

# Nanostructured Calcium Phosphate Dihydrate-Glutaraldehyde Complex as an Innovative Biocidal Film for Anti Sars-Cov-2 Protection of Inanimate Surfaces, Air and a Water Sanitization

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## Abstract

Glutaraldehyde 2% represents a significant anti-SARS-CoV-2 reference. Blocking one of the 1.5 pent-dial functions with nanostructured Dicalcium Phosphate Dihydrate (DCPD) yields a stable, brick-red compound. Antibiofilm tests on the reference bacterial layers of the Institute Pasteur (Algeria) and on resistant bacterial layers of some hospitals confirm that the "calcium phosphate-glutaraldehyde" complex retains the biocidal property. Electron microscopy reveals a porous material while the EDX microanalysis confirms the phosphor-organic structure. The biocidal property and the porous morphology of the material make it possible to consider protective films for inanimate surfaces, air purification filters and water purification filters against SARS-CoV-2 and fragments of viral genetic material.

## Keywords

SARS-CoV-2; Fragment genetic material; Protection of inanimate surfaces; Air purification; Water purification

## Introduction

Glutaraldehyde (GL) is a broad-spectrum biocide [1-3]. It inactivates the walls by interaction with the proteins of the active biological particles [4]. The internal proteins of bacteria and viruses can be denatured [5]. Furthermore, it is an excellent element for protein fixation (Figure 1) [6].

Glutaraldehyde is used as a cross-linking agent for collagen-based biomaterials. The mechanism is based on the action of amine on the aldehyde function. The reaction of a sugar with GL relates to the carbonyl group of the aldehyde as a cross-linking point with the amino group of the sugar [7]. This type of reaction is part of the carbonyl-amine spectrum. It follows the general reactivity mechanism of proteins and collagen.

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Glutaraldehyde reduces the virulence of the SARS-CoV-2. The 2% solution proved to be effective with a reduction to 3.0 Log<sub>10</sub> of the viral titer [8]. It also constitutes an excellent anti-SARS-CoV-2 and anti-genetic biocide for fragments found on surfaces. However, the issue with air, as reported by Liu Y [9], and water by Wurtzer S [10] remain unresolved.

In this work, we introduce a new biocide obtained by the action of calcium phosphate dihydrate on glutaraldehyde. The compound obtained provides protection against SARS-CoV-2 attached to inanimate surfaces and can be used for the decontamination of air and water.

## Material and Methods

Nanostructured calcium phosphate di-hydrate-Glutaraldehyde (DCPD-GL) complexation reaction on (Figure 2) [11].

## Hospital strains

The strains were isolated from patients hospitalized in the various departments of El Kettar University hospital center for Infectious Diseases of Algiers.

The young culture was prepared by seeding a few colonies of bacteria/yeasts on agar media (nutritive agar GN), incubated 24 h at 37°C for bacteria and 30°C for yeasts.

## Result

### Diffusion methods on discs (Antibiogram)

Inoculate the agar surface with the strain to be studied using a sterile platinum loop using the quadrant method. Impregnate sterile absorbent discs of 9 mm in the test solution using a sterile forceps; place the impregnated discs on the surface of the culture media, previously prepared, by pressing lightly on the disc, to ensure uniform contact with the culture medium. Incubation was carried out at 37°C for 24 hours for bacteria and 30°C for 48-72 hours for yeasts.

Reading is done by measuring the diameter of the muting zones around the disc using a ruler. The interpretation of the results is done according to the scale of estimation of antimicrobial activity given by the fascicle for standardization of the antibiogram on a national scale [12] (Figures 3 and 4, Tables 1 and 2).

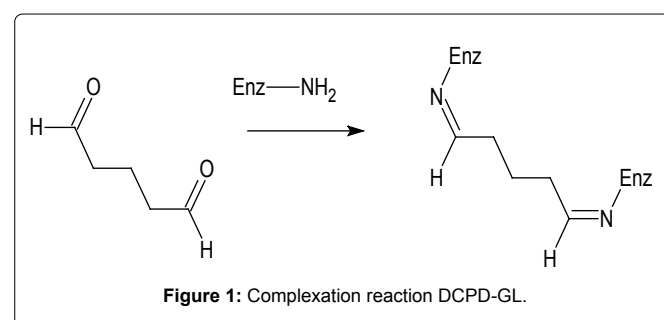


Figure 1: Complexation reaction DCPD-GL.

