v. 2, n. 10, 2022

Journal of
Engineering
Research

ELABORATION OF ANTISEPTIC GEL BASED ON CALENDULA OFFICINALIS AND TITANIUM OXIDE NANOPARTICLES DECORATED WITH SILVER

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Abstract: The nanometer size ($1x10^{-12}$m) confers the ability to penetrate different biological membranes, increasing the effectiveness for the use of treatments and combat diseases. People are susceptible to presenting skin infections that are derived from the increase in contaminating agents present in the environment and microorganisms, such as bacteria and fungi, which have direct contact with the skin. The goal of this paper is to develop an antiseptic gel based on *Calendula officinalis* extract and nanoparticles of TiO_2/Ag at 1 and 3%. TiO_2 was obtained by the photodeposition method to generate a synergy that increases its antimicrobial and fungicidal effects for its application. Titanium dioxide nanoparticles were decorated with 3% of their total weight in silver. Antimicrobial tests were performed in vitro media. The eradication of microorganisms was achieved, the gel presented antimicrobial activity, which is favored by increasing the concentration of nanoparticles and materials involved such as the extract of *Calendula officinalis* and TiO_2/Ag 3%.

Keywords: Titanium oxide, antiseptic, *Calendula officinalis*, nanoparticles, in vitro media, silver.

INTRODUCTION

There are products developed based on innovation and application in different fields of research, most of these fields give way to involving emerging sciences, thus maintaining a constant evolution. Nanoscience and nanotechnology offer wide possibilities for new uses and applicable technologies. These nanomaterials are not exempt from maintaining a relationship with organic compounds, both in their possible synthesis methods and in their application, giving rise to possible products that present economic and environmental advantages in their production. The problem that is planned to be solved is that people are susceptible beings to suffer from infections in our living tissue, since the skin is totally exposed to microorganisms that can be infectious. Whether it is an accident, insect bites, infected wounds, etc., the use of antiseptics is also required for clinical cases such as diseases and treatment of first-degree wounds.

Only in Mexico, there is a problem related to the epidemiology of wounds. The Ministry of Health, in 2018, mentioned the epidemiological characteristics and costs of wound care of the medical units and affected people in the area, because the price of antiseptics implies high costs for the treatment of acute and chronic wounds. It is estimated that 26.6% of the Mexican population has traumatic injuries and 23.4% corresponds to diabetic foot ulcers. Certainly, in cases of minimal severity, simple antiseptic treatments may be sufficient to meet the stated objective, although their costs in terms of quality-price should be mentioned. But, visualizing problems of greater difficulty, the application of antiseptics with a high bactericidal and fungicidal capacity is necessary. In addition, the need to generate products with these capabilities at a lower cost to allow their acquisition to a greater number of people.

Anjali John carried out a project together with his collaborators under the premise of green synthesis of silver nanoparticles using *Parkia biglandulosa* leaf extract as a reducing agent for antibacterial tests (Anjali John et al., 2021).

Within the process of testing and applying nanoparticles in the project, we find limitations, the main one of which is the current pandemic that prevents the development of the project as established by the scientific method. In addition to the difficulty of identifying the species of microorganisms growing in bacterial culture media.

OBJECTIVE

Prepare an antiseptic gel based on *Calendula officinalis* and TiO_2/Ag 1,2 and 3% nanoparticles to characterize the generate a synergy that increases its antimicrobial and fungicidal effects for its application.

EXPERIMENTATION

MATERIALS

Silver nitrate ($AgNO_3$), and Titania P25 in form of powder was purchased from Sigma-Aldrich. carbomer homopolymer and Glycerol was purchased from MERCK. Leaves of *Calendula Officinalis* was collected to obtain the extract used in the experiment. Solvents as ethanol, triethanolamine with high purity (>98%), and hydrochloric acid (Purity: 36.5%-38%) was purchased from Sigma-Aldrich. Commercial *Calendula officinalis hydrolate from lowers of pant were used.*

METHODS

Synthesis of TiO2 nanoparticles decorated with silver.

