Characterization of the antibacterial galactomannan/ Zn(OH)₂–ZnO composite material prepared *in situ* from a green process using mesquite seeds as a biopolymer source

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Abstract. In this work, the preparation of an antibacterial galactomannan/Zn(OH)₂–ZnO composite material by a green process is proposed and demonstrated. The galactomannan polysaccharide was extracted from the arid-native mesquite (*Prosopis* spp.) seeds through a hydrothermal technique, and it was used as an organic polymeric matrix for the *in situ* chemical precipitation of the Zn(OH)₂–ZnO inorganic particles from simple reactions between Zn²⁺ and NaOH. The preparation was performed under standard conditions in aqueous solutions. The composite material, which was obtained by centrifugation of the previous preparation, was characterized by X-ray diffraction, transmission and scanning electron microscopy, infrared spectroscopy and thermal analysis. By means of these techniques, it was deduced that the composite material is highly crystalline (about 94%), composed of about 92% inorganic and 8% organic portion, and has a predominant size of 760 nm and a mean size of 800 nm. The antibacterial activity of the composite material was also studied, which resulted better against *Escherichia coli* (Gram-negative bacterium) than against *Staphylococcus aureus* (Gram-positive bacterium), which was calculated in a difference of about 30%. This work presents the high potential of galactomannans to form functional composite materials with inorganic particles.

Keywords. Composite materials; galactomannan; zinc hydroxide; green synthesis; antibacterial activity.

1. Introduction

A composite material, or simply a composite, is defined by the International Union of Pure and Applied Chemistry as a multicomponent material comprising multiple different (non-gaseous) phase domains, in which at least one type of phase domain is a continuous phase [1]. Although it is not included in this definition, it is important to note that the constituent materials work together to give the composite unique properties [2]. This means that, for the case of organic/inorganic composites, the product can possess combined properties of the organic and inorganic components. Such is the importance of composite materials that they represent an emerging group of advanced materials that are gaining relevance day by day. Particularly, organic/inorganic composites have been extensively studied and widely employed in life science [3],

eco-environment [4], energy [5], electronics [6,7], polymer engineering [8] and many other fields. Therefore, utilization of organic/inorganic composites has become a common tool in the development of composite materials, thus allowing their application in fields as diverse as those mentioned above.

Among the fields mentioned previously, life science (in which health science is included) has received special attention from the scientific community. A notable problem case in health science, and particularly in medicine, is the bacterial resistance to antibiotics. Nowadays, the extensive use of traditional antibacterial drugs has caused the bacteria to become resistant toward these drugs, which further intensifies the problem. These antimicrobial agents are commonly classified into organic and inorganic agents. However, organic/inorganic antibacterial agents are reported to be advantageous over only organic or only inorganic

antibacterial agents due to their higher stability and improved safety [9].

In a recent paper, Kumar *et al* [10] reviewed the antimicrobial properties of zinc oxide (ZnO) nanomaterials, which gain attention due to their biocompatible nature. Similar antimicrobial properties have been reported for zinc hydroxide ($\text{Zn}(\text{OH})_2$) [11], which demonstrates the promising application of zinc compounds in the health science field. Here, it is important to note that the properties of zinc compounds can be improved when they are coupled with appropriate organic materials to form a composite, as previously stated. Starch/ZnO [12] and alginate/ZnO [13] are examples of these antimicrobial organic/inorganic composite materials. The question, however, is not only how to prepare both the organic and inorganic components, but also how to improve the method used for preparing these composite materials by taking into account, for example, environmentally friendly approaches.

