



# PREPARAÇÃO DE UMA PELÍCULA DE HIDROCOLÓIDE DEGRADÁVEL COM NANOPARTÍCULAS DE PRATA SINTOMAS COM AÇOUS EXTRATO DE ALHO (*Allium sativum*)

## PREPARATION OF A DEGRADABLE HYDROCOLOID FILM WITH SILVER NANOPARTICLES SYNTHETIZED WITH AQUEOUS GARLIC EXTRACT (*Allium sativum*)

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### RESUMO

A síntese verde de nanopartículas de prata visa unir sinergicamente as propriedades bactericidas do elemento com a composição química de extratos de plantas e potencialmente utilizá-los em materiais substituíveis de plástico biodegradáveis. Neste trabalho, foi preparada uma película de gelatina degradável com nanopartículas de prata sintetizadas com extrato aquoso de alho (*Allium sativum*); As nanopartículas foram previamente caracterizadas por espectrofotometria ultravioleta visível, microscopia eletrônica de varredura e microscopia eletrônica de transmissão. Para fazer o filme, gelatina pura, gelatina - carboximetilcelulose - glicerol e misturas de goma gelatina e arabina foram testadas, obtendo a textura desejada apenas com gelatina, em diferentes concentrações. O filme com maior flexibilidade foi 10% de gelatina, com resistência à punção de 47 N e densidade de 0,943 g / mL. Este filme foi analisado por espectroscopia infravermelha, encontrando modificações do espectro dependentes da presença de nanopartículas de prata - extrato de alho. A microscopia eletrônica de varredura revelou uma distribuição uniforme de prata no filme (74,4 nm) com uma abundância de 56,20% e uma concentração de 1,58 mg / g determinada por espectrofotometria de absorção atômica. Finalmente, a película de gelatina preparada é resistente, de cor âmbar, com possíveis propriedades bactericidas devido à presença de nanopartículas de prata e facilmente degradável a 50 ° C em água.

**Palavras-chave:** Película, gelatina, nanopartículas de prata, alho, *Allium sativum*

### ABSTRACT

The green synthesis of silver nanoparticles aims to synergistically bind bactericidal properties of the element with the chemical composition of plant extracts and potentially use them in biodegradable plastic substitute materials. In this work, a degradable gelatin film was prepared with silver nanoparticles synthesized with aqueous extract of garlic (*Allium sativum*); the nanoparticles were previously characterized by ultraviolet-visible spectrophotometry, electron scanning microscopy and electron transmission microscopy. To make the film, pure gelatin, gelatin - carboxymethylcellulose - glycerol, and gelatin - arabic gum mixtures were tested, obtaining the desired texture only with gelatin, at different concentrations. The film with the greatest flexibility was 10% gelatin, with puncture resistance of 47 N and density of 0.943 g/mL. This film was analyzed by infrared spectroscopy, finding modifications of the spectrum dependent on the presence of silver nanoparticles-garlic extract. The electron scanning microscopy revealed a uniform distribution of silver in the film (74.4nm) with an abundance of 56.20% and a concentration of 1.58 mg/g determined by atomic absorption

spectrophotometry. Finally, the prepared gelatin film is resistant, amber colored, with possible bactericidal properties due to the presence of silver nanoparticles and easily degradable at 50°C in water.

**Keywords:** Film, gelatin, silver nanoparticles, garlic, *Allium sativum*

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## INTRODUCTION

With the rise of nanotechnology, nanoparticles (NPs) of various elements have been developed that have a marked biocidal effect on a wide variety of microorganisms, such as viruses, bacteria and fungi. The biocidal capacity of certain nanoparticles is linked to their nature and to certain intrinsic characteristics such as its nanometric dimensions and the high area/volume ratio, which allows greater contact and interaction with said microorganisms.

Among the nanoparticles that have been shown to have biocidal properties, the most important are those of silver, zinc, copper and iron oxides (Seil and Webster, 2012; Hajipour *et al.*, 2013). Within the family of metal nanoparticles, the ones that have generated the greatest interest are the silver nanoparticles (AgNPs), since the salts of this metal have been used for several years in the treatment of specific diseases caused by viruses or bacteria (Ravindran *et al.*, 2014). In general, the preparation and stabilization of metallic nanoparticles are carried out through physical and chemical methods. Silver nanoparticles can be prepared by chemical reduction, electrochemical and photochemical techniques; the first being the most used strategy, since stable colloidal dispersions of the optimal shape and size are obtained (Flores, 2014).

