WATER RESISTANCE AND ANTIMICROBIAL IMPROVEMENT OF BAGASSE PAPER BY MICROWAVE MODIFICATION WITH FATTY ACID AND Ag-NPS NANOCOMPOSITE

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Environmentally friendly water-resistant and antimicrobial packaging materials have been developed. Paper sheets were coated with chitosan and stearic anhydride, and then were loaded with silver nanoparticles. A simple dipping method was performed to coat the paper sheets with chitosan. Stearic anhydride was used for surface hydrophobic modification assisted by microwave heating. The treated paper sheets were then characterized by FTIR, SEM and EDAX. Mechanical properties, air permeability, Cobb test, contact angle and antimicrobial tests against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were also investigated.

Keywords: paper sheet, chitosan, microwave, stearic acid anhydride, silver nano-particles, antimicrobial

INTRODUCTION

Water resistance is one of the most important properties of paper used in packaging. A low-cost coating with wax is traditionally used to offer a high degree of water resistance, but the difficulty in wax coated paper recycling limits its applications. A high level of water resistance for recyclable papers can be achieved by coating with synthetic polymers.[1] However, the main problem of using petroleum-based plastics for paper coating lies in the non-renewability of petrol.

Cellulose is the most abundant renewable natural polymer used in paper, textile and packaging industries.[2] Chitosan is derived from natural chitin, which is the second most abundant polysaccharide in nature after cellulose.[3] It is extracted from chitin by deacetylation of the N-acetyl glucosamine groups. Chitosan is a non-toxic, biocompatible[4] and biodegradable polymer.[5] Cellulose and chitosan are convenient for packaging, so they could be a good alternative to alleviate the environmental problems related fossil fuel depletion.[6] Long chain cellulose esters have been prepared by cellulose grafting with fatty acids.[7] These cellulose esters showed improved hydrophobic property with contact angles exceeding 90°.

Chitosan has been modified to produce a wide range of chitosan derivatives with different properties, for a variety of applications.[8] For example, esters are used as antimicrobial agents in the textile industry for producing cotton fabrics with antimicrobial properties and wrinkle recovery.[9] However, the esterification process significantly affects the solubility of chitosan. The process of chitosan esterification is usually performed in acidic medium.[10] Besides, high temperature, long reaction time, corrosion of equipment, the considerable amount of side products and the necessity for treatment of wastes are great disadvantages of this method.

Microwave heating is considered as a promising technique for enhancing chemical reactions. Microwave could provide selective heating, increase the reaction rate, accomplish better reproducibility of reactions and help in developing green syntheses. Several studies have been carried out by using microwave heating in cellulose esterification.[11-12]

Silver nanoparticles (AgNPs) could be utilized as a non-toxic benign antimicrobial material, but it has limited applications because of inferior binding with substrate surfaces. These nanoparticles have a high surface area, which increases their antimicrobial activity. Consequently, AgNPs are superior to bulk silver particles as antimicrobial agent.[13-14]

The aim of this work was to investigate the effect of surface modification of bagasse paper. Paper was modified by coating with chitosan, followed by microwave assisted grafting with long hydrocarbon chain fatty acid anhydride. AgNPs were loaded onto modified paper sheets to impart antimicrobial activity. The modified paper sheets were subjected to tests in order to investigate their mechanical properties, water absorption and air permeability. The study aimed to explore the antimicrobial activity of the modified paper against *Staphylococcus aureus* (Gram-positive bacteria), *Pseudomonas aeruginosa* (Gram-positive bacteria) and *Candida albicans* (yeasts) species.

EXPERIMENTAL

Materials

Unbleached kraft bagasse pulp was provided by IDFO Company, Egypt. Chitosan (purity of 90%, viscosity of 50-300 cps) was purchased from BioBasic Canada Inc. Acetic acid was used to adjust the pH of the prepared solutions of chitosan. Stearic acid anhydride, silver nitrate ($AgNO_3$) and trisodium citrate $C_6H_5O_7Na_3$ were of analytical grade purity, and were used without further purification.

Determination of the chemical composition of pulp

Chemical analysis was performed according to TAPPI standard methods to determine the percentage of the main chemical constituents of the kraft bagasse pulp. The used pulp had 5.65% lignin, 70.32% α-cellulose, 22.68% hemicelluloses and 0.67% ash.