Commercial TiO2 P25 nanoparticles were used. 9.9 g of TiO_2 and 0.158 g of silver nitrate were deposited in 150 mL of ethanol and mechanically stirred for 15 min to obtain 1% of dissolution. Subsequently, the dissolution was placed in an ultrasonic bath for 5 min. Subsequently, the dissolution is irradiated by ultraviolet light (15W type A, 250 nm) for two hours. Afterward, the resulting material is placed on a rotary evaporator at 80°C and 40 RPM for 90 min. Finally, the resulting solution is placed in a muffle at 80°C for 12 hours. The same process was done to obtain the 3% dissolution modifying the silver nitrate concentration (0.368 g $AgNO_3$).

X-ray characterization

The X-ray diffraction characterization technique is a characterization technique that allows the study of crystalline structures of solid materials, both natural and synthetic. Its lattice parameters and crystal size are analyzed with a fine monochromatic X-ray beam, which is passed through the collimator system that falls on the sample. (Ramírez et al., 2013). X-ray diffraction was performed by Siemens D-5000 diffractometer with CuKα1 radiation and monochromator graphite beam. Intensity was measured by scanning in the 2θ range between 10° and 70°.

FTIR characterization

To study possible structural changes in the material, infrared attenuated total reflectance was performed with a FTIR-ATR Nicolette Avatar 360 spectrometer.

SEM characterization

Cuenta 250 scanning electronic microscopy was used with Energy dispersive spectrometer (EDS) detector. As the acceleration voltage 10 kV were used and the SEM images were taken from the secondary electron signal.

Preparation of alcohol-based gel (ethanol) and *Calendula officinalis*:

For the preparation of the gel, 90 mL of ethanol were placed in a large container that would allow the materials to be mixed correctly with a balloon. 0.70 g of carbopol was added by passing through a fine strainer and shaken vigorously. Once the incorporation of carbopol is observed, 0.25 g of pure glycerin are added and stirring is continued gently. When no lumps are seen in the mixture, add 10 mL of extract of *C. officinalis* while stirring gently and we proceed to add 1 mL of triethanolamine drop by drop without stopping stirring. The result was kept in a cool and dry place.

Incorporation of TiO_2/Ag 3% nanoparticles to the gel:

Three 50 mL samples were separated to incorporate the nanoparticles. The amounts of TiO2/Ag 3% nanoparticles were weighed, and 0.0125 g (Concentration of 0.25 g/L) was added to the first sample, in the second sample 0.025 g (Concentration of 0.5 g/L) was added and in the third 0.05 g (Concentration of 1 g/L) was added. For its incorporation into the gel, when added, it was started by stirring with a sterilized stirrer until a homogenized mixture was obtained, and then it underwent a sonication process for 20 minutes. The color of the gel change from orange to pink as concentration increase.

Preparation of bacterial culture media:

The preparation of the growing medium consists of dissolving the thermolabile substances in distilled water following the instructions, sterilizing them by filtration and adding them to the rest of the components after they have been previously sterilized in an autoclave and cooled to room temperature or 40- 50 ºC in the case of media with agar. Before sterilization, the liquid medium are divided into the appropriate containers, such as flasks. If it is a solid medium and it is to be distributed in tubes or flasks, it will be necessary to melt the agar in a water bath or microwave oven. Once melted and homogenized, it is distributed hot to the tubes or flasks, covered, and sterilized in an autoclave.

Growing medium were cooled at room temperature (26° C and 28% humidity), until they acquired a gelatinous consistency. The Petri dishes are prepared by pouring the molten and sterile medium inside them and in a in an aseptic environment (for example, near the flame of a Bunsen burner), it is advisable to homogenize the medium during the operation to prevent the agar from settling at the bottom of the container.

RESULTS AND DISCUSSION

Synthesis of TiO2 nanoparticles decorated with silver.

X-ray characterization:

X-ray diffraction was performed using a siemens D-5000 diffractometer with CuKα1 radiation and a monochromator graphite beam. Intensity was measured by scanning in the 2θ range between 10° and 70°. The resulting diffraction spectra of the samples show the characteristic peaks corresponding to the crystalline phases of anatase and rutile, characteristic morphologies of titanium oxide. With respect to the titanium and silver oxide coating, it was observed that the peaks are characteristic of materials with a particle size on the nanometric scale. As shown in the diffractogram in Figure 2, the sample of TiO_2 nanoparticles decorated with 3% silver showed the tetragonal anatase crystalline phase with its space group of I41/amd (141) and tetragonal rutile phase with its space group P42/mnm (136), with cubic silver with its space group Fm-3m (225). diffractogram with 1% concentration is not shown due to concentration is not detected by the equipment.

