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Valorization of marine wastes in a preserving film based on chitosan for food applications

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Abstract
One of the best alternatives to reduce current packaging waste is the use of biodegradable films that allow the final replacement bags plastic packaging which are not recycled and are thus a pollution source. In this study, chitosan (CS)/bentonite (BT) nanocomposite was prepared by the intercalation of chitosan in bentonite to form miscible, biodegradable nanocomposite material used as packaging films for food preservation. In order to valorize the Moroccan marine wastes, chitosan was prepared from exoskeletons of shrimps. Furthermore, thanks to the excellent film-forming capacity and antimicrobial properties of chitosane, this material reduces the use of chemical preservatives. Chitosan/bentonite nanocomposites are economically interesting because they are easy to prepare and involve inexpensive chemical reagents. Chitosan is a deacetylated derivative of chitin, which is the most abundant polysaccharide in nature after cellulose; it is a natural polysaccharide, biocompatible and biodegradable in addition to the antibacterial properties that can be useful in many areas as the food packaging industry. In the same way bentonite are abundant and low-cost natural materials. Although chitosan/bentonite nanocomposites shape a natural films with a great potential and provide physical protection and they are biocompatible and biologically active towards microbial growth, while being non-toxic and biodegradable.

Keywords: Chitosan films, Biomaterials, Nanocomposite, Composite, Polysaccharide, Antibacterial activity.

Introduction
Current research is directed towards the development of new biocompatible film to reduce packaging waste and the use of biodegradable polymers. Chitin and chitosan are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications [1], as biomedicine [2], pharmaceuticals [3], metal chelation [4], food additives [5], and film-forming capacity (Fig. 1).

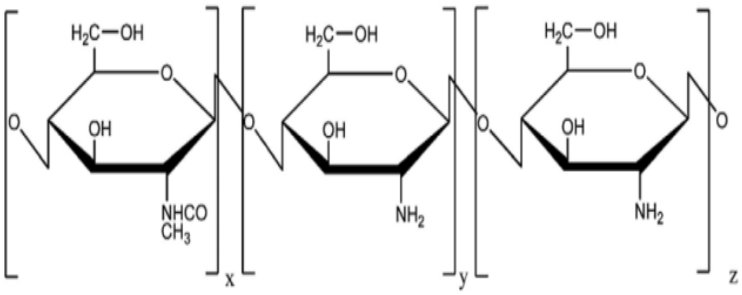

Figure 1: Chemical structure of chitosan.

Chitosan, a linear polyamine copolymer of ß-(1-4)-D-glucosamine and acetyl-b-(1-4)-D-glucosamine, is obtained by alkaline N-deacetylation of chitin, the second most abundant polysaccharide on the earth as a component of exoskeletons of crustaceans and insects [6]. The amino group NH_2 can be protonated to NH^{3+} and

readily form electrostatic interactions with anionic groups in an acid environment [7]. This property has been applied on edible films. Due to its natural origin, biocompatibility [8], biodegradability [9] or its antibacterial properties [10], chitosan is used in many areas such as food processing, biomedical, cosmetics, the treatment of water or agriculture. In the particular domain the food industry, chitosan is mainly used for its antibacterial properties and antifungal because it can prevent or slow the growth of bacteria or molds on the surface of food. The chitosan has a film-forming property which reflects the ability of chitosan to form a continuous film by curing, which makes him a suitable polymer for food packaging. Chitosan is very promising as a packaging material. However, given its cost of production, it seems preferable to combine with other substances to produce bio-active packaging at reasonable prices, so they can be widely used in food industries [11].
The objectives of this study were to prepare nanocomposite films based on chitosan and clay to gain the high mechanical strength of clay and the superior biological properties of chitosan [12].

2. Materials and methods

2.1. Materials

Chitosan with DDA ~ 87% in powder form was prepared in our laboratory. The degree of deacetylation (DDA %) was determined by conductimetric titration.
Analytical grade bentonite was purchased from Sigma-Aldrich. All the other chemicals used are of analytical grade and used as received.