Preparation of paper sheets

The paper sheets were prepared according to the S.C.A. standard, using a S.C.A. model sheet former (AB Worentzen and Wettre). In the apparatus, a sheet of 165 mm diameter and 214 cm^2 surface area was formed. The weight of oven dry pulp used for every sheet was of about 1.8 g. After sheet formation, the sheet was pressed for 4 min using a hydraulic press. Drying of the test sheets was carried out with the help of a drum (rotating cylinder) at 105 °C for 2 h.

Coating of paper sheets with chitosan

Paper sheets were dipped in a 2% chitosan solution for 60 s. After dipping, the paper sheets were pressed between two filter paper sheets to remove the excess chitosan, and then dried on the drum at 105 °C for 2 h.

Esterification of chitosan coated bagasse paper sheets

The esterification solution was prepared by adding stearic acid anhydride to a mixture of 50 mL CH_2Cl_2 and 2 mL pyridine under stirring at room temperature for 30 minutes. The esterification of the paper sheet was achieved by activating the paper sheet in a microwave at 640 W for 30 s and dipping the activated sheet quickly in the esterification solution at room temperature for 30 s. Then, the samples were activated again in the microwave at 640 W for 30 s and subsequently kept in acetone for 30 s to extract the residual acid before drying in an oven at 50 °C for 1 hour.

Loading of silver nanoparticles onto treated paper sheets

The AgNPs were loaded onto the stearic acid treated paper sheet by using the following method: the modified paper sheet was immersed into an $AgNO_3$ solution (15 mg $AgNO_3$ in 40 mL distilled water), it was removed and then immersed into a tri-sodium citrate solution (20 mg dissolved in 25 mL water) to reduce Ag^+ ions to AgNPs. The thus-prepared material was allowed to dry. In this way, the AgNPs loaded paper sheet was prepared.

Mechanical properties

The modified and unmodified paper sheets were tested for tensile strength and burst. Tensile testing was carried out according to TAPPI T494-06 standard method on 15 mm wide strips between jaws set 100 mm apart, using a universal testing machine (LR10K; Lloyd Instruments, Fareham, UK) with a 100-N load cell at a constant crosshead speed of 2.5 cm/min. Burst strength tests were conducted according to Tappi standard T403 on a Mullen tester (Perkins, Chicopee, MA, U.S.A.).

Air permeability

The air permeability test was carried out on a BENDTSEN Smoothness and Porosity Tester, Andersson and Sørensen, Copenhagen. Air permeability is the flow of air (cm^3 min^{-1}) passing through 1 cm^2 surface of the test piece at a measuring pressure of 1.00 kPa.

Water absorption (Cobb)

The water absorption test of the paper sheet was carried out as follows: the weighed paper sheet was put in a Cobb apparatus; 100 mL of water was added over the paper and left for 105 s, then poured. The paper sheet was dried well between two filter papers and weighed:

Cobb (g/m^2) = (Weight of wet paper sheet – Weight of dry paper sheet) X 100 (1)

Contact angle

The interface existing between a liquid and a solid (contact angle) is measured using a contact angle measuring system according to ASTM D724-99 Standard Test Method for Surface Wettability of Paper. It consists of a very fast image capturing system (500 image/second) based on the 3D Compact Video Camera Microscope (CVM), manufactured by SDL-UK. The measurements were carried out under environmental conditions of 22 °C temperature and 65% relative humidity. Every measurement was done 5 times for measuring the uncertainty of results and it was found to be ±1 (coverage factor k = 2 for statistical confidence level of 95%).

Infrared (IR) spectral analysis

The FT-IR spectra of the reference paper sheets, treated paper sheets and Ag-NPs containing paper sheets were recorded in the range of 400-4000 cm^{-1} on a Shimadzu 8400S FT-IR spectrophotometer.

Scanning electron microscopy (SEM) and EDX measurement

The surface morphology of the paper sheets and of the AgNPs introduced in the treated paper sheet was analyzed using a FEI INSPECT electron microscope, Philips, Holland. EDX analysis was carried out in order to acquire supporting information to confirm the presence of AgNPs in the modified paper sheets.

Assay of antimicrobial activity

The disc diffusion method was used to determine the antimicrobial activity of the paper sheets. A volume of 0.1 mL of the tested microorganisms was inoculated on Brian Heart Infusion Broth (at 42 °C for 24 h, 108-109 cells/mL), and then spread on the entire surface of the dish *via* a sterile spatula. Subsequently, sterile discs were placed onto agar at certain intervals by passing gently. After the plates were incubated at 42 °C for 24 h, the inhibition zones around the discs where no growth occurred were measured in millimetres. The experiments were repeated twice for all of the test strains.