In the previous context, excellent green methods were reported for different applications: Sui *et al* [14] used electrospinning to prepare photoluminescent polyethylene/ZnO fibres; Toommee *et al* [15], by means of film casting, prepared thermochromic polydiacetylene/ZnO thin films; Du *et al* [16] used dip-coating for the preparation of polyvinylpyrrolidone/ZnO thin films for potential use in the fabrication of sensors for superoxide radicals; Li *et al* [17] used physical mixing of poly(3-hexylthiophene-2,5-diyl) and ZnO for preparing solar cell components; Sebök *et al* [18] used layer-by-layer self-assembly to prepare films for optical sensors; Deshpande *et al* [19] used ionotropic gelation for the preparation of chitosan/zinc fertilizers; Matei *et al* [20] employed co-precipitation to prepare ZnO–poly(vinyl acetate) composites; and Chen *et al* [21] used polyol-mediated *in situ* preparation of ZnO nanoparticles for colorant degradation. Among all these techniques, the *in situ* synthesis of inorganic particles in a polymer matrix has the advantage of having control on the reaction conditions, which influence not only the final size and shape of the particles but also the distribution of the particles in the polymer matrix, which, in turn, can be achieved with the coordination of the precursors, such as the metallic ions or organometallic compounds, with the polymer, as observed in some of the previous studies [6,7,22].

On the other hand, it is indispensable to develop environmentally friendly methods that exclude the use of toxic materials throughout the synthesis process. In this way, parts of plants, such as leaves, roots, latex, seeds and stems, can be used to fulfil these requirements because of their natural origin. Additionally, these natural parts possess polyphenols, proteins, alkaloids, phenolic acids, carbohydrates and terpenoids, among others, which can stabilize the inorganic particles in the composite [23]. At this point, it is important to mention a relevant kind of carbohydrate: galactomannans, which are heterogeneous polysaccharides consisting of β -(1,4)-D-mannopyranose chains with D-galactopyranose ramifications linked to the mannose backbone by α -(1–6) bonds. They are classified according to the mannose/galactose

ratio. A particular property of galactomannans is their ability to join inorganic and organic substances *via* molecular interactions. For this reason, they can be used to prepare composite materials [24]. Also, they present important emulsifying, thickening and gelling properties. In addition, due to their biodegradability, galactomannans can be used as alternative sources for the production of ecological composites. The incorporation of functional inorganic particles into the galactomannan matrix to form the organic/inorganic composite, which combines the excellent biological and physicochemical characteristics of organic compounds with the antibacterial properties of the inorganic particles, can result in a new material with several advantages for pharmaceutical and food industries when compared with the synthetic alternatives.

Since galactomannans are obtained from the endosperm of the dicotyledon seeds, particularly from the Leguminosae family [25], the mesquite tree (*Prosopis* spp.), which belongs to the Leguminosae family and which is native of the arid and semi-arid zones, can be used as a source of galactomannan for the habitants of these regions. In this context, we reported an optimized hydrothermal methodology for the extraction of galactomannan from mesquite seeds [26], which was recently proven to be useful in the preparation of antibacterial galactomannan/ZnO nanocomposites [27]. Thus, in the exploration of new options for the sustainable utilization of natural resources from the arid zones, the present study encourages the use of mesquite seeds as a green source of galactomannan. Particularly, in the present research we report a green-prepared galactomannan/ $\text{Zn}(\text{OH})_2$ –ZnO composite, which was obtained from an alternative way as compared to the previously reported galactomannan/ZnO. Since we have previously observed that as higher the content of galactomannan in the composite the lower the antibacterial activity [27], in this alternative way we have reduced the content of the organic portion in the resulting composite; interestingly, the resultant material was chemically different: the inorganic portion is mainly composed of $\text{Zn}(\text{OH})_2$. Thus, we present below the structural and thermal characteristics, as well as the morphology, of the obtained galactomannan/ $\text{Zn}(\text{OH})_2$ –ZnO composite material. Additionally, the antibacterial effect of this green-prepared composite for Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) model bacteria is investigated. The results, both from material characterization and antibacterial tests, are thoroughly discussed with the literature.

2. Experimental

2.1 Materials

Zinc sulphate (ZnSO_4) was acquired from Golden Bell. Sodium hydroxide (NaOH) and sodium chloride (NaCl) were supplied by J.T. Baker. Ninety-six percent ethanol was purchased from the Mexican company Alcoholera de Zapopan. All the reactants were used without purification. Distilled

Table 1. Main physicochemical characteristics of the extracted galactomannan, according to our previous work [26].