Due to the impact of the synthesis of this material in the environment, currently, new alternatives are being searched for to aid the production of more environmentally friendly nanoparticles that result in less use of toxic substances aligned with the trend of "green chemistry". Flower petals and plant extracts have been successfully used; one of these techniques for the synthesis of silver nanoparticles that has been widely studied is garlic (*A. sativum*) as a reducing agent (Cardeño and Londoño, 2013). Silver nanoparticles have great applicability in food contact surfaces, especially in their packaging

due to its excellent antibacterial properties, in order to prolong the life of food (Trbojevich and Fernández, 2013).

Silver nanoparticles in colloidal form have the ease of forming part of new products through their functionalization or simple incorporation. If the objective is to form, a thin film there is a range of materials to which nanoparticles can be added, as is the case with hydrocolloids. These are macromolecules that have a great affinity for water, where they dissolve to a greater or lesser extent and modify their rheology, increasing the viscosity of the liquid and sometimes even jellifying, giving a solid appearance. The most common hydrocolloids are gelatin, cellulose and starch, and among those classified as additives are agar, alginate, arabic gum and pectin (Moreno, 2010).

Gelatin is a heterogeneous mixture of high molecular weight proteins, soluble in water and is a derivative of the partial hydrolysis of collagen of natural origin. Relatively it contains high amounts of non-polar amino acids, such as glycine, proline, valine and alanine, and is a triple-helical structure consisting of three extended protein chains that wrap around each other, and the gelatin is partially hydrolyzed in the form of collagen. The sequence of glycine, proline and alanine gives antioxidant and antihypertensive properties, in addition to increasing the bioavailability of calcium in the body. Gelatin is non-toxic and immunogenic; on the contrary, it is biodegradable and biocompatible, which is why it is used in the pharmaceutical industry in the administration of drugs in the form of capsules, hydrogels or microspheres (Ahmad *et al.*, 2012).

On the other hand, the current trend in the design of biodegradable materials based on hydrocolloids for biomedical applications focuses on the development of films with better properties of mechanical resistance and water, through the combination of biopolymers of

different characteristics (García and Martinelli, 2015).

Due to the bactericidal properties of silver, the tendency of green synthesis and the easy degradability of hydrocolloids, in this study a film was prepared using pure gelatin, a mixture of gelatin, carboxymethylcellulose and glycerol, and gelatin with gum arabic.

## MATERIALS AND METHODS

### 2.1. Reagents and Plant Material

All the reagents and solvents used were of analytical grade, which included Merck® silver nitrate, gelatin BHD Chemicals Ltd., UPS-grade arabic gum, Merck glycerol and BHD carboxymethylcellulose Chemicals Ltd. The water used was of ultra-pure quality produced by the Milli-Q® system (resistivity  $\cong$  18 M $\Omega$  / cm, Millipore, USA). As vegetable sample, fresh garlic cloves were purchased at a local store in Quito-Ecuador.

### 2.2. Equipment

The formation of the silver nanoparticles was verified using a UV-Visible spectrophotometer from Agilent Technologies; model Cary 60, operated at a resolution of 1 nm, with an optical path of 10 mm, within the wavelength range of 300 to 800 nm.

Electron Transmission Microscopy (ETM) measured the size of nanoparticles in a Hitachi H-600 ABS equipment. The SEM-EDX Scanning Electron Microscopy was performed on the Phenom ProX equipment and the results were analyzed using the ProSuite software. For the infrared spectroscopy studies with Fourier Transforms (FTIR), a Perkin Elmer spectrophotometer, Spectrum BX model with ATR coupling was used. The range of analysis was from 4000 to 400 cm<sup>-1</sup> with intervals of 2 cm<sup>-1</sup>, at a resolution of 4 cm<sup>-1</sup> and 10 scans for each sample.

