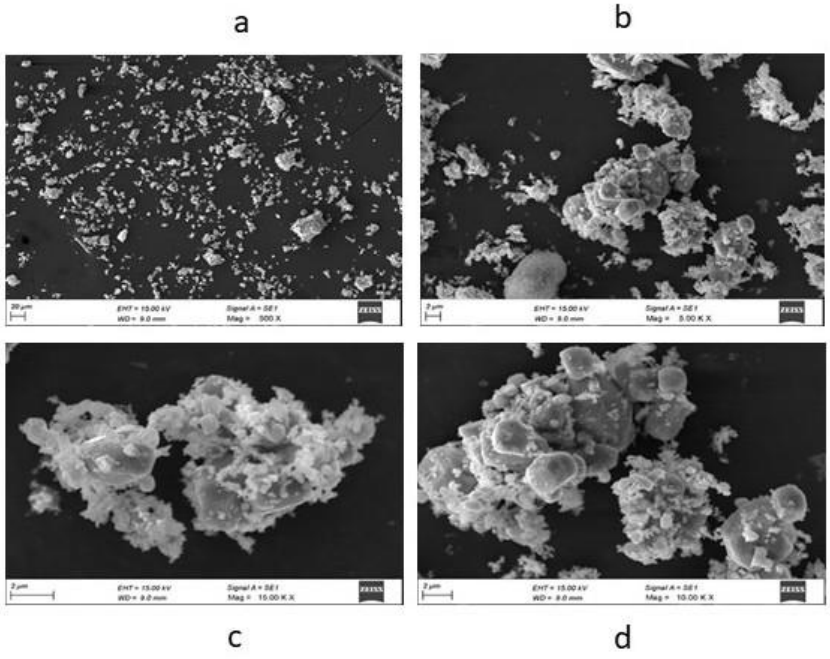

Figure 2: Scanning Electron Microscope CuONPs Synthesized from *Pleurotus ostreatus* with different magnification

At lower magnification (250x), a general overview shows loosely packed clusters of particles with varying sizes, suggesting a heterogeneous distribution. As the magnification increases to 250x and 5000x, more intricate surface details become visible, including faceted edges and smoother rounded surfaces, possibly indicating crystalline structures alongside amorphous debris. CuONPs synthesized using natural extracts exhibited irregular shapes and agglomerated formations, as observed through SEM analysis [26]. The presence of smaller particulates adhered to larger grains may suggest secondary particle formation or contamination. Overall, the images depict a complex microstructure with a mix of morphologies, indicating potential multi-phase composition or incomplete processing.

X-Ray Diffraction (XRD)

X-ray diffraction (XRD) analysis of Copperoxide (CuO) nanoparticles synthesized using *Pleurotus ostreatus* extract reveals a distinct crystalline pattern, confirming the successful biosynthesis of CuO in its tenorite phase. The XRD pattern of the sample shows several sharp peaks, indicating that the CuO sample is highly crystalline. The most intense diffraction peaks occur at 2θ values of 30.5° and 35.0°, which correspond to the crystallographic planes respectively. Additional peaks appear at other 2θ values such as 32.5°, 46.2°, 48.7°, 53.5°, 58.3°, 61.6°, 66.2°, 68.1°, 72.4°, 75.2°, 80.1°, and 83.2°, matching well with the standard JCPDS card for CuO (No. 45-0937). The high intensity and sharpness of the peaks indicate a well-crystallized phase without significant amorphous content or secondary phases, implying phase purity of CuO.

Figure 3 shows the XRD data illustrates the structural fingerprint of CuO, with the most intense peak observed at 38.7° and 35.5°, which corresponds to the (-111) and (111) plane. This peak was studied using Debye- Scherrer equation. Assuming a full width at half maximum (FWHM) of approximately 0.3°, the calculated crystallite size was found to be around 40–50 nanometers, confirming the formation of nanoscale particles. The absence of any extraneous peaks further emphasizes the phase purity and effective reduction of zinc precursors by the *Pleurotus ostreatus.* The overall XRD profile not only confirms the identity of ZnO but also supports its potential application in biomedical and antimicrobial fields due to its nanoscale features and structural integrity [19]

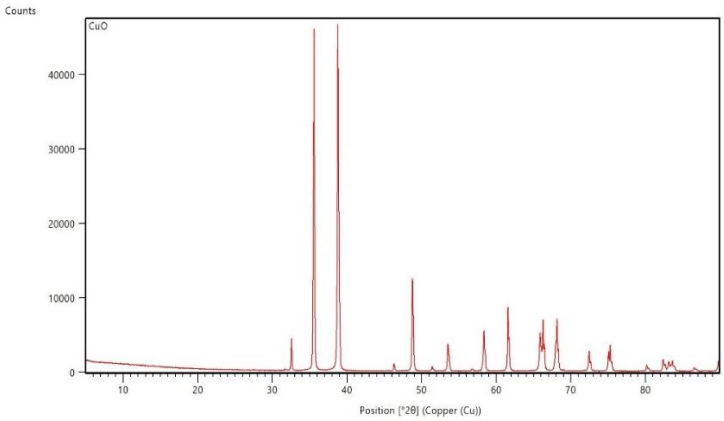

Figure 3: XRD Analysis CuONPs synthesized from *Pleurotus ostreatus*

Graphically, the pattern presents a well-resolved, symmetrical profile with no signs of amorphous humps, indicating a highly ordered crystal structure, which is essential for functional material performance [25]. The formation of CuONPs with desirable morphology and elemental structure was confirmed through various characterization techniques [26].