RESULTS AND DISCUSSION

Chitosan is a positively charged polymer due to protonation of the primary amino group in acidic medium, which could bind to negatively charged molecules in fats and lipids.[15] So, the stearic acid anhydride could react with precipitated chitosan molecules existing on the paper sheet surface upon microwave activation.

Chitosan could improve the bond strength between fibers forming paper sheet when used as a coating material. Therefore, it could improve the tensile strength and elongation rate before breaking.[16]

Breaking length

The mechanical properties of coated stearic anhydride treated and untreated paper sheets have been measured and the results are shown in Figure 1. The breaking length was slightly affected by stearic anhydride concentration up to 1% and then increased upon increasing anhydride concentration. During esterification, the paper sheets were subjected to the action of chemicals and solvents, as well as to microwave heating, which causes a deterioration of paper strength. Otherwise, the introduction of fatty acid moiety improved the paper strength, so at lower stearic anhydride concentrations, there was no remarkable improvement in breaking length. Meanwhile, at higher stearic anhydride concentration, more than 1%, the binding between stearic carboxylic groups and amino groups on the chitosan film compensated the esterification deterioration effect and formed a second coating layer, which explained the increase in breaking length. An increase in the amount of introduced stearic groups to the paper sheets as the stearic anhydride concentration was increased was evident from the decrease of air permeability (Fig. 3) and water absorption (Table 1).

Burst strength

The penetration of chitosan into the fibers and the compatibility between chitosan and cellulose fibers increased the strength of fiber bonding, which enabled higher burst strength values for the coated paper sheets. The treatment with stearic anhydride had no effect on the burst strength of the bagasse sheet at 0.25 and 0.5% stearic anhydride. By increasing the concentration from 0.5 to 1.5%, the burst strength increased by ≈40%, as shown in Figure 2.

Air permeability

Paper sheets were coated with chitosan and stearic acid to obtain a packaging material with superior barrier properties towards air. This property is significant to achieve a long shelf-life for the packaged product. As can be seen from Figure 3, the air permeability decreased with increasing stearic anhydride concentration. Decreasing air permeability indicates a more compacted structure, which was confirmed by the morphological study.

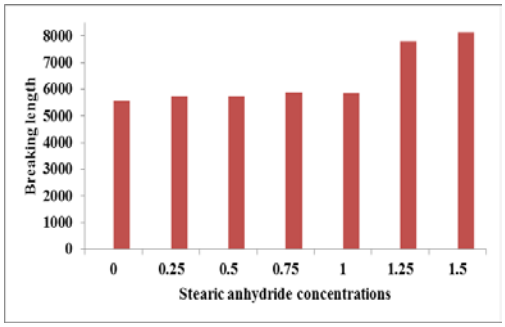

Figure 1: Effect of stearic anhydride concentration on breaking length of the paper sheet coated with chitosan

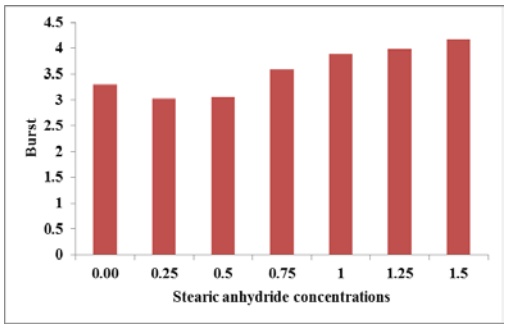

Figure 2: Effect of stearic anhydride concentration on burst strength of the paper sheet coated with chitosan

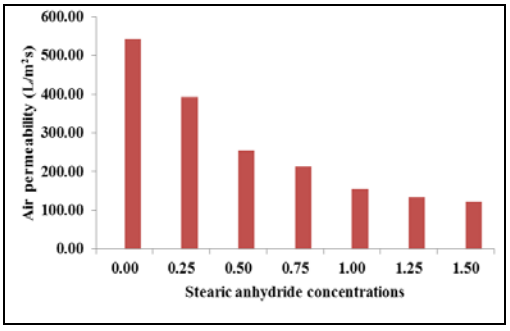

Figure 3: Effect of stearic anhydride concentration on air permeability (L/m^2s) of the paper sheet coated with chitosan