The quantification of the silver content was carried out by Flame Atomic Absorption Spectrophotometry (AA) with a Perkin Elmer brand equipment, model AAnalyst 400. The density was measured on a digital Perkin Elmer Densito 30PX and the membrane puncture analysis was performed on the Tinius Olsen Super L-60/602 Universal Physical Testing Machine.

### 2.3. Preparation of the aqueous garlic extract

Whole teeth of the white garlic variety (A. sativum) were used according to the suggested protocol (Cardeño and Londoño, 2014). They were washed with distilled water to eliminate the presence of dust particles or contaminants. Each clove weighed 25 g that were crushed in a mortar and transferred to an Erlenmeyer, where 100 mL of distilled water was added. This mixture was heated at 90°C for 5 minutes (Rastogi and Arunachalam, 2013; Von White *et al.*, 2012). The resulting solution was finally filtered in a vacuum system.

### 2.4. Synthesis of AgNPs silver nanoparticles

For the synthesis of nanoparticles, 50 mL of a 2.36 mM aqueous solution of silver nitrate (AgNO<sub>3</sub>) was heated to a temperature between 50 and 60 °C. 5 mL of the previously prepared garlic extract was added dropwise and the solution at 60 °C was kept under constant stirring for 1 hour. The formation of nanoparticles was evidenced by the change in the color of the solution from slightly yellow to brown (Cardeño and Londoño, 2014). It was allowed to stand at room temperature and was stored in an amber glass bottle under cooling for further analysis.

### 2.5. Preparation of the hydrocolloid film

#### 2.5.1. Gelatin and carboxymethylcellulose film

A gelatin solution was prepared at 10, 15, 20, 30 and 50% respectively with 0.1 to 0.5% carboxymethylcellulose and 15% glycerol in each test, as indicated in Table 1. It was heated to 90 °C and kept under constant stirring for 30 minutes (Valle-Guadarrama, *et al.*, 2008). The resulting solution was placed in a Petri dish at room temperature.

**Table 1.** Weight/volume percentages of the components used for the gelatin-carboxymethylcellulose film.

Gelatin (% w/v)	Carboxymethyl cellulose (% w/v)	Glycerol (% w/v)
10	0,1	15
15	0,2	
20	0,3	
30	0,4	
50	0,5	

### 2.5.2. Gelatin and arabic gum film

They were weighed and dissolved in 20 mL of distilled water of 0.1 to 0.5 g of gelatin and 0.2 to 1.0 g of arabic gum respectively, as indicated in Table 2. It was kept under constant stirring for 2 hours. The resulting solution was placed in a Petri dish and dried in an oven at 70 °C.

**Table 2.** Amounts of the components used for the gelatin-arabic gum film.

Gelatin (g)	Arabic gum (g)
0,1	0,2
0,2	0,4
0,3	0,6
0,4	0,8
0,5	1,0

### 2.5.3. Gelatin film

A gelatin solution of 10, 15, 20 and 25% respectively was prepared. To dissolve the gelatin it was heated to a temperature of about 70°C, with constant stirring. The solution was placed in a Petri dish, and dried in an oven at 40 °C for 24 hours (Ahmad, *et al.*, 2012).

### 2.5.4. Hydrocolloid film with silver nanoparticles AgNPs-A. sativum

The colloidal solution of AgNPs-A. *sativum* was used as solvent for each film according to the concentration expressed as a percentage.

## 2.6. Resistance analysis

The strength of each film was determined by the membrane puncture technique under the ASTM D 4833-13 performed in triplicate in the Materials Laboratory of the Faculty of Civil Engineering of the Pontifical Catholic University of Ecuador. For each formulation, a sample of 10 cm in diameter and 0.25±0.03mm in thickness was prepared.