Physicochemical parameter	Result
Molecular weight (g mol^{-1})	82.465
Solubility in water at 25°C (%)	12.64
Intrinsic viscosity (dl g^{-1})	1.77
Galactose:mannose ratio	2.05

water was used for the preparation of all the aqueous solutions. The mesquite seeds, obtained from trees located at 29°N and 110°W , were purchased from the Mexican Comisión Nacional Forestal (CONAFOR), in Hermosillo, Sonora, Mexico.

2.2 Extraction and purification of galactomannan

The extraction of galactomannan from mesquite seeds was performed by means of the optimized hydrothermal method reported previously by us [26]. For this, mesquite seeds were ground as received. The germ and the husk were discarded. The endosperm was kept for the galactomannan extraction. Then, the endosperm was mixed with water in a proportion of 1:20 endosperm:water (w/v) and the mixture was poured into a flask. Then, the mixture was placed in an autoclave at 121°C and 100 KPa (15 psi) for 15 min. Later, the previous preparation was filtered and the galactomannans were retrieved from the filtered solution by ethanol precipitation using 2 ml of alcohol per 1 ml of filtered solution. Next, the precipitate was dried at 50°C for 16 h.

The precipitated powder extracted by means of the previous method was then purified by following the next procedure: 2.5 g of the retrieved galactomannan was dissolved in 1 l of distilled water using constant stirring at room temperature, later this solution was centrifuged at 4000 rpm for 10 min. Then, the galactomannan was precipitated from the supernatant by using 2 ml of ethanol per 1 ml of supernatant. The precipitated phase, which was separated by filtration, was dried at 50°C for 16 h and then it was stored until the preparation of the organic/inorganic composite material. The main physicochemical properties of this galactomannan, which were analysed and reported in a previous work [26], are summarized in table 1.

2.3 Preparation of the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite

The galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material was prepared by means of an *in situ* precipitation technique [28,29]. For this, 0.3 g of the galactomannan extracted and purified according to that described in the previous section were added to 50 ml of distilled water and the mixture was then stirred until the galactomannan is dissolved. Immediately

after the complete dissolution of galactomannan, 0.359 g of ZnSO_4 were added to the mixture in order to obtain a zinc concentration of 0.025 M. Then, this preparation was stirred for 2 h. Afterwards, 50 ml of 0.2 M NaOH were added to the previous preparation, which was left under stirring for 24 h.

We have identified two ways to treat the previous preparation in order to obtain a composite between galactomannan and the zinc compound: (a) precipitation with ethanol in static mode (without centrifugation) and (b) mechanical precipitation by centrifugation without ethanol. According to the previous report [27], the inorganic portion of the composite precipitated with ethanol is ZnO, and the galactomannan is present as a considerable constituent of the composite. On the other hand, as presented in this paper, the inorganic portion of the composite material precipitated by centrifugation is mainly $\text{Zn}(\text{OH})_2$, and it is present as the main component of the whole composite. For the precipitation by centrifugation, the preparation described in the previous paragraph is centrifuged at 4000 rpm for 10 min, and the obtained precipitate—the composite—is washed several times until the washing-water reached a pH of 7. After this washing process, the prepared composite is dried at 35°C for 24 h. By following this procedure, 0.098 g of composite powder is produced.

2.4 Characterization techniques

X-ray diffraction (XRD) measurement was performed at room temperature by employing a Bruker D8 Advance diffractometer equipped with a $\text{Cu K}\alpha$ ($\lambda = 1.541 \text{ \AA}$) X-ray source. The 2θ range was between 15 and 65° with a 2° min^{-1} collection rate. Phase identification was performed by matching powder diffraction file (PDF) cards with the experimental diffraction peaks. Quantitative analysis of the diffractograms was performed by employing Rietveld refinement using the FullProf suite [30,31]. All the necessary initial crystallographic parameters for the Rietveld refinement were obtained from the Crystallography Open Database [32] and the peaks were modelled by employing the Thompson-Cox-Hastings pseudo-Voigt profile with axial divergence asymmetry [33].