Table 1
Effect of stearic acid anhydride concentration on water resistance, contact angle and surface free energy of the chitosan-coated paper sheet

Stearic anhydride %	Blank	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Cobb g/m^2	22.6	5.58	4.41	4.96	5.52	4.84	5.08	5.03
Contact angle	Completely absorbed	112	129	120	--	120	--	125
Surface free energy (mN/m)	145.6	45.53	26.99	36.40	--	36.40	--	31.04

Water absorption (Cobb test)

Liquid water sensitivity was evaluated by the Cobb test. The Cobb values of the chitosan-coated composite sheets treated with stearic anhydride were compared with respect to the stearic anhydride % used in the paper sheets coating, as shown in Table 1.

The treatment with chitosan reduces the Cobb values considerably from 22.6 for blank paper to 5.58 g/m^2 for the paper treated with 2% chitosan, which confirmed the superior compatibility between chitosan and cellulose in paper. Water resistance increases upon the treatment with fatty acids until it reaches a steady level at about 80% decrease of water absorption, compared with that for untreated paper sheets. This could be elucidated by the effect of the differences in the paper sheet microstructures. Certainly, the distribution of stearate groups on the paper sheet surface reduced water absorption and then reduced Cobb values. Water resistance increased by 9-21% after coating the chitosan-treated paper sheets with stearic acid anhydride.

Contact angle

Contact angle measurements were carried out by depositing water drops on the paper sheets. As expected, the blank paper sheet was hydrophilic, the contact angle of the water droplet deposited on its surface could not be determined because it

was completely absorbed. The contact angle increased after coating with chitosan, due to the decrease of the accessible voids and pores on the paper sheets.[17] Water affinity was reduced significantly after coating with chitosan, from completely absorbed to 112° contact angle. After esterification, the contact angle increased again by 7-15% (Table 1); it reached 120-129° for different samples treated with stearic anhydride. There was no significant difference observed in the contact angles of the samples treated by higher stearic anhydride concentration.

It is known that the surface free energy (ΔG) of paper and cellulosic fibers is an important factor affecting the liquid penetration rate and adhesion to other polymers, in painting and printing. One of the important functions of the contact angle measurement is the calculation of the ΔG of the solid, which corresponds to the surface tension of the liquid and has the same unit (mN/m). Contact angle methods are still preferred for determining the ΔG of paper by using Young's equation:[18]

Surface free energy (mN/m) = solid-liquid interface + liquid surface tension X cos θ (2)

In the case of paper as a solid and water as a liquid, the equation could be simplified as:

Surface free energy (mN/m) = 72.8 (1+ cos θ) (3)

Surface free energy values (Table 1) show a clear reversible relation with contact angle values. As the paper sheet surface becomes homogeneous and smooth, the ΔG values decrease.

FTIR spectroscopy

A characteristic band at 2915 cm^{-1}, which is attributed to C-H elongation in alkyl chains, could be used to evaluate the penetration of the hydrophobic component in the paper sheets. It distinguishes the alkyl chains presence in hydrophobic components. This band was observed as a weak band for bagasse paper and became sharper for the paper modified with stearic anhydride.

The main characteristic peaks of the bagasse paper sheet are located at 3350 cm^{-1}, assigned to O-H stretching, and at 2916 cm^{-1}, due to CH stretching. The band at 1649 cm^{-1} is attributed to deformation vibration of the absorbed water molecules. Also, the spectra of the modified sheets showed similar patterns. Focusing on the region between 1000 and 2000 cm^{-1}, the carbonyl absorption band could be observed at 1739 cm^{-1}, which confirmed the presence of fatty acid on the modified paper sheets.[19]

The spectrum of the sheet coated with chitosan shown in Figure 5 exhibits a band at 3367 cm^{-1}, which is due to amine NH symmetric vibration. A further strong broad vibration at 3442 cm^{-1} is assigned to the stretching vibration of O-H and N-H groups. The peak of 2927 cm^{-1} is typical of C-H vibration.[20] The characteristic absorption of chitosan is the band at 1333 cm^{-1}, assigned to the vibration of C-H, and the band at 1559 cm^{-1}, which is assigned to the stretching vibration of the chitosan amino group. The broad peak at 1080 cm^{-1} indicates C-O stretching vibration.[21]

Figure 5 presents the spectrum of the bagasse sheet in comparison with that of the sheet impregnated with the chitosan solution. Even if the sheet was only impregnated with chitosan, some differences are visible in its spectrum.