## RESULTS AND DISCUSSION

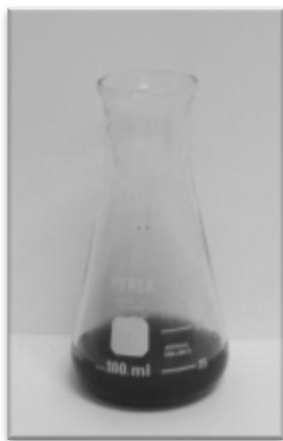
### AgNPs synthesis using aqueous garlic extract (A. sativum)

In this work, the synthesis of AgNPs is reported through a "green" method, which consists of the use of garlic extract as a reducing agent. Garlic (*A. sativum*) contains high levels of organosulfur compounds that are divided into two groups. One group is fat-soluble and includes compounds such as diallyl sulphide (DAS), di-allyl disulfide (DADS) and di-allyl trisulfide (DATS). The other water-soluble group contains compounds such as S-allyl cysteine (SAC) and S-allylmercaptocysteine (SAMC). From the garlic extract, SAC and SAMC are the possible compounds involved in the reduction reaction of the metal ions of the aqueous nitrate solution from Ag<sup>+1</sup> to Ag<sup>0</sup> (Rastogi and Arunachalam, 2013). The mechanism of reaction is not clearly defined because there are studies that indicate that the sugars present in garlic are responsible for the reduction process (Von White *et al.*, 2012).

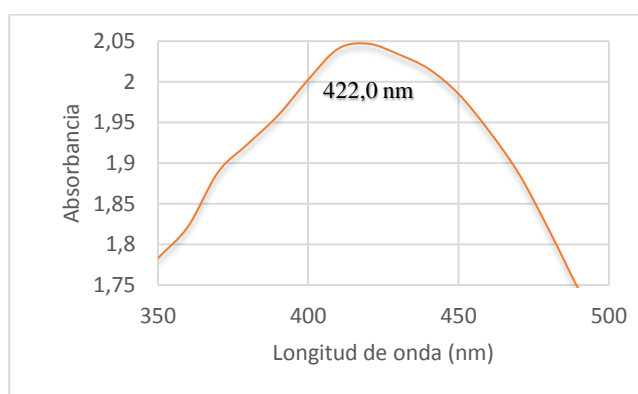
During the synthesis process, the change of the solution from yellow to brown was observed (Figure 1a), indicating the formation of AgNPs, as reported by other authors (Khan *et al.*, 2013, Amin *et al.*, 2012).

For the characterization, the AgNPs are ideal candidates for the study with UV-Visible spectroscopy, due to their surface plasmon resonance properties and absorption in the visible region (Basavaraj, *et al.*, 2015). The formation and stability of the nanoparticles in the colloidal solution was monitored by UV-Visible spectroscopy analysis; one of the obtained spectra, which is presented in Figure 1b, indicates the maximum absorbance at 422 nm.

Table 3 shows the average maximum absorption value obtained at 422.40 nm. According to several authors, the absorbance around 430 nm is characteristic of silver nanoparticles without organic additives (Vilchis *et al.*, 2008); while at 420 nm, it indicates the existence of coated nanoparticles (Shankar *et al.*, 2015)



**Figure 1 (a).** Colloidal solution of AgNPs-A. sativum



**Figure 1 (b).** UV-Vis spectrum

**Table 3.** Maximum absorption wavelengths of AgNPs-A. sativum

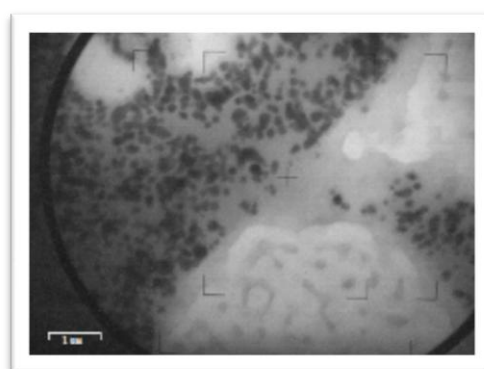
Synthesis	$\lambda$ of maximum absorption (nm)
1	422
2	423
3	423
4	422
5	422
<b>Average</b>	<b>422,40</b>

Additionally, it was estimated that the particle sizes are in the range of 60-80 nm due to the average absorption peaks at 422.40 nm, according to the estimate proposed by Pradeep (2012).