2.2. Methods

2.2.1. Preparation of chitosan film

Chitosan film was prepared by dissolving 2% (W/V) chitosan solutions in 2% of acetic acid at room temperature. After the chitosan was dissolved completely, the solution was filtered to remove the insoluble residue. This solution was cast into glass plate which had been previously cleaned with methanol and dried at room temperature for 20 h to evaporate the solvent and form the films.
The dried films were soaked with an aqueous solution of 1 M NaOH for 30 min to remove residual acetic acid, followed by rinsing with distilled water to neutralize, and then dried at room temperature.

2.2.2. Preparation of chitosan/bentonite film

Chitosan solutions were prepared by dissolving chitosan in a 2% aqueous acetic acid solution at a concentration of 2 wt% under continuous stirring at room temperature. After the chitosan was dissolved completely, the solution was filtered to remove the insoluble residue.
Bentonite solution was prepared in water at different concentrations while varying the ratio (bentonite / chitosan) to study the films properties. The heat treatment of bentonite solution aims to exfoliate the clay particles. A series of chitosan/bentonite composite films were prepared by mixing 100 ml of 2% chitosan solution with 100 ml of 1, 2, 3, 5% bentonite solutions.
Using a film maker apparatus, the mixtures were spread onto glass plates already cleaned with methanol and the film thickness was maintained constant during this study. The dried films were soaked with an aqueous solution of 1 M NaOH for 30 min to remove residual acetic acid, followed by rinsing with distilled water to neutralize, and then dried at room temperature.

2.2.3. FTIR analysis

A Nicolet 6700 FTIR -ATR crystal spectrometer, Canada, connected to software of the OMNIC operating system (version 8.2 Thermo Nicolet) was used to obtain FTIR spectra of chitosan. Powder of chitosan samples were placed in contact with attenuated total reflectance (ATR) on the small sampling area of ZnSe crystal at controlled ambient temperature (25°C). FTIR spectra were collected in the frequency range of 4000-450cm^{-1} by co-adding 64 scans and at a resolution of 4 cm^{-1}. The spectrum was rationed against a background of an air spectrum. The sampling area was carefully cleaned by wiping with acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as transmittance values at each data point in triplicate.

2.2.4. NMR analysis

The ^{1}H NMR spectra were acquired on a Varian Mercury 500 MHz spectrometer, Canada. The experiments were performed at 70 °C, at which temperature the solvent peak (HOD) does not interfere with the peaks of chitosan.

For the test, the chitosan solution was prepared from 10 mg of chitosan in a solution composed of 1.96 ml of D_2O and 0.04 ml of DCl stirred for 30 minutes at room temperature. In these solutions, DCL is in excess relative to the chitosan amino groups so that the polymer readily dissolves. After dissolution, approximately 1 ml of the chitosan solution was transferred into a NMR tube of 5 mm. The sample tube is inserted into the magnet and the thermal equilibrium is allowed to meet for 10 minutes before starting the experiment. 1H-NMR experiments were a unique sequence of pre-saturation pulses solvent. A 90° pulse corresponding to a pulse width of 11 ms was used. The delay before application of the pulse is 6 s and the acquisition time is 2 seconds for a total relaxation time of 8 seconds between each pass.

2.2.5. Thermal analysis

The thermal gravimetric analysis was carried out on a TGA 2950 Hi-Res Thermogravimetric Analyzer Canada, at a heating rate of 10°C/min under nitrogen atmosphere. The mass of the samples was generally in the range of 2-3 mg. The sample pan was placed in the balance system equipment and the temperature was raised from 0 to 600°C.

2.2.6. Traction analysis

Mechanical properties of chitosane/clay nanocomposite were measured with a Universal Testing Machine Ludwig mpK, Morocco. The initial grip separation was set at 35mm and the crosshead speed was set at 5 mm/min.

A tensile test involves subjecting a sample to a longitudinal extension at constant speed. A force diagram shows the change in elongation function of the force. These curves provide information on the sample mechanical properties. From the traction analysis, we can deduce parameters such as the modulus of elasticity, the voltage rupture which is the maximum stress reached during the test, the elongation at break that corresponds to the deformation of the affected active area upon breakage, the coefficient of fish characterizes the contraction of the material perpendicular to the direction of the force applied, or the necking coefficient corresponding to the area reduction of the location area of the deformation at rupture.