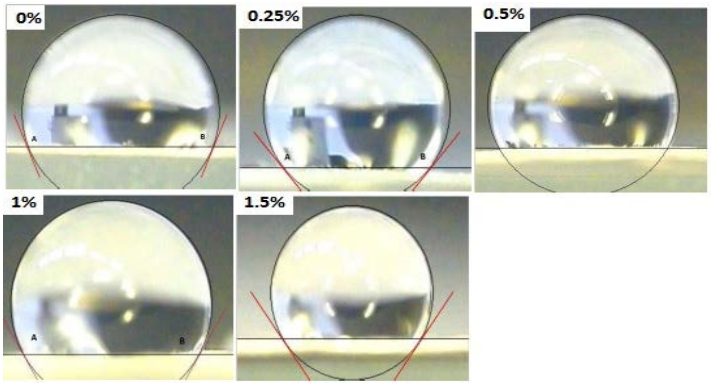

Figure 4: Effect of stearic anhydride concentration on contact angle of bagasse paper coated with chitosan and treated with stearic anhydride

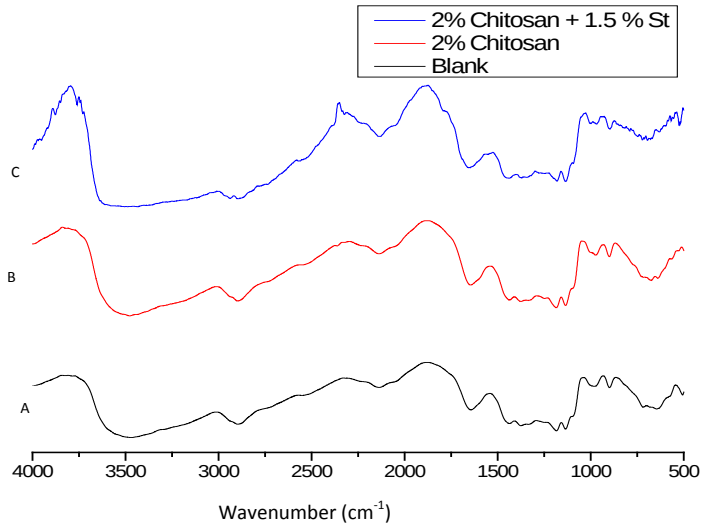

Figure 5: Infrared spectra for (A) reference bagasse paper sheet, (B) bagasse paper sheet coated with chitosan, and (C) bagasse paper sheet coated with chitosan and treated with stearic anhydride

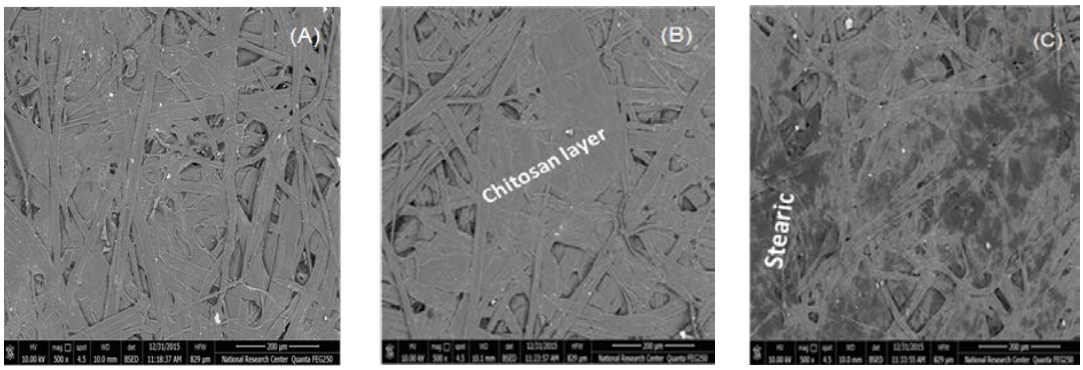

Figure 6: SEM of (A) reference bagasse paper sheet, (B) bagasse paper sheet coated with chitosan, and (C) bagasse paper sheet coated with chitosan and treated with stearic anhydride

The absorption band at 3350 cm^{-1} shifted to 3342 cm^{-1} and became a border indicating a possible overlapping stretching of hydrogen bonded –OH and NH_2. The characteristic bands at 2916 cm^{-1} for bagasse and at 2927 cm^{-1} for chitosan are assigned to CH stretching.