For transmission electron microscopy (TEM) imaging we employed a JEOL JEM-2010F field emission transmission electron microscope. For this, the samples were suspended in distilled water, and one drop of the sample was placed in a carbon film supported by a copper grid in order to obtain micrographs operating at 200 kV. On the other hand, for scanning electron microscopy (SEM), performed in order to investigate the shape and size of the prepared product, we employed a JEOL JSM-5410 LV scanning electron microscope. For this, the samples were covered with a gold thin film and scanned using an acceleration voltage of 5 kV.

The Fourier-transform infrared spectroscopy (FT-IR) measurement was performed with a PerkinElmer Spectrum Two FT-IR spectrometer. For this process, 1 mg of the sample was mixed with potassium bromide in order to form pellets, and the spectrum was obtained in a transmission mode from 4000 to 500 cm^{-1} with a resolution of 2 cm^{-1} .

The thermal degradation study was performed by means of thermo-gravimetric analysis (TGA) using a PerkinElmer Pyris 1 TGA apparatus. For this, 5 mg of sample were placed in the sample slide and was measured from room temperature up to 600°C with a temperature increment of 10°C per minute. The process was performed under a nitrogen atmosphere.

2.5 Microbiological tests

Escherichia coli (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria were used for the microbiological tests. They were provided by Rubio Pharma y Asociados. A first culture of both microorganisms was prepared in a trypticase soy broth as a growing medium and later the bacteria were cultured in Petri dishes with trypticase soy agar. They were incubated at 37°C overnight with the aim of reaching a concentration of 10^9 colony forming units per ml (CFU ml⁻¹).

The antimicrobial activity of the composite material was determined following the ASTM E2149-01 standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions [34]. For this, three concentrations of the composite (0.1, 0.3 and 0.5 mg ml⁻¹) and one control with a concentration of 0.5 mg ml⁻¹ of purified galactomannan were tested. The test solutions were prepared by dissolving 15, 45 and 75 mg of the composite material in 15 ml of water using an ultrasonic bath; similarly, 75 mg of galactomannan were dissolved in 15 ml of water for preparing the control. Then, 1 ml of the control solution and 1 ml of each of the three composite solutions, differing in concentration, were added (separately) into four glass tubes containing 9 ml of a saline solution (8.5 g of NaCl in 1 l of distilled water) containing a microbial inoculum that provided an initial number of bacteria of $\sim 10^5$ CFU ml⁻¹. Then, the control and the three test tubes were agitated for 1 h at 37°C. Later, 0.1 ml aliquots were taken for each case and placed in Petri dishes with trypticase soy agar as growth media, and were incubated at 37°C for 24 h. After this incubation time, the number of viable bacteria was counted. The percentage of bacterial reduction (%Red) was calculated using the equation [34]:

$$\%Red = [(C_0 - C)/C_0] \times 100, \quad (1)$$

where C_0 , expressed in CFU, is the number of bacterial colonies from the control and C , also in CFU, is the number of bacterial colonies counted from the samples. This process was performed equally for both *E. coli* and *S. aureus*.

3. Results and discussion

The following text is divided into four subsections: the first and second ones are for the basic characterization and thermal analysis of the extracted galactomannan and the prepared composite material; the third subsection is aimed at discussing

the antibacterial activity of the prepared composite material; and the fourth subsection is for additional comments regarding a short evaluation of the use of galactomannan in the preparation of the composite.

3.1 Basic characterization

3.1a XRD characterization: The XRD pattern of the composite is presented in figure 1. All the diffraction peaks in the pattern can be clearly attributed to two phases: one corresponding to Zn(OH)₂, with a wulffingite crystalline structure, as referenced to PDF #38-0385 and the other one corresponding to ZnO, with a zincite crystalline structure, according to PDF #36-1451. From preliminary whole-pattern fitting analysis, we obtain that the inorganic part of the composite possesses high crystallinity, because the experimental data matching required an amorphous profile that accounted for just 6% of the total integrated area of the diffraction pattern.