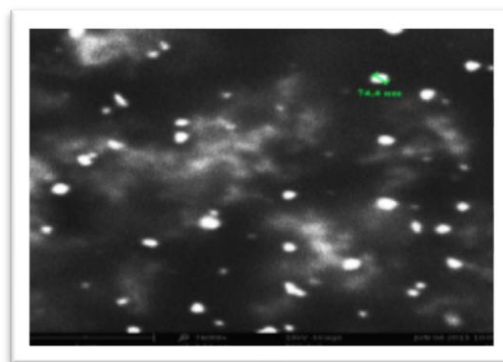
The displacement of the absorption maximum is related to the size of the nanoparticle, understanding that as it increases

the interaction with the radiation beam changes, which translates into a bathochromic effect. In this case, the correlation between the maximum absorption and the particle size was valid, based on the analysis carried out by Electron Transmission Microscopy (TEM) and Scanning Electron Microscopy (SEM).

Figure 2 shows the dispersion of the silver nanoparticles in the solution. Quality obtained by TEM (a) and in the SEM image (b) the average nanoparticle size of 74.4 nm is appreciated, estimated value in the ProSuite software, evidencing the correlation of the prediction of nanoparticle size as a function of the maximum absorption obtained by UV-Vis spectrophotometry.



**Figure 2a.** TEM image of AgNPs-A. sativum



**Figure 2b.** SEM image of AgNPs-A. sativum

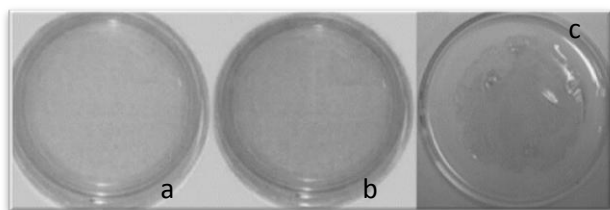
### Preparation of the hydrocolloid film

Gelatin hydrocolloid was chosen for its mechanical properties, and high solubility in water. Additives of the same species (arabic gum and carboxymethylcellulose) were used to improve the appearance of the film based on studies carried out (Enríquez, *et al.*, 2012). In all cases, gelatin amounts of less than 25% w/v

were used due to the saturation of the preparation. The proportions of each reagent (arabic gum and carboxymethylcellulose) were used based on previous tests.

The drying of gelatin films is important, because at low temperatures, a film of helical conformation is obtained, while at higher temperatures to the environment a ball-like structure is obtained and are generally more brittle (García and Martinelli, 2013).

The films obtained are shown in Figure 3. The gelatin and carboxymethylcellulose films (Fig. 3a) were very brittle, so glycerol was added (Kavoosi *et al.*, 2013), however in different proportions it did not provide the desired texture. The gelatin and arabic gum films (Fig. 3b) were equally brittle and the very thin sheets are impossible to manipulate to the touch. The gelatin films (Fig. 3c) showed very good results in terms of resistance to touch and sharpness. When increasing the concentration of gelatin, the film presented greater opacity and hardness.

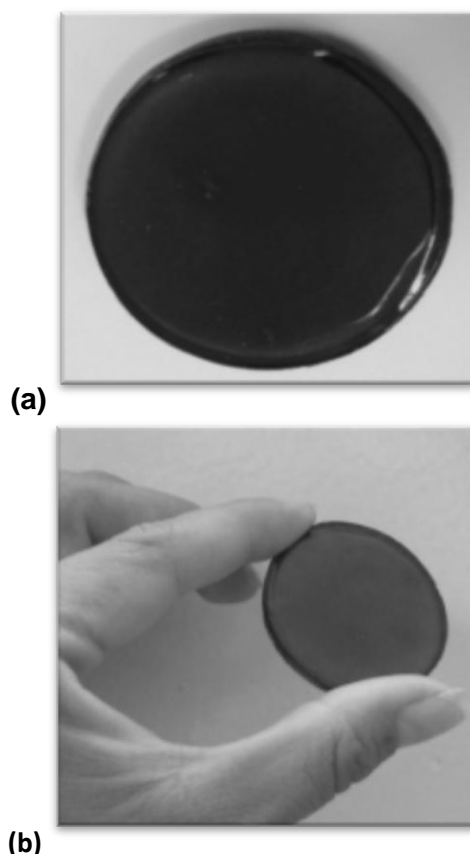


**Figure 3.** Films obtained with different mixtures: gelatin-carboxymethyl cellulose film (a), gelatin film - gum arabic (b) and gelatin film 15% (c)

#### Preparation of AgNPS-*A.sativum*-gelatin film

To prepare the gelatin film at 10, 15, 20 and 25%, the colloidal solution of the silver nanoparticles synthesized with garlic extract was used as solvent.