Scanning electron microscopy

Scanning electron microscopy is a most suitable tool for morphometrical studies of fibers and paper. Figure 6 shows SEM images for the untreated bagasse paper sheet, the bagasse paper sheet coated with chitosan, and the bagasse paper sheet coated with chitosan and treated with stearic anhydride.

From Figure 6 (a), it is clear that the bagasse paper sheet had a rough surface with many pores, while the bagasse paper sheet coated with 2% chitosan (Fig. 6 (b)) had few pores and the cellulosic surface was covered by chitosan. Figure 6 (c) shows that the cellulosic surface was well covered by stearic acid (seen as dark splotches), while the paper sheet had a smooth surface with minute pores. Thus, SEM analysis reveals a clear-cut distinction between the appearance of the untreated and treated paper sheets.

Figure 7(a) demonstrates the dispersion of AgNPs on the surface of the bagasse paper sheets coated with chitosan and treated with stearic anhydride. Figure 7 (b) shows the elemental mapping corresponding to different elements in the treated paper sheet and the distribution of C, O and Ag in the treated paper sheet can be observed in Figure 7 (c-e). The EDX spectrum of the paper sheet coated with chitosan and treated with stearic anhydride, emphasizing the presence of AgNPs with a strong peak is shown in Figure 7 (f).

Antimicrobial study

Thanks to the presence of the free amino group, chitosan can inhibit the growth of bacteria, and

due to its ability to absorb water from the environment and form a gel, it exhibits a significant antimicrobial activity.[22] Antimicrobial testing confirms the presence of inhibition zones on the surface of the chitosan coated paper, mainly attributed to the AgNPs, which performs very well against microorganisms (Fig. 8).