From the previous qualitative analysis, it is very clear that the composite has two distinct crystalline phases. Quantification of the weight percentage of each phase comprising the crystalline fraction (94% of the total composite material), employing Rietveld refinement [30,31], shows an estimated phase percentage that yields 76% for the Zn(OH)₂ phase and the remaining 24% for the ZnO phase. The result of this Rietveld refinement is shown in figure 2, where the overall χ^2 has a value of 1.88. This result is acceptable, because an improvement of the χ^2 value requires the inclusion of an amorphous profile at around 20.4°, which, according to our previous observations about XRD of pure galactomannan [26], is related to the galactomannan polysaccharide. Moreover, this improvement also requires the incorporation

Figure 1. XRD pattern of the galactomannan/Zn(OH)₂-ZnO composite material. All the diffraction peaks can be related to the presence of the Zn(OH)₂ (PDF #38-0385) and ZnO (PDF #36-1451) phases.

Figure 2. Rietveld refinement for the XRD pattern of the galactomannan/ Zn(OH)_2 -ZnO composite material.

of additional models designed to reproduce the difference in the peak broadening and intensity between the peak located at around 34.5° and the rest of the peaks pertaining only to the ZnO phase.

Table 2 shows the output of the Rietveld refinement. The microstructural analysis from FullProf includes the crystallite size and the maximum strain, which were determined from the full width at half maximum evaluated with the Scherrer and

Stokes–Wilson formulae, respectively. The results show that Zn(OH)_2 has an average crystallite size of ~ 44.1 nm, while the ZnO phase has an average size of ~ 9.8 nm. This indicates that ZnO not only has a smaller fraction, but also possesses lower crystallinity with respect to the Zn(OH)_2 phase (this observation also arises from the comparison of the maximum strain values corresponding to each phase).

3.1b Transmission and scanning electron microscopy characterization: The high crystalline nature of the inorganic portion is undoubtedly appreciable in the TEM images of figure 3, which agrees with the previous XRD results. Particularly, figure 3c shows that almost the totality of the material is crystalline. Here, it is important to note that the galactomannan is unstable under the electron beam used for TEM measurements, which was noticed by the immediate disappearance of a part of the material during the measurement; thus, the organic portion could not be shown in the TEM images.

Additionally, SEM analysis was performed in order to determine the morphology and particle distribution of the composite product. In this regard, the SEM images of the composite are presented in figure 4, from which it can be observed that the hexagonal bar is the main shape preferred by the particles. Also, a high polydispersity of the particle size is observed according to the histogram presented in figure 5, the particle size varies from 0.4 to $2.3 \mu\text{m}$, with a predominant size of 760 nm and a mean size of 800 nm (practically similar values).

Table 2. Parameters determined through Rietveld refinement.

Phase	Crystal structure	Space group	Lattice parameter ($\text{\AA}$)			Crystallite size ($\text{\AA}$)	Maximum strain (%)	Phase percentage (%)	R_{wp}	R_p	χ^2
			a	b	c						
Zn(OH)_2	Wulfingite	P 21 21 21	4.911	5.150	8.476	441	18	76	14.5	10.6	1.88
ZnO	Zincite	P 63 m c	3.254	3.254	5.210	98	34	24

Figure 3. TEM images of the galactomannan/ Zn(OH)_2 -ZnO composite material at different magnifications: (a) 500, (b) 100 and (c) 10 nm scale.

Figure 4. SEM image of the galactomannan/ Zn(OH)_2 -ZnO composite material.

Figure 5. Histogram of the galactomannan/ Zn(OH)_2 -ZnO composite material particles.

3.1c FT-IR spectroscopy characterization: The FT-IR analysis was performed to corroborate that the previously discussed inorganic portion is really interacting with the galactomannan biopolymer, thus the composite material was formed. For a better comparison, figure 6 shows the FT-IR spectra of the prepared galactomannan/ Zn(OH)_2 -ZnO composite and the extracted and purified galactomannan as a reference (the same that was used for preparing the composite material, and whose main characteristics were reported previously [26]). For the reference case, it is observed that the FT-IR spectrum shows the characteristic bands usually reported in the literature for galactomannan polysaccharides [35–52]. In this way, the broad band at around 3360 cm^{-1} observed in the reference spectrum corresponds to the O–H stretching vibrations of the hydroxymethyl ($-\text{CH}_2\text{OH}$)

Figure 6. FT-IR spectra of the (a) extracted galactomannan and (b) prepared galactomannan/ Zn(OH)_2 -ZnO composite material.

