NANOCIÊNCIAS E NANOTECNOLOGIA:

Pesquisa e Aplicações

Juan Ramón Collet-Lacoste
(Organizador)

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PRÓLOGO

Las propiedades particulares de las Nps, muy diferentes en muchos aspectos a las de sus sólidos masivos, han abierto nuevos campos de estudio e investigación a todo nivel: teóricos y aplicados. Son más inestables que los sólidos masivos de los que se diferencian principalmente por su estructura electrónica que no suele ser continua. Esto es una ventaja a nivel de su reactividad y suelen presentar superficies específicas altas que son muy propicias para los procesos de catálisis, un ingrediente muy importante en los procesos cinéticos. Otra propiedad interesante es que no presentan defectos estructurales en su volumen como vacancias o dislocaciones, a diferencia de sus correspondientes sólidos masivos.

Las presentes monografías forman parte del título: "Nanociências e Nanotecnologia: Pesquisa e Aplicações". Los artículos están ordenados de lo más general (*e.g.*, producción y caracterización de las Nps) a los relacionados con aplicaciones prácticas (*e.g.*, foto catálisis y a su relación principalmente con aplicaciones de origen biológico).

Estos muestran la potencialidad de las nanotecnologías en la comprensión de nuevas aplicaciones en campos tan variados como la catálisis, fotocatálisis, bio-remediación, contaminantes, ambientes acuáticos, antisépticos, bactericidas, virucidas, compuestos bio-activos, biosíntesis extracelular e intracelular, estudio de suelos, vegetales y probióticos, etc.

Juan Ramón Collet-Lacoste

SUMÁRIO

CAPÍTULO 10

ELABORATION OF AN ANTISEPTIC GEL BASED ON BIOACTIVE COMPOUNDS OF *ORIGANUM VULGARE* AND *ALOE VERA* ENCAPSULATED IN SiO_2 Y ZnO-SnO_2 NANOPARTICLES FOR CONTROLLED RELEASE

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ABSTRACT: The purpose of this project was to develop an antiseptic gel based on bioactive compounds of *Origanum vulgare* and *Aloe vera* incorporated with SiO_2 and ZnO - SnO_2 nanoparticles, to obtain a broad spectrum of bactericidal and virucidal action. Currently, in Mexico, the sanitary crisis caused by SARS-CoV-2 has had a significant impact on Mexican families, since hygiene and disinfection is the main concern of Mexicans. This problem has led the population to purchase antiseptic gels with a narrower spectrum of action, in order to reduce the cost of disinfection products. ZnO nanoparticles present antimicrobial properties, which together with SnO_2 nanoparticles, which have photocatalytic properties, generate a synergy. Likewise, the liberating properties of the SiO_2 nanoparticles show a better antimicrobial activity as a function of the antiseptic gel based on bioactive compounds of *Origanum vulgare* and *Aloe vera*.
KEYWORDS: Nanotechnology. Nanoparticles. Controled Release. Antiseptics. Bactericidal. Virucidal and bioactive compounds.

1 INTRODUCTION

Nanotechnology is the study and development of systems at the nanometer scale (1 nm is equivalent to 10^{-9}m), in which totally new properties and phenomena are observed, which are governed by the laws of quantum mechanics, these new properties

are those that scientists take advantage of to create new materials (nanomaterials) or nanotechnological devices. Ortiz Aguilar (2019) explains that nanomaterials can occur naturally, however, there are also manufactured nanomaterials, which are intentionally designed with specific properties (mechanical, electrical, optical and catalytic) and these nanomaterials can be presented in the form of nano-objects, materials that are characterized by having one, two or three external dimensions at the nanoscale.

SiO_2, SnO_2 and ZnO nanoparticles are nanostructured materials that have been studied for their properties, which include antibacterial action. For the fabrication of these nanomaterials, different synthesis methods have been used, either chemical or physical, which provide good properties.

In Mexico, the health crisis caused by SARS-CoV-2 had a significant impact on Mexican families, as the unemployment rate in Mexico increased, indicating a slow recovery of the country's economy. The Mexican population is applying sanitary measures to avoid contagion in their homes, with an estimated increase in the use of antiseptic gels in homes. However, due to the sanitary crisis, the prices of sanitizers, such as antiseptic gels, have risen, since hygiene and disinfection is the main concern of Mexicans. This problem leads the population to purchase antiseptic gels with a lower spectrum of action, in order to reduce the cost of disinfection products. On the other hand, there is a problem related to the epidemiology of wounds in Mexico. The health sector (2018) mentions the epidemiological characteristics and costs to wound care in medical units, as well as for the affected population in this area, since the prices of antiseptics present high costs for the treatment of acute and chronic wounds. It is estimated that 26.6% of the Mexican population presents traumatic injuries, and 23.4% corresponds to diabetic foot ulcers.