Physical Property Test

Swelling Test

The water absorption behaviour of the chitosan–CuO hydrogel was studied by adding 1 g of dried hydrogel was immersed in PBS (pH 7.4). At predetermined intervals, the hydrogel was taken out, weighed, and the percentage of swelling was determined, it was given in Figure 4.

Figure 4: Swelling behaviour of Chitosan Copperoxide Nanoparticle- Hydrogel

The hydrogel demonstrated a steady increase in swelling, reaching a maximum swelling of 40% after 24 hours. This significant water uptake capacity suggests that the chitosan–CuO hydrogel has promising moisture-retention properties, making it suitable for biomedical applications such as wound dressings and drug delivery systems. Recent studies have highlighted similar findings. For instance, a study reported that chitosan hydrogels absorbed substantial amounts of PBS, with equilibrium water content values ranging from 23 to 30 times their mass after 24 hours of immersion [27]. Swelling studies demonstrated its excellent water absorption capacity, essential for moisture regulation in biomedical use. his significant water uptake capacity is indicative of the hydrogel's ability to maintain a moist environment, essential for effective wound healing and controlled drug release.

***In vitro* Degradation Test**

To assess biodegradability, a 1g of dried hydrogel sample was immersed in PBS containing 1 mg/mL lysozyme and incubated at 37 °C with gentle shaking. The hydrogel was taken out, rinsed, dried, and weighed at certain intervals (Days 1, 3, 7, 14, and 21). Degradation was calculated as a percentage, it was given in Table 1.

Table 1 Degradation profile of chitosan–CuO hydrogel in lysozyme-containing PBS

DAYS	WEIGHT AFTER DEGRADATION	DEGRADATION (%)
0	**1.000**	**0.00%**
1	**0.985**	**1.50%**
3	**0.945**	**5.50%**
7	**0.885**	**11.50%**
14	**0.765**	**23.50%**
21	**0.645**	**35.50%**

The hydrogel exhibited gradual enzymatic degradation, reaching 35.5% degradation after 21 days. This confirms the biodegradable nature of chitosan in enzymatic environments and highlights its suitability for biomedical applications, such as drug delivery and tissue regeneration, where controlled degradation is essential. Similar study investigated the *in vitro* degradation of chitosan–genipin hydrogels in PBS with lysozyme. The results demonstrated that lysozyme effectively degrades the secondary amide linkages in the hydrogel network, leading to significant mass loss over time. This behaviour is consistent with the observed 35.5% degradation of the chitosan–CuO hydrogel over 21 days, highlighting the role of lysozyme in facilitating chitosan degradation in physiological conditions [28].

Antimicrobial Testing

The antimicrobial activity of the chitosan–CuO hydrogel was assessed using the agar well diffusion method against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The hydrogel was put into wells, which were incubated for 24 hours at 37 °C. To assess the suppression of bacterial growth, the zone of inhibition was evaluated and compared with standard Gentamycin discs, it was given in Table 2.

Table 2 Zone of inhibition of Chitosan-CuO Hydrogel

MICROORGANISM	ZONE OF INHIBITION HYDROGEL	ZONE OF INHIBITION Gentamycin
Staphylococcus aureus	1.7 mm	1.5 mm
Escherichia coli	0.9 mm	0.3 mm

Antimicrobial testing showed notable inhibition zones against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, highlighting its potential in infection control. The resulting chitosan– CuO hydrogel exhibited promising functional properties shown in Figure 5.

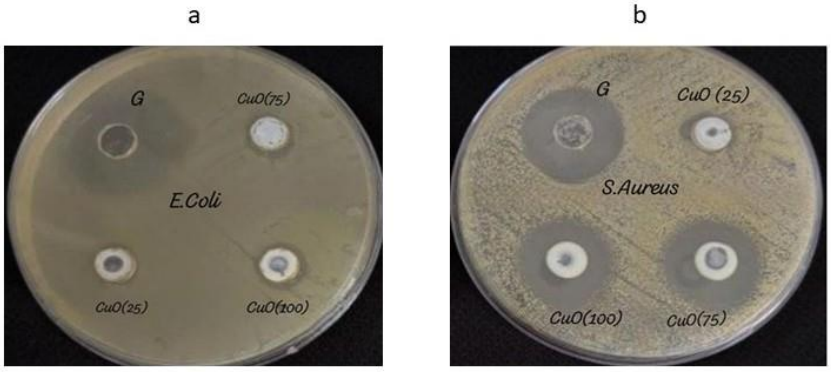

Figure 5: Antibacterial activity of Chitosan– CuO hydrogel

Another research demonstrated that chitosan/polyvinyl alcohol (PVA)/CuO nanocomposites showed notable inhibition zones against *S. aureus* and *E. coli*, attributed to the synergistic antibacterial effects of the constituents [29].

CONCLUSION

Chitosan was extracted from *Pleurotus ostreatus* mushrooms, offering a natural and biodegradable polymer base. Simultaneously, CuONPs were synthesized through a green method using mushroom extract, which acted as a reducing and stabilizing agent. Chitosan–CuO nanocomposite hydrogel developed in this study presents a sustainable, green-engineered solution with applications in wound care, drug delivery systems, personal care products and environmental remediation. Its multifunctional characteristics make it a promising for future research and development in advanced bio functional materials.

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Competing Interest

There are no competing interests among the authors.

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