and the abundant hydroxyl ($-\text{OH}$) groups present in the galactomannan biopolymer, along with the contribution from the molecules of adsorbed water. The vibration bands at $2990\text{--}2800\text{ cm}^{-1}$ (identified at 2938 and 2888 cm^{-1}) are characteristics of the C–H stretching of the methanediyl ($-\text{CH}_2-$) and the abundant methanetriyl ($>\text{CH}-$) groups in the polysaccharide. The signals observed at 1416 and 1371 cm^{-1} are assigned to CH_2 deformation modes, while the signals observed at the $1190\text{--}920\text{ cm}^{-1}$ interval (which includes the peaks at 1145 , 1064 , 1021 and 976 cm^{-1}) are associated with several vibration modes involving mainly the C–O bond. Finally, the bands at 868 and 810 cm^{-1} are reasonably caused by the D-mannose residue [35], although these signals are usually referred in the literature as an indication of the presence of β -linked D-mannopyranose units (868 cm^{-1}) and α -linked D-galactopyranose units (810 cm^{-1}) [36].

The FT-IR spectrum of the galactomannan reference also presents two bands at 1739 and 1645 cm^{-1} . The first of them, at 1739 cm^{-1} , is attributed to the C=O stretching vibration of the carbonyl group ($>\text{C}=\text{O}$), which could originate from impurities remaining in the sample. On the other hand, the signal at 1645 cm^{-1} could be associated with the amide I characteristic of proteins, which is governed by the C=O stretching vibration of carbonyl in the peptide bond. However, the low intensity (or absence) of the amide II at about 1530 cm^{-1} suggests a low content of protein in the galactomannan reference. For this reason, the signal at 1645 cm^{-1} is rather caused by bending vibrations of adsorbed water molecules.

All the previous signals due to the galactomannan polysaccharide remain in the FT-IR spectrum of the prepared galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite, although all of them show a high reduction of intensity. For example, the absorption bands corresponding to the O-H stretching (appreciated here at around 3270 cm^{-1}), the CH_2 deformation (1435 and 1372 cm^{-1}) and vibrations related to the C-O bond (the 1200 – 925 cm^{-1} interval), and even the bending vibration of adsorbed water by the galactomannan (1652 cm^{-1}) are still appreciable; but originally weak signals, such as the C-H stretching vibrations, almost disappear in the FT-IR spectrum of the composite. This high reduction in the absorption intensity is an indication of the low content of galactomannan in the composite material, which is present mainly as a capping agent of the inorganic particles. The absence of impurities, evidenced by the disappearance of the absorption band related to the C=O stretching vibration, at 1739 cm^{-1} , is related to this low content of organic fraction in the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material (fraction quantification will be inferred from the thermal analysis that will be addressed in the next subsection).

Additionally, it is important to note the absorption contribution from the inorganic material in the FT-IR spectrum of the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite. For example, according to Srivastava and Secco [53], the $\text{Zn}(\text{OH})_2$ material presents two absorption bands at about 1081 and 1027 cm^{-1} ; therefore, signals in the 1200 – 925 cm^{-1} interval (related to the C-O bond in galactomannan) could be increased due to the overlap with signals owing to the presence of $\text{Zn}(\text{OH})_2$ in the composite (this can be of particular relevance for the signals at 1064 and 1021 cm^{-1} , related to galactomannan polysaccharide). In a similar way, other signals due to $\text{Zn}(\text{OH})_2$ and ZnO could increase, in a more or less extent, the different absorption bands in the whole FT-IR spectrum. Finally, it is important to mention the high-intensity absorption band observable at 500 cm^{-1} , which begins at about 580 cm^{-1} and may continue beyond 500 cm^{-1} (the abscissa scale finishes at 500 cm^{-1}). This high-intensity signal has been attributed to vibrations related to the bond between metal and oxygen, in this case, probably the $\text{Zn}=\text{O}$ transverse optical stretching mode [54]. This confirms the presence of the inorganic fraction in the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material.