In all four cases, an amber, touch-resistant film was obtained as shown in Figure 4. The depth of the color increased proportionally to the concentration of the nanoparticles and the opacity of the gelatin. The color provided by the silver nanoparticles with the garlic extract provides an additional advantage of light protection.



**Figure 4.** Front view (a) of AgNPs-*A. sativum*-Gelatin film and against light (b)

#### Resistance analysis

The resistance results obtained are shown in Table 4.

**Table 4.** Resistance to membrane puncture

Sample	1	2	3	4
Concentration	10%	15%	20%	25%
Nominal thickness (mm)	0.24	0.23	0.22	0.22
Maximum load (N)	47	45	31	26

As can be seen, as the concentration of gelatin increases, flexibility and puncture resistance decrease, because this biopolymer tends to crack due to its strong cohesive energy density, and because of the interactions in the hydrogen bonds and polar groups of amino acids in its structure (Sifuentes-Nieves, *et al.*, 2012; Gama, 2014). The film with 10% of gelatin, presented the greatest flexibility in relation to the other concentrations, a result that is similar to the study carried out by Quintanilla (2016), where a 12.5% gelatin film has a

mechanical strength of 53.26 N with a thickness of less than 0.13mm.

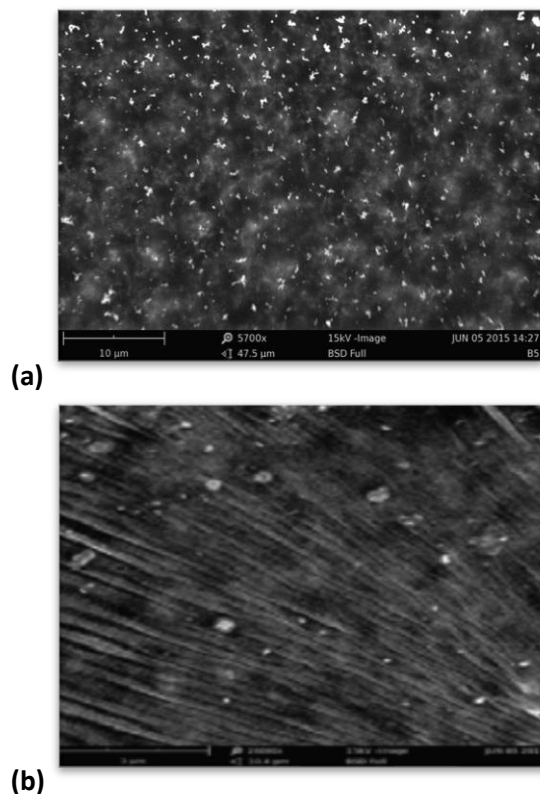
### Characterization of the AgNPs-*A.sativum*-Gelatin film

The AgNPs-*A.sativum*-Gelatin film was analyzed by infrared spectroscopy FT-IR with ATR coupling. Figure 5 shows the infrared spectra of garlic extract *A. sativum* (Figure 5a) together with the spectrum of the silver nanoparticles synthesized (Figure 5b), where a good coincidence between both spectra is observed with the exception of the band at 1337  $\text{cm}^{-1}$  which is exclusive of the AgNPs spectrum; said band corresponds to the tension vibration C-N corresponding to one of the nitrogenous compounds SAC or SAMC that act as reducers, which seem to be bound on the surface of the nanoparticle. The presence of a nitrogenous compound, probably amino, is consistent with the bands at 3280 and 1621  $\text{cm}^{-1}$ , which indicate the tension and flexion of the N-H bond, respectively (Stejskal, 2011).