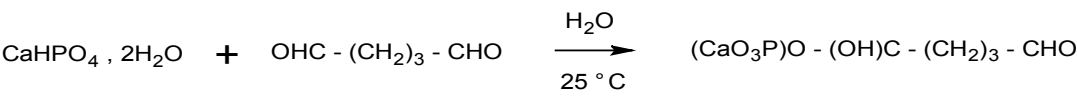


Figure 2: Complexation reaction DCPD-GL.

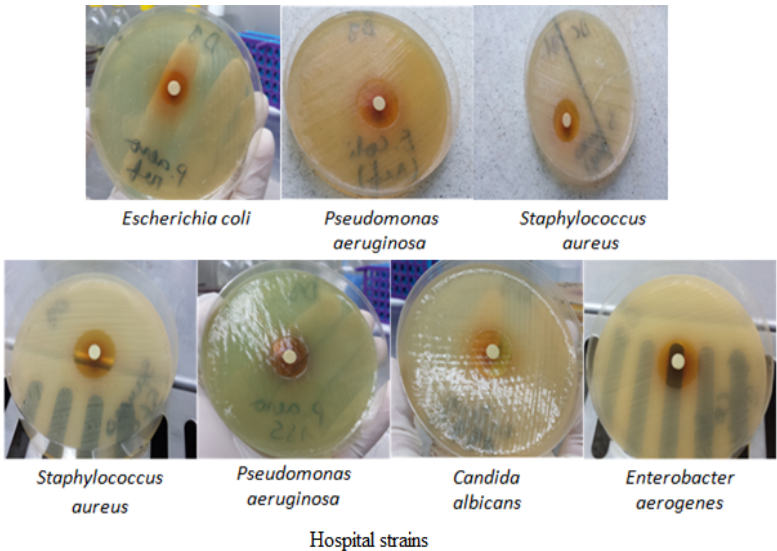


Figure 3: Glutaraldehyde antibiogram on reference strains and hospital strains.

Table 1: GL antibiogram results.

Reference strains				
GL	Test 1	Test 2	Test 3	Mean
Staphylococcus aureus	22	22	24	22
Escherichia coli	23	24	23	23.3
Pseudomonas aeruginosa	16	20	16	17.3
Hospital strains				
GL	Test 1	Test 2	Test 3	Mean
Staphylococcus aureus	24	30	25	26,3
Escherichia coli	21	22	22	21,6
Pseudomonas aeruginosa	20	20	20	20
Candida albicans	19	16	20	18,3
Enterobacteraerogenes	21	20	20	20,3

Table 2: DCPD-GL antibiogram results.

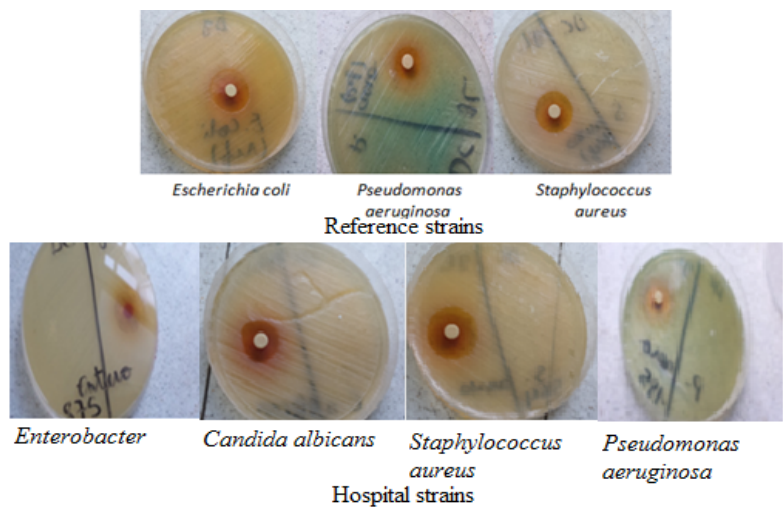
Reference strains				
DCPD-GL	Test 1	Test 2	Test 3	Mean
Staphylococcus aureus	20	21	24	21,6
Escherichia coli	24	22	20	22
Pseudomonas aeruginosa	15	18	15	16
Hospital strains				
DCPD-GL	Test 1	Test 2	Test 3	Mean
Staphylococcus aureus	19	25	24	22.6
Escherichia coli	20	22	20	21.3
Pseudomonas aeruginosa	17	19	17	17.6
Candida albicans	17	15	19	17
Enterobacter	20	17	19	18.6