3.2 Thermal analysis

Figure 7a shows the TGA curves and figure 7b shows the first derivative of the purified galactomannan and the prepared galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material. As seen from the thermograms, the purified galactomannan, analysed as a reference, shows two visible stages of mass loss. The first one, beginning at room temperature and finishing at around 125°C , is attributed to the loss of adsorbed water of the biopolymer, causing a decrease of 8.0% . The second mass loss begins at around 200°C and continues along the total range of the temperature tested. Also, it is appreciable that this stage occurs by means of several diffusion steps,

Figure 7. (a) Thermogravimetric curves and (b) the first derivative of the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material and extracted galactomannan from 25 to 600°C .

the step occurring between 280 and 350°C being the most obvious one, with an inflection point at 326°C , according to the first derivative of the thermogram. This step, which represents a mass loss of up to 50% of the galactomannan mass, can be attributed to the decomposition of the polysaccharide [55].

On the other hand, the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite presents three stages of mass loss. The first stage, which is attributed to the release of adsorbed water, occurs between room temperature and 135°C , and represents a mass loss of about 2.0% . The second stage, which is characterized by a sharp loss of mass, which is very similar to that reported by Srivastava and Secco for $\text{Zn}(\text{OH})_2$ [56], occurs, in our case, between 135 and 170°C , with an inflection point at 139°C . This stage represents a loss of 10.5% of the composite mass, and can be attributed to the loss of water molecules produced during the thermal transformation of $\text{Zn}(\text{OH})_2$ to ZnO [56]. Finally, the third stage, which occurs above 170°C , represents a mass loss of 8.5% , and it can be associated with the degradation of the galactomannan, according to the observation from the thermogram of the galactomannan used as a reference. Therefore, it is inferred that the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material is composed of about 8.5% of galactomannan polysaccharide and about 90% of inorganic material. This is almost consistent with the results obtained from XRD calculations.

3.3 Antibacterial test

The percentage of microbial reduction, as calculated from equation (1), is shown in figure 8. The photographs of the antibacterial test on agar plates are shown in figure 9. The results confirmed the antimicrobial activity of the

Figure 8. Percentage of bacteria reduction by testing the galactomannan/Zn(OH)₂-ZnO composite material for *E. coli* and *S. aureus*.

galactomannan/Zn(OH)₂-ZnO composite, which presents evident activity even with a dilute concentration of 0.1 mg ml⁻¹. On the other hand, the extracted galactomannan, tested in a concentration of 0.5 mg ml⁻¹, did not show antibacterial properties. According to the excellent reviews on the matter [10,57,58], the antimicrobial activity of ZnO-based materials can be attributed to the following three mechanisms: (1) the physical interaction of ZnO particles with the bacterial cell, (a) through accumulation in the bacterial membranes after electrostatic interactions, (b) through particle internalization, or (c) simply due to the particle abrasiveness; (2) the release of Zn²⁺ ions, thus leading to a chemical interaction of this antimicrobial ion with bacterial cells; and (3) the photo-induced generation of reactive oxygen species, such as hydroxyl radicals ($\cdot\text{OH}$), superoxide anion radicals ($\cdot\text{O}_2^-$), and hydrogen peroxide (H₂O₂), among which H₂O₂ is generally considered as the primary species being responsible for the antimicrobial actions, and which sometimes is separated to constitute a fourth antibacterial mechanism. Determining the exact operative antimicrobial mechanism is difficult, since, as rightly concluded by Kumar *et al* [10], the biocidal activities are mainly affected by the ZnO morphology, the presence of surface defects on the ZnO particles, the concentration of the ZnO material, types of the bacterial strain, the method applied for exploring the antibacterial activities and the features of the bacterial cell envelope. Therefore, by considering some similarities to our case, the previous three mechanisms

Figure 9. Photographs of agar plates with (a) *E. coli*; (b) *E. coli* cultured in the presence of 0.1 mg ml⁻¹ of composite; (c) *E. coli* cultured in the presence of 0.3 mg ml⁻¹ of composite; (d) *E. coli* cultured in the presence of 0.5 mg ml⁻¹ of composite; (e) *S. aureus*; (f) *S. aureus* cultured in the presence of 0.1 mg ml⁻¹ of composite; (g) *S. aureus* cultured in the presence of 0.3 mg ml⁻¹ of composite and (h) *S. aureus* cultured in the presence of 0.5 mg ml⁻¹ of composite material.

are considered, to a greater or lesser extent, as the operative ones to attack the bacteria tested in this work.