3. Results and discussion

3.1. Infrared spectroscopy (FTIR)

The IR spectrum of chitosan was recorded in the region of 4000–450 cm^{-1} and is shown in Fig.2. The spectrum of chitosan shows wide band around 3450 cm^{-1} corresponding to amine N–H symmetrical vibration and H bonded O–H group. The peaks present on the range 3400–3800 cm^{-1} were also corresponding to combination of characteristic peaks of O-H, NH2 and intra-molecular hydrogen bonding. The peaks at 2920 and 2320cm^{-1} are assigned to the symmetric and asymmetric –CH2 vibrations of carbohydrate ring. The absorption peak at 1650cm^{-1} (C=O in amide group, amide I vibration), 1545cm^{-1} (–NH2 bending of amide II) and 1390 cm^{-1} (N–H stretching or C–N bond stretching vibrations, amide III vibration). The peak at 1092 cm^{-1} corresponds to the symmetric stretching of C–O–C groups. The absorption peaks in the range 900–1200 cm^{-1} are due to the antisymetric C–O stretching of saccharide structure of chitosan.

3.2. NMR spectroscopy

The ^1H NMR spectrum of the polymer is a superposition of the spectra of monomers which are slightly modified because of their linkages between them. The peak at 2.0 to 2.1 ppm represents three protons of N-acetylglucosamine (GlcNAc) and the peak at 3.5- 3.6 ppm represents the H-2 proton of the glucosamine residue (GlcN). Protons, which are connected to the glycosidic ring, have densities similar electrons remains the same chemical shift. The signals of ring protons with the C6-H partially overlap and produce a large signal envelope in the middle of the spectrum. All signals were observed between 3.8 and 4.4 ppm. The protons (H_1) are observed at the highest chemical shift. This is due to their vicinity and glycosidic oxygen ring. The protons H-1 [GlcN (H-1D) and GlcNAc (H-1A)] resonate at 4.9 and 5.3 ppm, respectively. Between the different 1H NMR spectra, the methyl protons that go toward 2.0 -2.1 ppm, has the highest resolution. The protons H-1 [H1 (GlcNAc) (δ = 5.3 ppm) and H1 (GlcN) (δ= 4.9 ppm) have the least resolution. Resolutions of protons H_3, H_4, H_5, H_6 are also low. The signals of the last protons normally overlap with solvent HOD signal (D_2O / DCI) to 4.05 ppm. In our case, they are not seen because we have established a solvent in the pre-saturation.