2 OBJECTIVE

To elaborate an antiseptic gel based on bioactive compounds of *Origanum vulgare* and *Aloe vera*, incorporated with SiO_2 and ZnO-SnO_2 nanoparticles, which allows having a broad bactericidal and virucidal spectrum to be used as a sanitary measure and improve the antimicrobial action against microorganisms present in wounds.

3 EXPERIMENTAL PART

3.1 OBTAINING THE AQUEOUS EXTRACT OF *ORIGANUM VULGARE*

Origanum vulgare leaves were collected on the federal highway in Fortín, Ver. The leaves were selected complete and free of pests. They were then washed with abundant tap water and the leaves were dried by dehydration for 6 h at 40°C in a smoking oven. Once

dried, they were ground in a grinder. The crushed material was subjected to continuous extraction with 95% ethanol using the Soxhlet method. Finally, a simple distillation was performed to obtain the pure extract.

3.2 OBTAINING THE EXTRACT OF *ALOE VERA*

Aloe vera leaves were collected and selected homogeneously and representatively in shape, size and color, discarding those that showed damage or other alterations. The *Aloe vera* leaves were washed in a bath with water and sodium hypochlorite to eliminate microorganisms. Once the leaves were washed and dried, they were cut longitudinally on a tray, then with a spoon, the mucilage was gently scraped until it was completely separated from the cortical parenchyma, then the gel was stored in airtight containers.

3.3 PREPARATION OF NANOPARTICLES

SiO_2 nanopartciles

400 mL of distilled water and the surfactant were placed in an agitation of 700 rpm for 5 min. Subsequently, 16 mL of NH_4OH were added by drip, leaving the solution to agitate for 10 min. The temperature was raised to 95°C, leaving it to react by a constant agitation at 700 rpm. To maintain the temperature, a sand bath system was mounted. The product was then allowed to cool to room temperature. It was centrifuged at 15,000 rpm for 15 minutes with three washes of an ethanol-water solution (1:1). Once obtained, 15 mL of *Origanum vulgare* extract was added, allowing the mixture to rest. Subsequently, the mixture was placed in a water bath at 40°C until a pasty consistency was obtained. It was calcined at 400°C for 1h. At the end of the process of obtaining nanoparticles, a light green dry material was obtained.

SnO_2 nanoparticles

Two g of tin chloride ($SnCl_2 \cdot 2H_2O$), used as tin precursor, was added to 42 mL of *Origanum vulgare* extract and left in agitation until its complete dissolution; obtaining a mixture. Subsequently, the sample was placed in a water bath at 60°C until a pasty consistency was obtained. It was then calcined at 400°C for 60 min. At the end of the process, a dry light gray material was obtained.

ZnO nanoparticles

2 g of zinc nitrate was dissolved in 50 mL of distilled water in a beaker with constant stirring for 10 min, using a magnetic stirrer. After stirring, 5 mL of *Origanum vulgare* extract

was added. The mixture was heated from 60°C to 90°C. The color of the resulting solution changed from a transparent white to a light green paste confirming the formation of ZnO NPs. The obtained paste was transferred to a ceramic crucible and kept in a muffle furnace heated at 400°C for 2h. The resulting powder was used for characterization and preparation of the antiseptic gel.

3.4 CHARACTERIZATION OF NANOPARTICLES

UV-Vis Spectroscopy Analysis

UV-Visible spectrophotometer was used to confirm the formation of SiO_2, SnO_2 and ZnO nanoparticles. The UV-Vis spectra of the samples were performed in Perkin Elmer spectrophotometer, in the range of 200 to 800 nm.

3.5 PREPARATION OF ANTISEPTIC GEL BASE

2 g of carbopol were dissolved in 120 mL of alcohol in a beaker with a stirring of less than 150 rpm. Subsequently, 2 mL of glycerin was added, maintaining a constant agitation. When the mixture was homogenized, 1 mL of trihydroxyethylamine was added. Finally, when the gel obtained consistency, it was bottled.