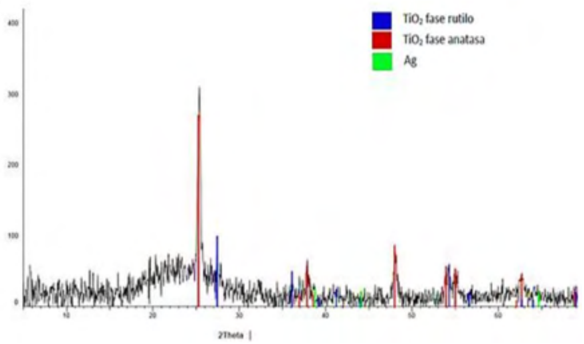


Figure 1. X-ray diffractogram of the TiO_2/Ag 3% nanoparticle sample.

FTIR Characterization

Figure 2 shows the FTIR spectrum of the comparison of the concentrations of titanium nanoparticles decorated with silver, at 1%

and 3. The vibrational elongation in the O-H band at a wavelength of 3423 cm^{-1} is observed, it comes from the occluded water and is generated due to the bending vibration of the hydrogen atoms attached to the oxygen atoms, presenting in the two concentrations. On the other hand, OH groups linked to Ti atoms (Ti-OH) can also be seen, corresponding to 3662 cm^{-1} and 1659cm^{-1}, which are the vibrations of hydroxyl groups, mainly in water adsorbed on the surface of Titania as can be seen in Ti-C catalysts in the band 1132cm^{-1} – 1238cm^{-1}, in the wavelength 1392cm^{-1} sulfate ions and Titania (Ti-O-SO) are observed, as in the wavelength at 890 and 450cm^{-1} are assigned to the Ti-O-Ti groups, their separation wave is due to the asymmetric and symmetric sections, such as the absorption peak of the ethanol functional group at the wavelength 1091cm^{-1} .it is observed vibration elongation bands and the reflection assigned to the C-H bond at 2926 and 1464 cm^{-1} respectively, coming from alkoxides that were used in the synthesis.

SEM Characterization

In morphological analysis and EDS of the specimens, they show that the concentration of 3% presents a better dispersion on the surface of the substrate, the EDS analysis confirms that the silver is superficial. It is confirmed that at a concentration of 1%, silver is found in small proportions on the surface, causing the detector not to identify this material as Figure 3 shows.

Antimicrobial test of the gel with incorporated TiO_2/Ag 3% nanoparticles:

For the *in vitro* tests, four media were used, which consist of one for each test of the concentration of TiO_2/Ag 3% nanoparticles that are 1 g/L, 0.5 g/L and 0.25 g/L, and a test of white gel, which consists of the preparation of ethanol-based gel without adding *C. officinalis* extract or nanoparticles. In the samples a marked difference can be seen between the tonality of the samples with the bioactive compound and the white, while between them the change in tonality is slightly perceptible.

The tests consisted in the bacterial colonies grew, a dose of gel (approximately 0.3g-0.5g) was added with a sterile filament in the center of the plate, and the diameter of the disinfected area measured in millimeters was constantly recorded. Table 1 shows the record of the disinfected area for each of the samples over time. The antiseptic gel without added compounds (Blank) exhibits an inhibitory activity derived from ethanol base against colonies grown on agar.

The antiseptic gel contains 10% *Calendula officinalis* extract, and as the concentration of silver-decorated titanium oxide nanoparticles increases, the antimicrobial activity of the gel increases. This is due to the synergy that occurs between the antiseptic effects of ethanol, demonstrated in the first test, added to the effects of the *C. officinalis* extract and the TiO_2/Ag 3% nanoparticles.

The total test lasted 22 hours, where all the tests were successful in eradicating the microorganisms, but it should be noted, the superior efficiency of the gel by incorporating the nanoparticles in the first hours of the test. Certainly, the best results were provided by the sample with the highest concentration, but seeing a simple relationship between results and concentration, the most effective sample compared was that of 0.5 g/L, considering a lower use of synthetic chemical compounds, benefiting both the biological and the economic part.