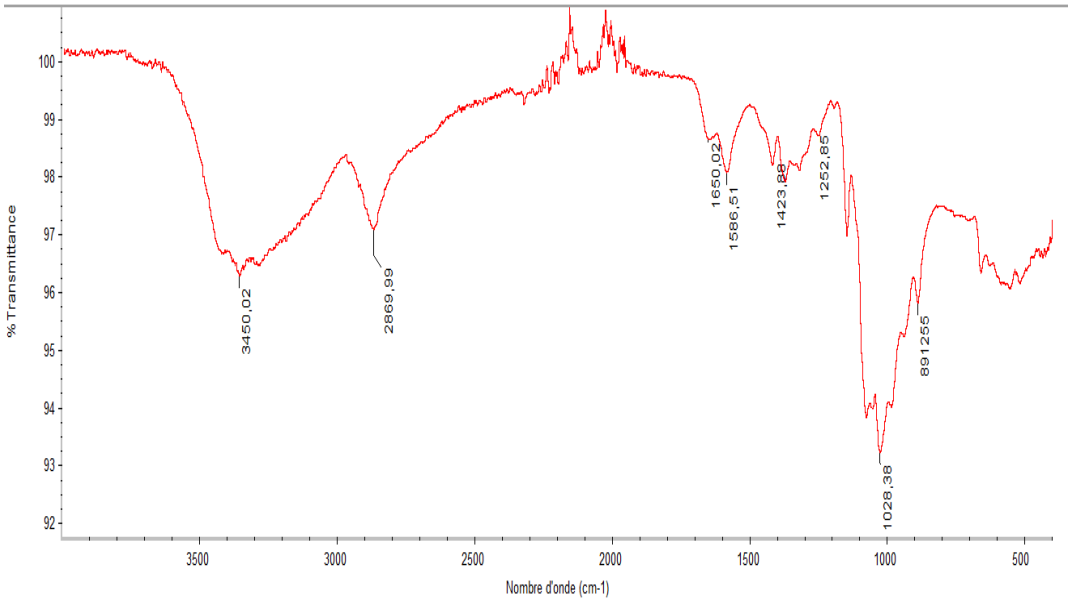

Figure 2: The infrared spectrum of chitosan.

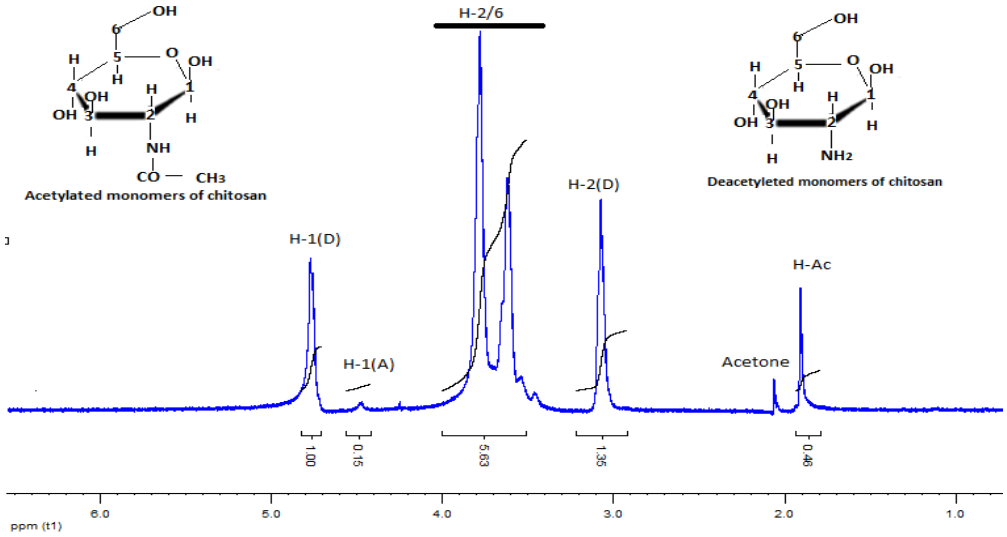

Figure 3: ^{1}H NMR spectrum at 70°C of chitosan.

3.3. Thermal analysis

ATG of chitosan showed a weight loss in two steps. The first step is around 80 °C and about 4% of weight loss, which corresponds to the water loss. The second step of the weight loss began at 242 °C; the weight loss was 42% due to the degradation of chitosan.

3.4. Mechanical properties of the film

The tensile strength (TS) and elastic modulus (EM) of chitosan films increase by the formation of nanocomposites, particularly for chitosan/bentonite prepared from different ratios. The increase in the TS and EM of such nanocomposite films can be attributed to the high rigidity and aspect ratio of the nanoclay as well as the high affinity between the biopolymer and the bentonite. On the other hand, the chitosan/bentonite nanocomposites have shown significant decrease in elongation at break (EB).This reduction can be attributed to the restricted mobility of macromolecular chains.