3.5 PREPARATION OF AN ANTISEPTIC GEL BASED ON NATURAL EXTRACTS AND NANOPARTICLES

2 g of carbopol were dissolved in 120 mL of alcohol in a beaker with a stirring of less than 150 rpm. Subsequently, 2 mL of glycerin was added, maintaining a constant agitation. When the mixture was homogenized, 1 mL of trihydroxyethylamine and 5 mL of *Aloe vera* extract were added. After the gel obtained consistency, it was packaged. To impregnate the SiO_2 and ZnO - SnO_2 nanoparticles, a sonicator was used with a duration of 30 min. Finally, the gel samples were left to rest.

3.6 PREPARATION OF CULTURE MEDIA

In the preparation of the culture media using nutrient agar, 23 g of the medium was rehydrated in 1L of distilled water. It was then allowed to stand for 10 to 15 min. Once rested, it was heated at constant stirring to boiling point for 1 min to dissolve it completely. It was then autoclaved at 121°C for 15 min.

In casein peptone culture media, 20 g of the medium was rehydrated in 1 L of distilled water. It was then allowed to stand for 15 to 20 min. Once resting, it was heated

by stirring constantly to boiling point for 2 min to dissolve completely. It was then sterilized in autoclave at 121°C for 17 min.

Once sterilization was complete, the media were poured into each of the corresponding petri dishes, where they were allowed to cool to room temperature. Finally, the media were labeled for use in other tests.

3.7 DETERMINATION OF ANTIMICROBIAL ACTIVITY

To analyze the antimicrobial activity of the antiseptic gel based on extracts with the incorporation of SiO_2 and SnO_2 - ZnO nanoparticles, disc diffusion methods were performed, where gram-positive and gram-negative bacterial species were used. The sterile discs loaded with different concentrations of nanoparticles solution (1 mg/ mL) were aseptically maintained in petri dish media, previously cleaned with a broth solution of test bacteria.

In addition, the base antiseptic gel was used as a positive control, while samples of the natural extract-based antiseptic gel with the incorporation of nanoparticles were used as negative controls. The antimicrobial activity was scored in the clear zones surrounding the corresponding discs.

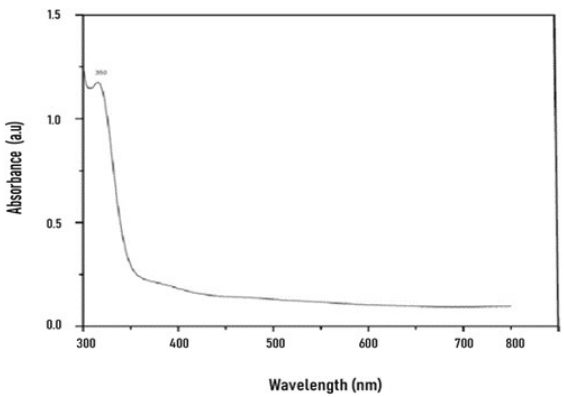
4 RESULTS AND DISCUSSION

UV-Vis Spectroscopy Analysis

SiO_2 nanoparticles

The SiO_2 nanoparticles were analyzed using UV-Vis spectroscopy in the range between 300 to 800 nm. In Figure 1, the peak absorbance of these nanoparticles is observed at 350 nm, due to their surface plasmon resonance.

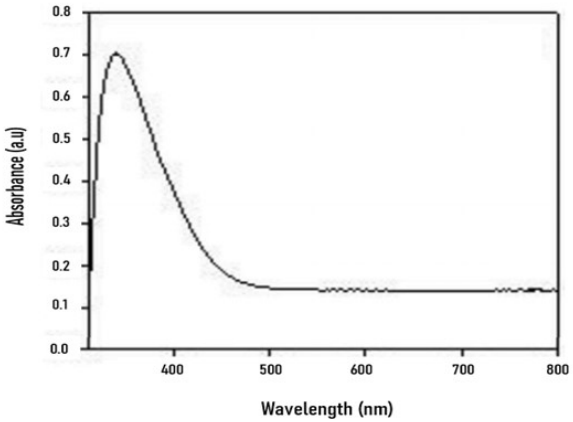
Figure 1. UV-Vis spectroscopy of the SiO_2



SnO_2 nanoparticles

UV-Vis spectroscopy analysis was performed at room temperature. The absorption spectrum of SnO_2 nanoparticles was recorded in a wavelength range between 200 to 800 nm. In Figure 2, the absorbance peak of SnO_2 nanoparticles at 260 nm is observed. The energy value of the forbidden band is 2.84 eV.