From the antimicrobial test, with the results shown in figure 8, it was also observed that the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite shows better antibacterial activity against *E. coli* than against *S. aureus*. This result is consistent with those reported by Yamamoto [59], Applerot *et al* [60] and Trandafilović *et al* [13]. However, according to that reviewed by Pérez-Espitia *et al* [58], there are also several studies reporting that *S. aureus* is more susceptible to ZnO particles when compared to *E. coli*, which is caused by a strong affinity between ZnO nanoparticles and *S. aureus* cells. This uncertainty can be understood if we consider the conclusions by Kumar *et al* [10], mentioned in the previous paragraph, in which the biocidal activities can be affected by several factors, including the particle size. According to Zhang *et al* [61], the ZnO material with large-sized particles or aggregates exhibits antibacterial activities through the production of H_2O_2 . Given that the particles of the composite material prepared in the present work are relatively large, we consider that the H_2O_2 -mediated mechanism is the main antimicrobial mechanism operating in our case. With this consideration and taking into account that *S. aureus* can produce antioxidant and detoxification agents, such as carotenoid pigments and the enzyme catalase, we can explain the 30%-resistance presented by the *S. aureus* bacterium, which, based on this result, can be catalogued as a more antioxidant protected bacterium [60].

3.4 Additional comments

The galactomannan extracted from mesquite seeds was used in a green process for the *in situ* preparation of the antibacterial composite material, keeping in mind, at least, two important purposes for this polymeric material. The first one is to serve as a polymer matrix that helps in controlling some final characteristics of the inorganic portion, such as the particle size, shape and distribution. The second one is to serve as a stabilizer of the inorganic particles, especially to help in the preparation of solutions or suspensions that can be managed, particularly during the preparation of samples for the antibacterial tests and for future application as an antibacterial product (we are now exploring ways to prepare topical antimicrobial products). Although it is well known that pure ZnO and $\text{Zn}(\text{OH})_2$ particles show antibacterial activity, the previous advantages completely disappear when these inorganic compounds are synthesized in the absence of the polymer matrix. Importantly, we tried in parallel to study ZnO for reference purposes, but the product formed agglomerates, was insoluble, and, in general, was completely different from the prepared composite material. Thus, it was difficult to employ it for reference purposes. It was more convenient to compare the composite with respect to the pure galactomannan.

4. Conclusions

This study demonstrates the preparation of the galactomannan/ $\text{Zn}(\text{OH})_2$ -ZnO composite material by means of an *in-situ* precipitation method at room temperature.

Based on XRD analysis, it was deduced that about 6% of the composite material is amorphous, which tentatively corresponds to the galactomannan polysaccharide. By means of TGA, it was observed that about 8.5% of the composite material can be associated with galactomannan.

XRD confirmed that the inorganic portion of the composite material (at least 92%, by excluding water, according to the TGA results) is composed of 76% $\text{Zn}(\text{OH})_2$ and 24% ZnO, presenting a crystallite average size of 44.1 and 9.8 nm, respectively. The high crystallinity of the composite material was also confirmed by TEM.

According to SEM measurements, the prepared composite material is predominantly presented in particles with a predominant size of 760 nm and a mean size of 800 nm. The shape of these particles is like hexagonal bars.

FT-IR confirmed the low content of the galactomannan polysaccharide, but as an important part of the composite material. The presence of $\text{Zn}(\text{OH})_2$ and ZnO was also confirmed.

Regarding the antibacterial properties, the prepared composite showed more efficient activity (30%) against *E. coli* (Gram-negative bacterium) than against *S. aureus* (Gram-positive bacterium). The antibacterial activity of the composite material was noticeable even at a concentration of 0.1 mg ml^{-1} .

The previous points support the idea that mesquite seeds are a source of galactomannans that can be used in the near future to prepare organic/inorganic composite materials with many different applications in areas such as materials, food, environmental, and, of course, health sciences.

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