Figures 4A, B, C and D show the antimicrobial activity of the gel on the colonies presented in the petri dishes after the inhibition tests, and as recorded in Table 1, the disinfected areas are of different sizes depending on the concentration. The gel

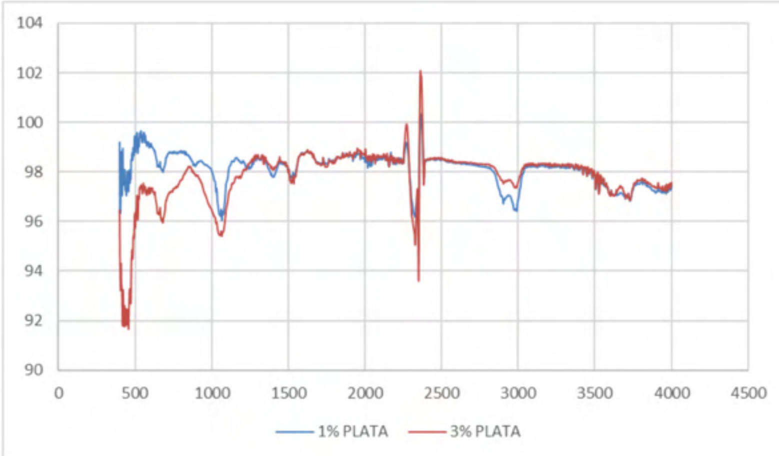


Figure 2. FTIR comparison of 1% and 3% concentrations.

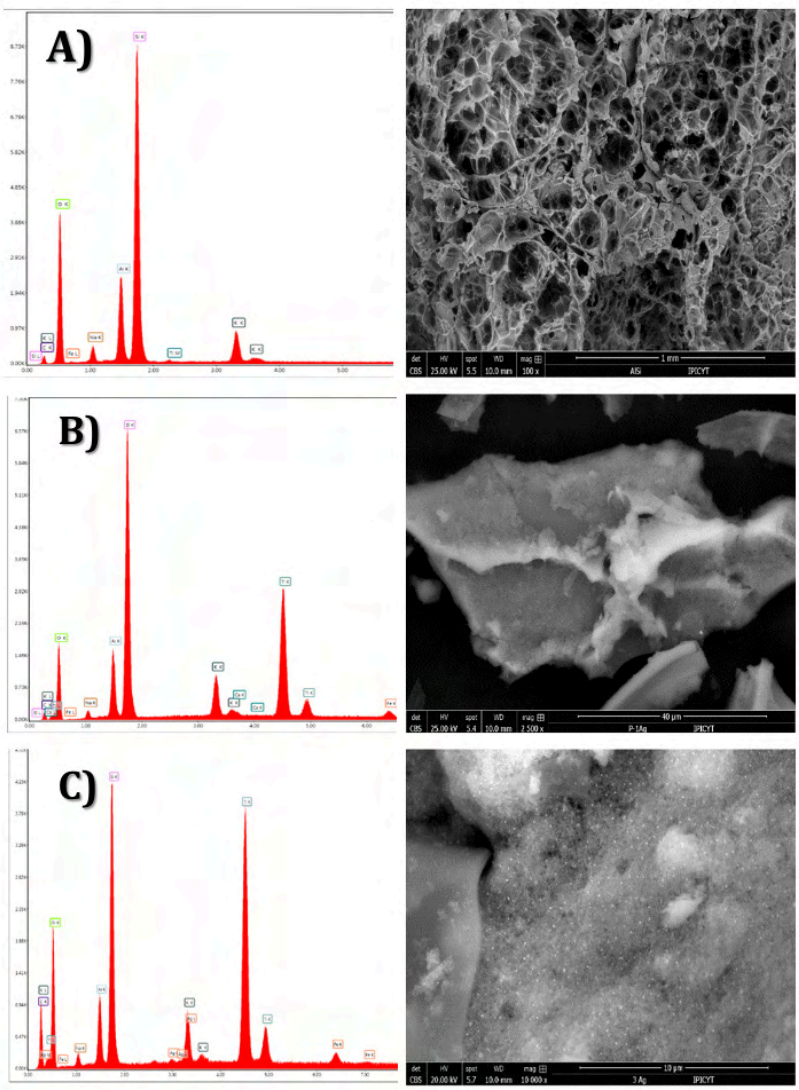
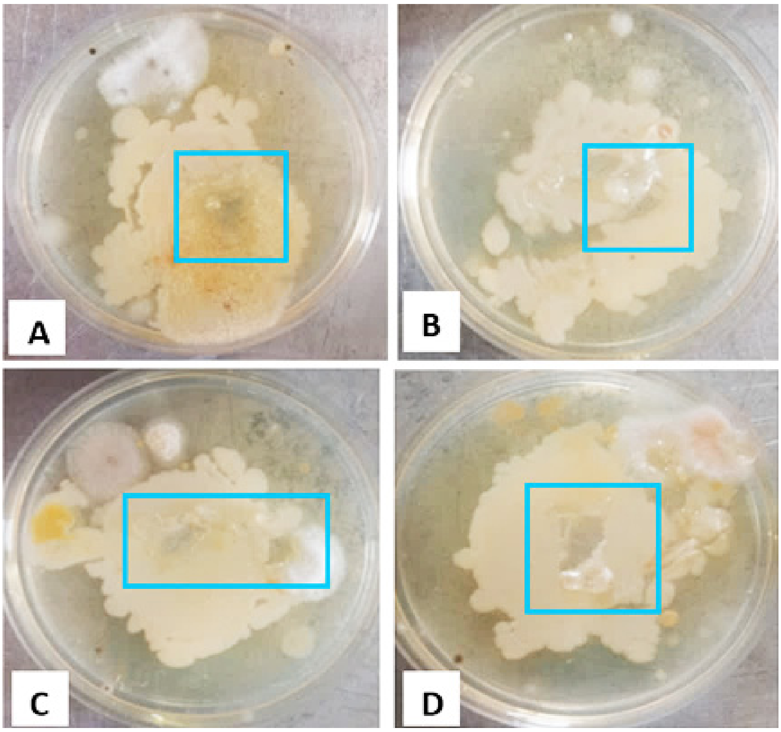