On the other hand, the FTIR-ATR spectra of the gelatin hydrocolloid with and without nanoparticles are shown (Figure 5c and 5d). In both cases, the main gelatin bands, indicative of their peptide composition, are observed at 3290, 1635, 1539 and 1238  $\text{cm}^{-1}$ , corresponding to the tension vibrations N-H, amide I, amide II and amide III, respectively; these bands coincide with what was reported by the literature for the characterization of animal source gelatin (Almeida, 2014). Between 1335 and 1500  $\text{cm}^{-1}$ , differences between both spectra are observed, thus, it is observed that the band at 1453  $\text{cm}^{-1}$  practically disappears in the spectrum of Figure 5d. If it is taken into account that in this region the bending vibrations of methyl groups are present. It can be inferred that the presence of the nanoparticles slightly influences the structural conformation of the colloid.

On the other hand, in the whole spectrum of the AgNPs-*A. sativum*-Gelatin film, a decrease is observed in the intensity of the bands, which can be attributed to a limited interaction of the film with the infrared radiation due to the presence of the metallic particulate material, which constitutes a barrier for the penetration of the evanescent wave coming from the ATR.

The SEM images of Figure 6 show a uniform dispersion of the silver nanoparticles in film (a) and the smooth surface in the gelatin in which the AgNps (b) are supported. Film morphology depends on several factors including solubility, solvent evaporation, total thickness and molecular weight.



**Figure 6.** SEM images of the AgNPs-*A. sativum*-Gelatin at 76000X and 26000X

The EDX spectra of Figure 7 confirmed the elemental composition of the garlic extract *A. sativum* (a) in relation to the gelatin (b) and the AgNPs-*A. sativum*-Gelatin film. (b). The fact that the film does not present nitrogen on the surface indicates an adequate coating of the gelatin, an effect confirmed by infrared spectroscopy.

In the film, the silver composition equals 56.20%. All samples of EDX were coated with gold to prevent the accumulation of static electric fields during scanning.

The quantification of the silver content in the colloidal solution of nanoparticles and the AgNPs-*A. sativum*-Gelatin film, was performed by flame atomic absorption spectrophotometry. The silver content in the aqueous suspension resulting from the synthesis of the garlic extract was 204.46 mg / L, indicating that the yield of

the reaction was 88.73%. The final concentration of silver nanoparticles in the film obtained was 1.58 mg / L.

The degradability of the film was determined from 50°C using water as solvent, observing its complete solubility.

## CONCLUSION

In this study, a degradable gelatin film was prepared in concentrations of 10, 15, 20 and 25%. Garlic extract *A. sativum* 25% was used to prepare silver nanoparticles of average size of 74.4 nm measured by SEM and TEM, and analyzed by UV-Vis. The film with the greatest flexibility is 10% in gelatin concentration, with a puncture resistance of 47N and density of 0.943g/mL. The film was analyzed by infrared spectroscopy FT-IR and showed the variation in the IR spectra of gelatin, *A. sativum*, AgNPs-*A. sativum* in relation to AgNPs-*A. sativum*-Gelatin

The SEM-EDX analysis demonstrated the uniform distribution of the nanoparticles and that the silver composition equals 56.20%. The concentration of silver in the film measured by flame atomic absorption spectrophotometry was 1.58mg/g. The film degrades from 50°C using water as solvent. The method is simple and environmentally friendly, with potential industrial applications.