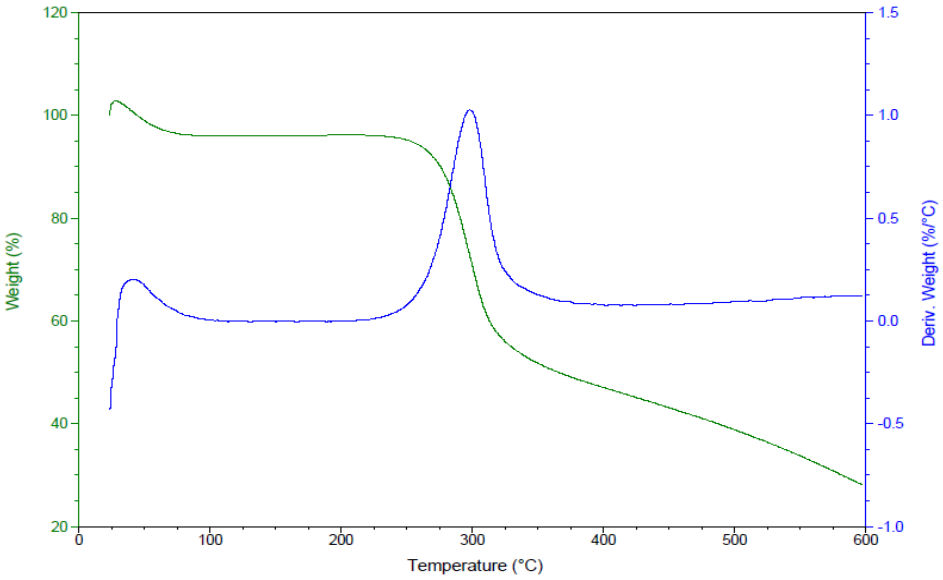

Figure 4: The TGA spectrum of chitosan.

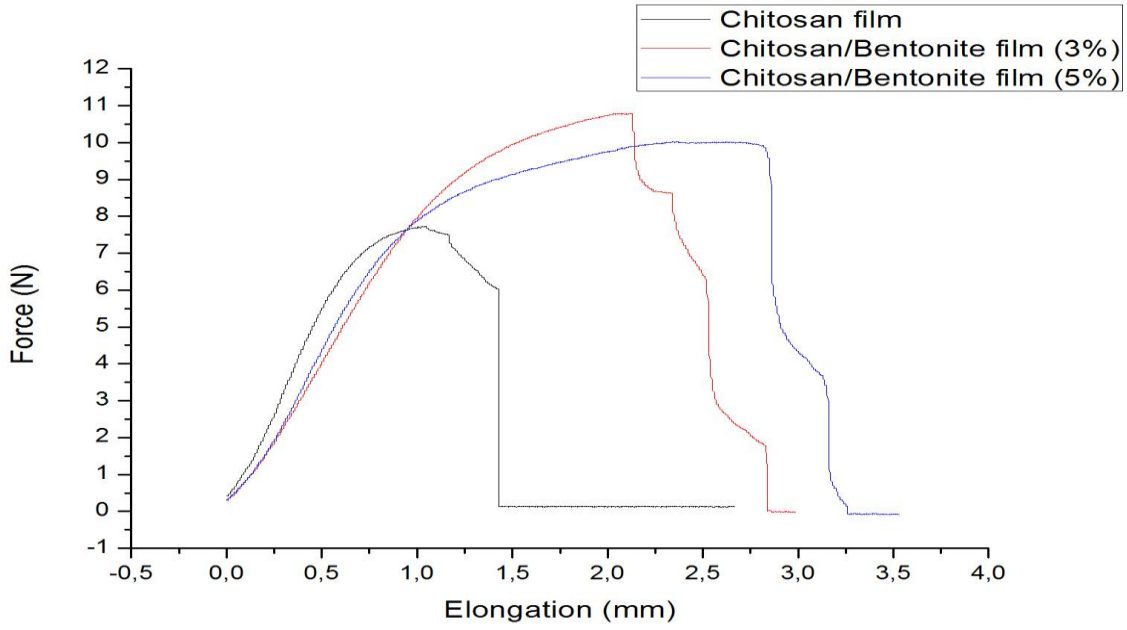

Figure 5: Traction diagram films of chitosan and chitosan / bentonite at different ratios.

Conclusion

In this study, chitosane/bentonite nanocomposite films were successfully prepared. The intercalation of chitosane into the bentonite interlayer spacing was achieved by the electrostatic attraction between NH^{3+} groups and the negatively charge of bentonite surface. The tensile curve obtained shows that the film has developed an interesting force. The nanocomposite film prepared combines two inexpensive resources available and biocompatible.

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