Discussion

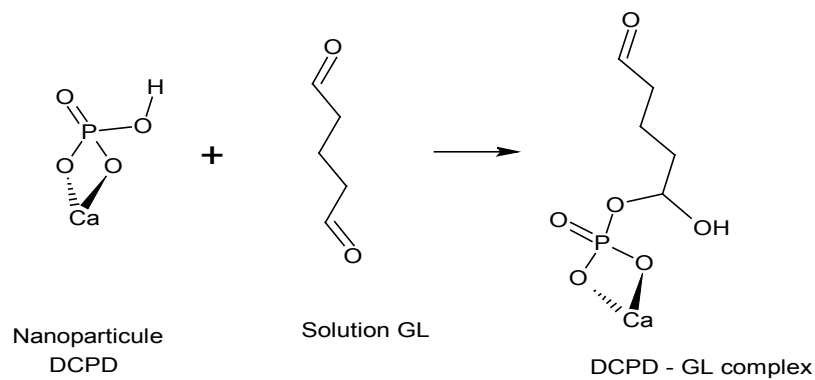
The anti-biograms of both GL and DCPD-GL complex on hospital strains and reference strains show microbiological activity on the various germs. Indeed, the diameters of the inhibition zones obtained are in the range 16 cm-25 cm. Furthermore, as reported [13], It is used in the disinfection of thermo-sensitive instruments in highly pathogenic medium. A 2% activated glutaraldehyde solution may be the most effective biocide agent for disinfection and decontamination of fibro-optic bronchoscopes [14] and in particular, the deactivations of inanimate surfaces at risk ofSARS-CoV-2 [8]. Glutaraldehyde

represents an appreciable reference as a bactericide and virucide in the design of molecules adapted to the requirements of hospital hygiene (Figures 5-7) [15,16].

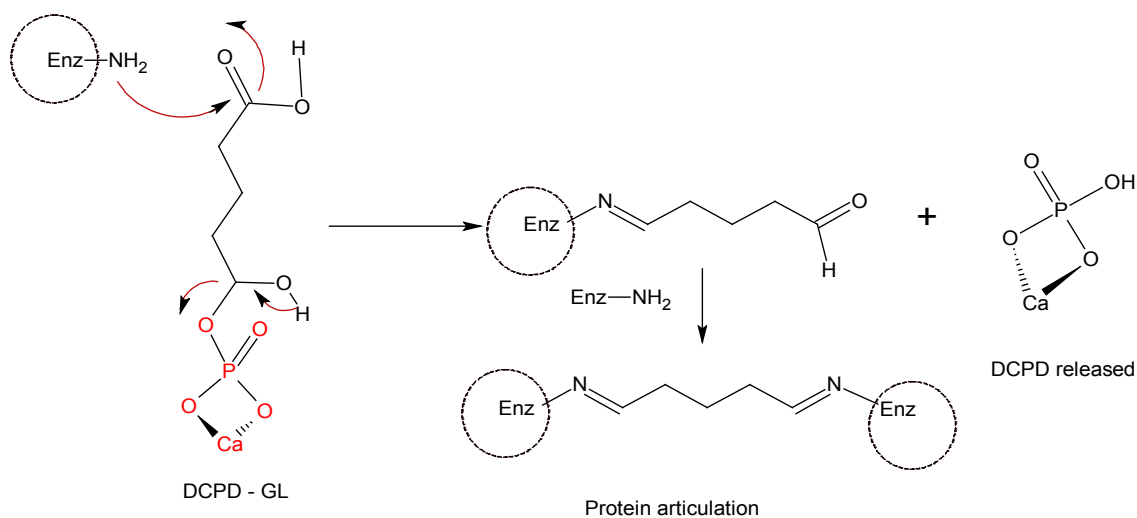
The complexation of glutaraldehyde by DCPD gives a stable brick-red compound (Figure 8). Electron microscopy shows a porous compound (Figure 9). While a coupled X-ray microanalysis confirms its phospho-organic structure (Figure 10). The microbiological activity of the DCPD-GL complex is similar to that of glutaraldehyde as shown by comparing the means (Figures 3 and 4, Tables 1 and 2). This result can be chemically explained: The Phosphate dihydrate