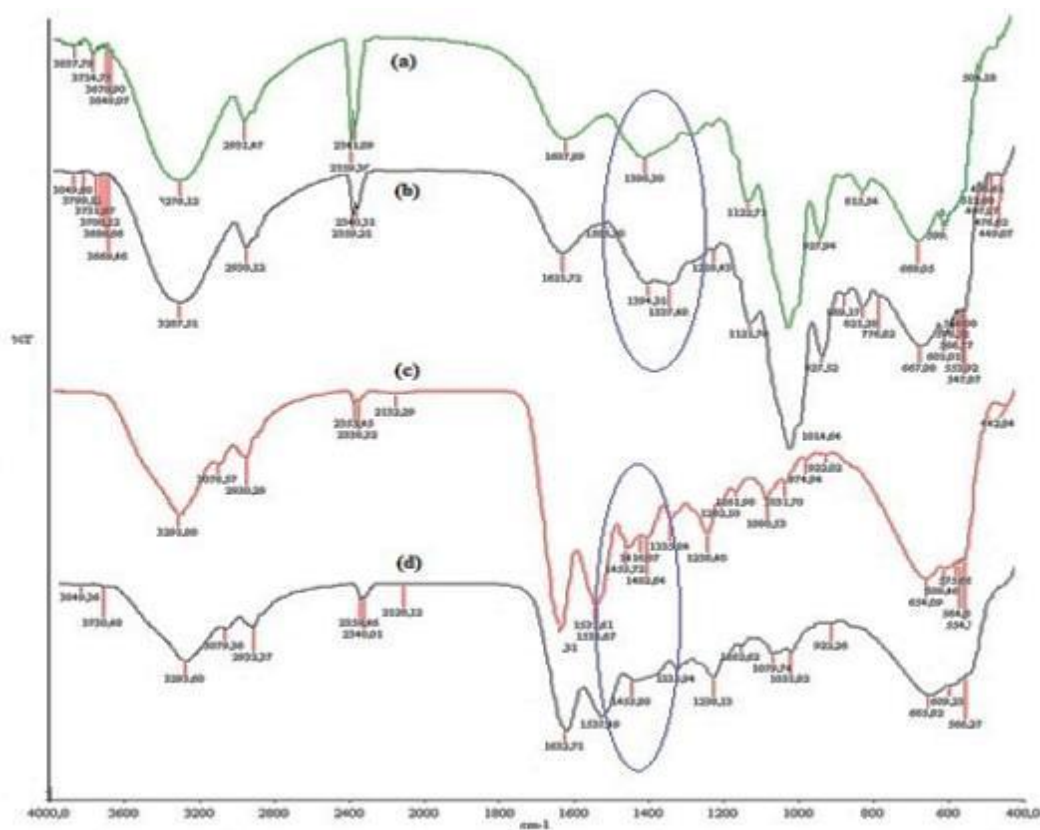
## ACKNOWLEDGEMENT

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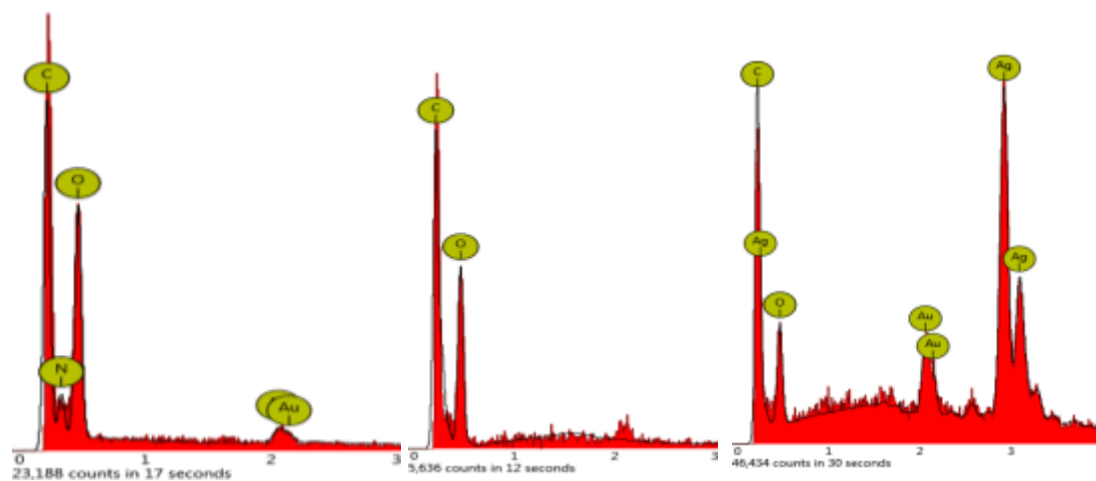
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**Figure 5.** IR spectra of: garlic extract *A. sativum* (a); AgNPs (b); gelatin (c); Hydrocolloid film with AgNPs (d)



**Figure 7.** SEM-EDX spectra of *A. sativum* (a), gelatin (b) and AgNPs-*A. sativum*-Gelatin film (c)