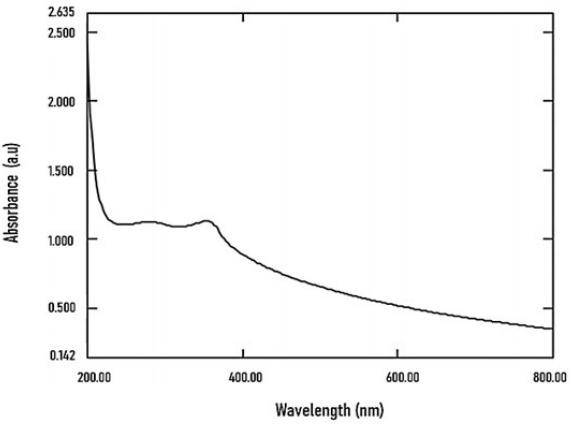
Figure 2. UV-Vis spectroscopy of the SnO_2.



ZnO nanoparticles

The confirmation of Origanum vulgare extract of ZnO nanoparticles was observed by the color change from white to light green. In Figure 3, the UV spectrum of the synthesized ZnO nanoparticles is shown. The absorbance peak is at 354 nm, due to the surface plasmon resonance of these nanoparticles.

Figure 3. UV-Vis spectroscopy of the ZnO.

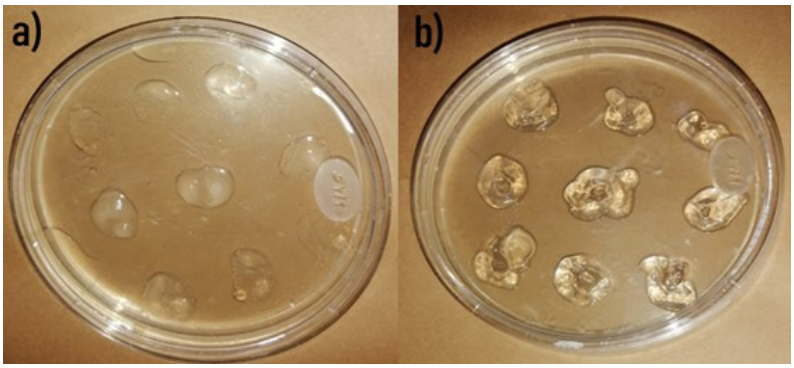


Determination of antimicrobial activity of antiseptic gel

The nanoparticles used for the preparation of the antiseptic gel are used in various applications due to their antimicrobial properties. In the present study, agar disc diffusion test was used to measure the efficiency of the antiseptic gel based on natural extracts with the incorporation of SiO_2 and ZnO - SnO_2 nanoparticles against gram negative and gram positive bacteria. The inhibition zone was analyzed by the disk diffusion method for 4 h with the corresponding nanoparticles, using different concentrations for the preparation of the antiseptic gel.

Figure 4 shows the antimicrobial activity of the antiseptic gel samples, in the case of Figure 4(a) it can be determined that the base gel presents a superficial antimicrobial activity against the positive bacteria, this is attributed to the large surface area to volume ratio of the nanoparticles used, providing a better inhibitory contact with the microorganisms. The antimicrobial properties of the nanoparticles interact with the microorganisms by adhering to the surface of the bacterial cell membranes, penetrating and affecting the permeability of the bacterial membranes.

Figure 4. Antimicrobial activity (a) base gel and (b) antiseptic gel with ZnO-SnO_2 nanoparticles.



Tables 1 and 2 show the size of the disinfected diameter using the antiseptic gel based on natural extracts with the incorporation of SiO_2 and ZnO - SnO_2 nanoparticles.

Table 1. Recording of the diameter of the disinfected área of the ZnO-SnO_2 nanoparticles.