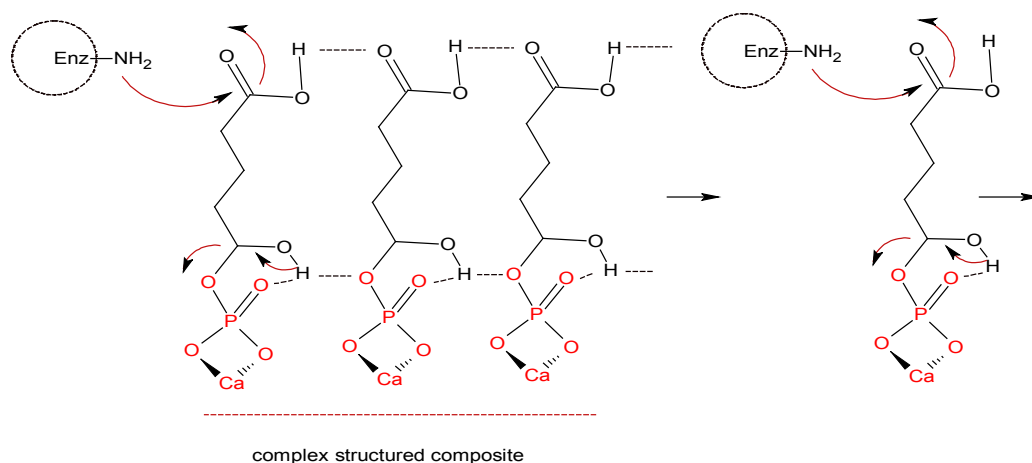
**Figure 4:** Antibiotic results of DCPD-GL on reference and hospital strains.



**Figure 5:** DCPD-GL complex.



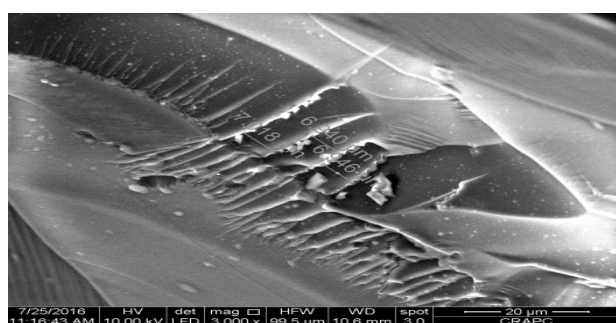
**Figure 6:** DCPD-GL complex protein articulation mechanism.



**Figure 7:** Mechanism of action of the protective film of the complex structured DCPD-GL.



**Figure 8:** Aspect of the complex structured DCPD-GL complex film.



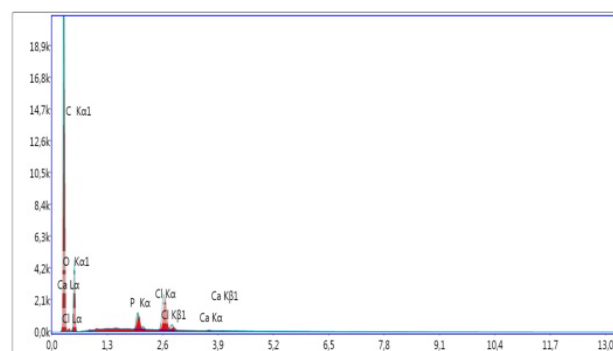
**Figure 9:** SEM Complex structured DCPD-GL.

blocks one of the functions of pent-1.5 dial in water. The material can be obtained in solution, in the form of a thin film or in the form of crystals.

The action of the DCPD-GL complex on the bacteria (Enz-NH<sub>2</sub>) results in a release of DCPD in the medium after formation of the schiff base.

## Conclusion

The 2% glutaraldehyde solution is a broad spectrum biocide. It



**Figure 10:** EDX confirms the constitution of the complex structured DCPD-GL.

deactivates the SARS-Cov-2 Strain-229E. The action of nanostructured dicalcium phosphate dihydrate on the glutaraldehyde molecule gives a stable brick-red, neuter, odorless, and porous material. Biocidal property remains similar to the glutaraldehyde molecule. The DCPD-GL complex has pressing applications as a protective film for sensitive inanimate surfaces, as a filter for cleaning air and for purifying water in biologically active environments SARS-Covid-2. Complexation of glutaraldehyde with nanostructured dicalcium phosphate dihydrate blocking one of the two functions makes it possible to obtain a stable solid form. The porous material shows a biocidal property similar to glutaraldehyde in solution.

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