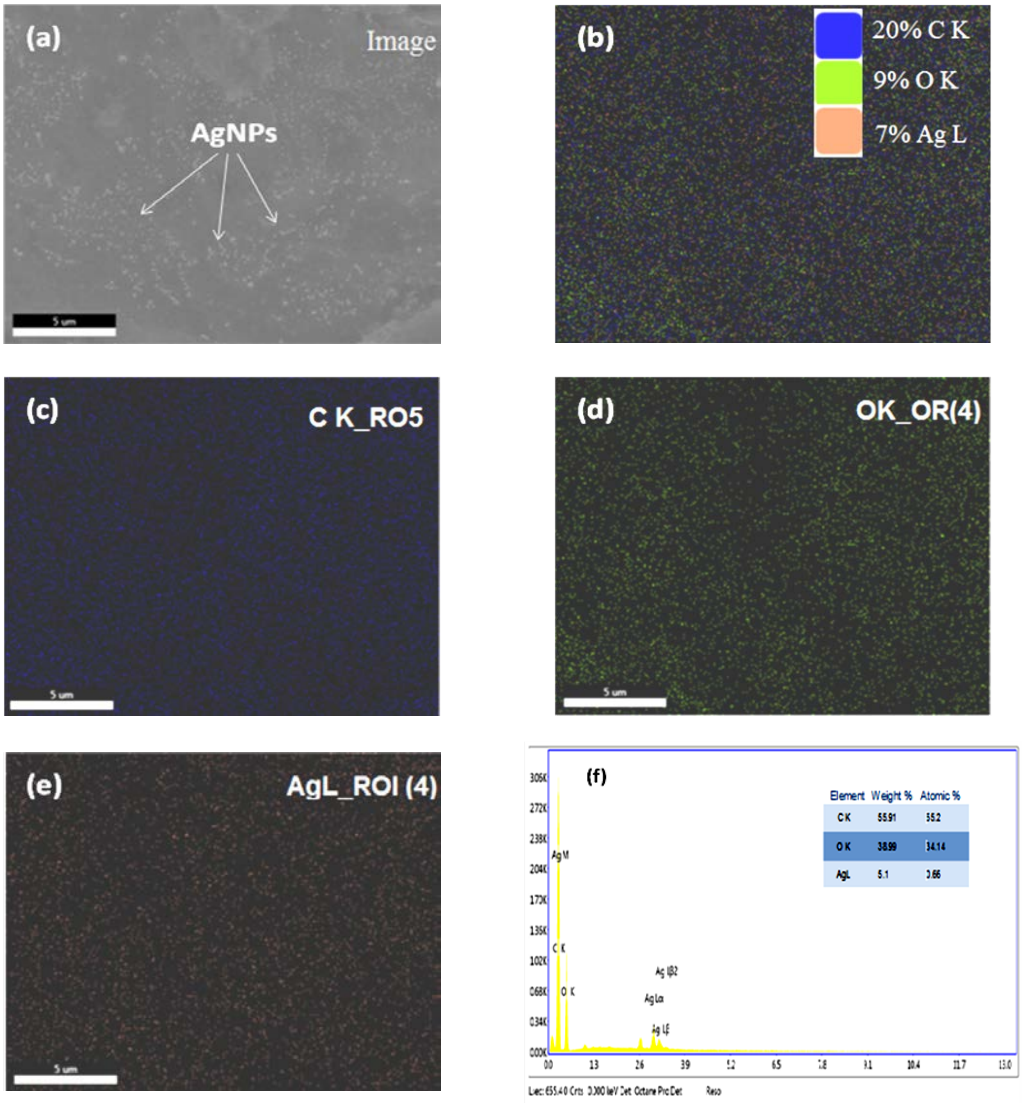

Figure 7: SEM and EDAX analyses of chitosan films loaded with AgNPs: (a) SEM; (b) elemental maps; (c-e) C, O and Ag distribution in treated paper, respectively, and (f) EDX spectrum

Paper sheet	Microorganism		
	S. aureus	*Pseudomonas aeruginosa*	*Candida albicans*
Untreated			

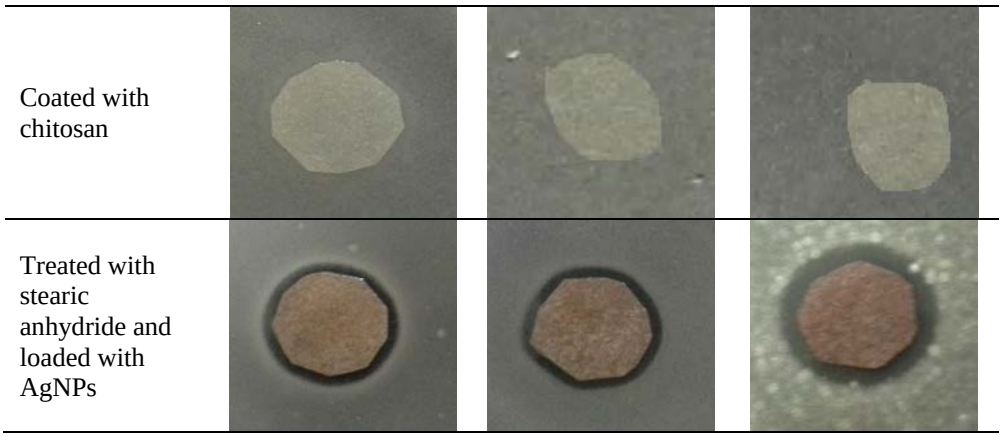

Figure 8: Antimicrobial activity of differently treated bagasse paper sheets

AgNPs, which are considered as a non-toxic environmentally friendly antimicrobial material, were incorporated into the chitosan coated bagasse paper sheet treated with stearic anhydride. A high inhibition zone against all the investigated strains was observed for the AgNPs containing paper sheets, in contrast to other paper sheets without AgNPs, which did not exhibit any antimicrobial activity. The mechanism of inhibition of microorganisms by AgNPs is not fully recognized. It has been supposed that AgNPs damage the DNA of microorganisms, which loses its reproduction ability and cellular proteins become inactivated. Furthermore, it has been affirmed that Ag attached to proteins' functional groups, resulting in their denaturation.[23] Also, the interaction between Ag and the outer membrane constituents causes structural changes, degradation and final cell death.

CONCLUSION

This study achieved an improvement in the hydrophobic character of chitosan-coated papers. The combination of chitosan with stearic acid and the compatibility of this acid with chitosan and paper sheets were achieved by the microwave technique. AgNPs were loaded onto the chitosan coated paper sheet treated with stearic acid, giving excellent antimicrobial effects against *S. aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, and yielding antimicrobial papers with hydrophobic surface suitable for packaging applications. Also, the mechanical tests of the paper sheets illustrated an increase in the coated paper strength, compared with that of uncoated paper. Thus, the present study developed a material based on biodegradable polymers, which is appropriate for packaging applications and can be disposed of after use in an ecologically acceptable way.

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