Suitable-Biocide Formulation for Hospital Sanitization and Surface Protection

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ABSTRACT
The objective of this study was to develop a long-acting biocide spray to protect surfaces in hospital environments. The efficacy of the spray was tested against poliovirus, bacterial and fungi. The resulting was the deactivation of viral particles and excellent performance against bacterial and fungi. The formulation was utilized by the Ministry of Health of the Province of Buenos Aires, Argentina, during the pandemic in swab centers and vaccination facilities.

Keywords: Biocide, Nanoparticle, Hospital, Disinfectant

Introduction
Surface contamination in hospitals can serve as a potential reservoir for nosocomial pathogens. Therefore, it is crucial to understand the impact of different sanitization strategies on the clinical microbiomes in order to select the appropriate methodology. Hospital-associated infections (HAIs) continue to pose significant challenges in healthcare worldwide [1]. Consequently, sanitization programs for clinical environments are considered essential for the prevention and control of hospital infections [1-3]. However, traditional disinfectants have several limitations. Primarily, they are not effective against recontamination of cleaned surfaces, as disinfection is only temporary. Recontamination has been reported to occur as early as 30 minutes after disinfection [4-5]. Additionally, these disinfectants have adverse effects on the environment [6-7]. Concerns exist about human exposure to carcinogenic volatile organic compounds (VOCs), such as benzene. Given the extensive use of sanitizers during the COVID-19 pandemic, it is crucial to investigate the potential exposure to toxicants present in these products [8-9].

In recent years, there has been a reevaluation of hospital surface disinfection, driven by the urgent need for effective and sustainable sanitization solutions. A new concept of surface disinfectants is in demand. This paper presents the development of a surface disinfectant spray that is environmentally friendly (benzene-free) and has the ability to remain effective on surfaces for over 24 hours. This innovative technology ensures longer-lasting disinfection of various surfaces in hospitals, thereby reducing the incidence of HAIs.

Methodology
An aqueous-based biocide formulation was developed, consisting of a solution of alcohol and silane, along with a dispersion of silver and copper nanoparticles, the final chemical composition was as follows: 1% silane (10 ml/1000 ml) (potassium methylsiliconate 2.4 M; SILRES BS16; CAS 31795-24-1); 1.6% nanosilver (16 ml/1000 ml); 0.8% nanocopper dispersion (8 ml/1000 ml); 70.9% 96° ethanol (CAS 64-17-5) (709 ml/1000 ml); and 25.7% distilled water (257 ml/1000 ml). For the alcohol-based biocide, the final chemical composition was 1% silane (10 ml/1000 ml) (potassium methylsiliconate 2.4 M; SILRES BS16; CAS 31795-24-1); 1.6% nanosilver (16 ml/1000 ml); 0.8% nanocopper dispersion (8 ml/1000 ml); 70.9% 96° ethanol (CAS 64-17-5) (709 ml/1000 ml); and 25.7% distilled water (257 ml/1000 ml).

Results
Bactericidal Activity
A bacterial suspension of 10-7 CFU/mL was used for the experiment (S. aureus and E. coli). The biocidal efficiency was assessed by mixing the antibacterial emulsion with an equal amount of bacterial suspension. This mixture was spread on nutrient agar plates with and without biocide formulation and incubated at 37 °C for 24 hours. The number of viable cells (colonies) was manually counted and expressed as the mean CFU/mL. The results are presented in Figure 1.

Figure 1: Bacterial cultures, where A represents S. aureus and B represents E. coli: A1 and B1 are controls for each strain; A2 and B2 are treated with a commercial biocide, while A3 and B3 are treated with the developed formulation.

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Figure 1 shows the inhibition of bacterial growth by the formulation, where no colony forming units were observed in comparison with the control or the commercial biocide.

**Fungal activity**

This technology was tested using the product on wood, exposing it to specific fungi to obtain a more demanding biocidal assay. For this purpose, wood samples were exposed to three species of wood-decay fungi: Coniophora puteana (brown rot) and Pleurotus ostreatus (white rot), following the general guidelines of ASTM D 2017 standard.