Figure 3. Microscopic analysis of the different concentrations A) reference sample, B) sample with 1% and C) sample with 3% of TiO2/Ag.

Sample concentration	Register of disinfected area (in mm)				
	Time	1 h	3 h	8 h	22 h
BlanK		0.9 mm	1.5 mm	3.9 mm	7.8 mm
0.25 g/L		1.2 mm	2.3 mm	5.5 mm	9.3 mm
0.5 g/L		1.8 mm	2.8 mm	7 mm	13.5 mm
1 g/L		2.3 mm	3.5 mm	9 mm	15.5 mm

Table 1. Registration of the disinfected area by adding gel in different concentrations of TiO_2/Ag 3%.



Figures 4. Bacterial colonies after 22h of antiseptic test. Blank (A), 0.25g/L (B), 0.5g/L(C), 1g/L(D).

maintains a stable efficiency after several days of storage, since the *in vitro* inhibition tests were carried out one week after its preparation, which is an indication that, during its incorporation and storage, there was a dispersion of nanoparticles in the gel.

There is some irregularity in the shape that the disinfected area adopts, this is because the infusion of the antimicrobial product is not regular as disc infusions would be, for example. It is because of this situation that the disinfected areas take on this appearance.

With these results and discussions, carried out under the premise of using a material such as titania (titanium oxide) on a nanometric scale and independent of its morphology, to be incorporated into a gel made from ethanol and *Calendula officinalis* plant extract, and carry out tests that subdue its qualities, it is possible to give way to the conclusions of this work.

CONCLUSIONS

- The X-ray diffraction technique confirms the presence of TiO_2 in the anatase and rutile phase, and in the case of the catalyst with 3% by weight of silver, the presence of characteristic peaks for metallic silver.
- The TiO_2/Ag 3% nanoparticles maintain their properties within the gel based on alcohol and *Calendula officinalis* extract, and the nanoparticles did not present segregation within the gel.
- Based on the results of the inhibition tests, it is shown that the gel has antimicrobial activity, which is favored by increasing the concentration of nanoparticles, and due to the materials involved in the gel, such as *Calendula officinalis* extract and TiO_2/Ag 3%.
- There is a synergy between them that allows a greater efficiency of the final product as the concentration of TiO_2/Ag 3% nanoparticles increases.

ACKNOWLEDGMENTS

We thank the Universidad Tecnológica del Centro de Veracruz (UTCV) for allowing the temporary use of the infrastructure with the necessary health measures.

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