Registro de área desinfectada (mm)				
	1 h	***2 h***	***3 h***	***4 h***
Blanco	5 mm	6 mm	7 mm	8 mm
0.25 g / L	5 mm	7 mm	9 mm	11 mm
0.5 g / L	9 mm	10 mm	15 mm	17 mm
1 g / L	10 mm	11 mm	14 mm	17 mm

Table 1 shows the records regarding the diameter of the disinfection area using ZnO-SnO_2 nanoparticles in the antiseptic gel samples. The record of the disinfection area was recorded in millimeters. As can be seen in the table, the antiseptic gel without the bioactive components of *Origanum vulgare* and *Aloe vera* extracts with the incorporation of nanoparticles at different concentrations, presented a lower area than the samples of the antiseptic gel incorporated by natural extracts already mentioned and ZnO-SnO_2 nanoparticles.

The study to evaluate the effectiveness of the antiseptic gel had a duration of 4 h, where the samples showed an effectiveness in reducing microorganisms present in the medio de cultivo of gram-negative and gram-positive.

The sample with a concentration of 0.5 g/L showed a higher effectiveness in the antimicrobial activity on gram-negative and gram-positive bacteria. However, the samples with a concentration of 1 g/L of ZnO-SnO_2 nanoparticles also presented a greater effectiveness, however, there was not a great difference with respect to the samples with a concentration of 0.5 g/L, which is why it was analyzed that this concentration allows a prolonged release of the bioactive compounds of the *Origanum vulgare* and *Aloe vera* extracts.

Table 2. Recording of the diameter of the disinfected of the SiO_2 nanoparticles

Registro de área desinfectada (mm)				
	1 h	***2 h***	***3 h***	***4 h***
Blanco	5 mm	6 mm	7 mm	8 mm
0.25 g / L	5 mm	6 mm	8 mm	9 mm
0.5 g / L	5 mm	7 mm	8 mm	10 mm
1 g / L	6 mm	8 mm	9 mm	11 mm

Table 2 shows the record corresponding to the antimicrobial evaluation of the antiseptic gel based on the bioactive components of *Origanum vulgare* and *Aloe vera* extract with different concentrations of SiO_2 nanoparticles with respect to the antiseptic gel without components, which was considered as a blank.

The sample that presented the best effectiveness for the reduction of microorganisms was the one with the concentration of 1 g/L of SiO_2 nanoparticles with the bioactive compounds of the *Origanum vulgare* and *Aloe vera* extracts. However, this sample did not present the same effectiveness with both gram-negative and gram-positive microorganisms.

5 CONCLUSIONS

UV-Vis spectroscopy confirmed the formation of SiO_2, ZnO and SnO_2 nanoparticles.

The study of the antimicrobial activity showed that the antiseptic gel based on *Origanum vulgare* and *Aloe vera* extract with the incorporation of 0.5 g/L of ZnO - SnO_2 nanoparticles developed a synergy, increasing the efficiency of the antimicrobial activity due to its photocatalytic properties, allowing the reduction of gram-negative and gram-positive bacteria.

The release character of SiO_2 can be observed through the increase of the inhibition area with respect to the release of Origanum vulgare and *Aloe vera* extracts over time.

Therefore, it is concluded that the antiseptic properties of alcohol, as well as those of *Origanum vulgare* and *Aloe vera* extracts, the SiO_2 releasing character, and the photoactive properties of ZnO-SnO_2, present a synergy resulting in a better antimicrobial activity.

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SOBRE EL ORGANIZADOR

El Dr. Juan Ramón Collet-Lacoste es licenciado en ciencias químicas de la Universidad de Buenos Aires (UBA) y PhD de la Universidad de Paris Sud (XI). Su especialidad es la físico química, en la rama de la termodinámica de los procesos irreversibles (TPI), especialmente en el estudio de los procesos cinéticos en los sistemas electroquímicos.

Ha desarrollado varios trabajos relacionados a los mecanismos de reacción y transporte de materia sobre electrodos metálicos, así como el desarrollo de electrodos para celdas de combustible de baja temperatura (fuel cells).

Es un especialista en la técnica de impedancia electroquímica, en la cual ha publicado varios artículos en revistas internacionales.

Desde el punto de vista experimental, ha trabajado en el desarrollo de celdas de combustible con Nps de platino y paladio y de electrolizadores alcalinos de baja temperatura.

Actualmente realiza trabajos sobre la oxidación acuosa del aluminio en gradientes de temperatura. Este trabajo esta relacionado a los elementos combustibles de los reactores experimentales multipropósito para la fabricación de radioisótopos de uso médico.

ÍNDICE REMISSIVO

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