Once inoculated, the specimens were cultivated under controlled humidity and temperature conditions (25±2 °C, 60±5%RH) for 16 weeks. The resulting deterioration was measured gravimetrically. As shown in Figure 2, the formulation exhibited antifungal activity (lower percentage of mass loss) against both fungal species.

**Virucidal Activity**

The virucidal assay was conducted according to the ASTM E 1053-1 standard (surface test) using poliovirus type 1 (PV-1) Sabin strain. The test was performed at the Faculty of Pharmacy and Biochemistry, University of Buenos Aires. A stock of PV-1 was placed in sterile Petri dishes and treated with the evaluated product. The product was allowed to act, and the liquid from each plate was collected using a sterile brush and placed in a tube on ice for viral plaque counting. The counting was performed by infecting confluent monolayers of Vero cells (in quadruplicate). Additionally, 0.5 ml of sterile infection medium was added to the product to test for cytotoxicity. The results demonstrated that the disinfectant product reduced the number of infectious viral particles by more than 99.9983% compared to the untreated control virus. Furthermore, it did not result in cytotoxicity for Vero cells.

**Ecotoxicity Tests**

Acute toxicity tests were performed on plant cultures exposed to high concentrations of the product in order to see differences in growth versus a normal plant. It is expected that there will be no differences in stem growth in order to corroborate that these compounds are neither harmful nor ecotoxic. They were evaluated at five nominal concentrations with four replicates, plus the control, in a 6 x 4 Completely Randomized Block Design (CRBD). The efficacy of the treatments was evaluated through a one-way Analysis of Variance (ANOVA), after transforming the data to arcsine square root. In the case of significant differences between replicates, a Turkey test was performed.

<table>
<thead>
<tr>
<th>Species</th>
<th>A. cepa</th>
<th>A. sativa</th>
<th>B. napus</th>
<th>S. alba</th>
<th>S. lycopersicum</th>
<th>P. vulgaris</th>
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<tr>
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<td>Control</td>
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<td>≥5.00</td>
<td>≥5.00</td>
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<td>(0.0-0.12)</td>
<td>(n.d.)</td>
<td>(0.03-0.25)</td>
<td>(-)</td>
<td>(0.13-1.13)</td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>≥5.00</td>
<td>1.67</td>
<td>0.56</td>
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<td>≥5.00</td>
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<td>≥5.00</td>
<td>1.51</td>
<td>0.44</td>
<td>0.43</td>
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<td></td>
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<td>(0.04-303)</td>
<td>(n.d.)</td>
<td>(0.09-0.36)</td>
<td>(0.09-0.85)</td>
<td>(-)</td>
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<td>(0.36-1.12)</td>
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</tbody>
</table>

In all the cases it is observed that there is no difference in each of the stages with the addition of the compound in the growth and survival of the plants, obtaining as a result that the formulation is not harmful or toxic.

**Leaching**

Leaching tests were performed on different materials immersed in water for various periods (ranging from 1 hour to 72 hours with intermediate measurements), and the residual residues in the water were measured using UV spectrophotometry. Specific wavelengths were employed for each individual material in the mixture. The results indicated that none of the active components of the formulation were detected in the water after the immersion period, indicating the sustained action of the product and the absence of residual toxicity.

**Conclusion**

The developed formulation acts through three mechanisms: 1) sanitizing the applied surface, 2) creating impermeability to prevent the retention of microdroplets or secretion droplets carrying pathogens, and 3) containing active ingredients that deactivate pathogens upon contact, reducing or eliminating their pathogenicity.

This environmental-friendly formulation offers prolonged protection on the surfaces to which it is applied, preventing infections resulting from contact with contaminated surfaces. It can be easily applied through spraying or atomization due to its low viscosity and does not require drying or curing time, allowing for quick application and reaplication.
References


