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Preparation and Investigation of Mechanical and Antibacterial Properties of Poly (ethylene terephthalate)/Chitosan Blend

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Abstract

The aim of this work was to evaluate the anti-bacterial and mechanical properties of chitosan based solvent-cast blends with synthetic Poly (ethylene terephthalate) (PET). The amount of chitosan loading was varied from 1% to 9% (W/W). Chitosan and PET were homogeneously dissolved in a ternary solvent system with different mass ratios in a trifluoroacetic acid, chloroform, and acetic acid solution and processed into uniform films. Molecular interactions between chitosan and PET were investigated using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. Morphology and tensile properties of these blend films were investigated. The antibacterial activity of the samples was evaluated utilizing the colony forming unit method against three typical human pathogenic microorganisms, *Escherichia coli, Klebsiella pneumonia*, and *Staphylococcus aureus*. The results indicated that the PET/chitosan films showed a significantly higher growth inhibition rate compared with the PET film. Chitosan release from a wide range of blends was studied using the Ninhydrin method. The release tests revealed that dissolution of the biocide glucosamine groups, i.e. the chitosan water soluble fractions, also increased with the increase in the amount of chitosan content. Results obtained from ATR-FTIR spectra

suggested that there exist pronounced interactions that probably resulted from hydrogen bond formation between different components. SEM micrographs showed that the compatibility of the two polymers was reduced when the fraction of chitosan was increased. Tensile strength and elongation at break of the blends reduced with the increase in chitosan content. These results indicated that the blends became brittle with increasing the chitosan content.

Keywords: Chitosan; Poly (ethylene terephthalate); Antibacterial; Mechanical Properties; Release.

1. Introduction

Recent studies have focused on the development of antibacterial surfaces and bulk to attain high functionality and high valued products[1,2]. Since microbial contamination of foods occurs primarily at the surface, direct applications of antibacterial substances onto foods have limited benefits as they could be neutralized upon direct contact or diffuse rapidly from the surface into the food mass[3]. The use of packaging or coating films containing antimicrobial agents could be more efficient by slow migration of the bactericidal agents from the carrier film structure to the food surface, maintaining a high concentration where it is needed[4]. As the release occurs during a continuous period, the antimicrobial action can also be maintained during the transport and storage period of the product[5]. In this sense, chitosan (CS) is a linear cationic natural polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is derived from chitin, the second most abundant polysaccharide on earth after cellulose[6-9]. Chitin is one of the most abundant natural biopolymer derived from exoskeletons of crustaceans. It can be also obtained from cell walls of fungi which becomes a basis for biotechnological production of this material. Chitosan is a product derived from N-deacetylation of chitin in the presence of hot alkali. The degree of deacetylation and the degree of polymerization(DP), which in turn decides molecular weight of polymer, are two important parameters dictating the use of

chitosan in many applications, in pharmaceutical, cosmetics, biomedical, biotechnological, agricultural, food, and non food industries as well(water treatment, paper, and textile)[10]. Excellent properties such as non-toxicity[11, 12], biocompatibility [13, 14], antimicrobial properties[5, 15-20], biodegradability and bioactivity mean that CS is widely used in biomedical fields[5, 21]. This biopolymer is mostly available from waste products in the shellfish industry, and therefore, abundant commercial supplies are currently offered. It can also be obtained from the chitin component of fungal cell walls. Several studies have already demonstrated the antibacterial and antifungal action of this compound for both bioactive preservative and bioactive packaging applications [22-24]. On the other hand, many efforts have been tried to produce new bio functional materials from chitosan in non-woven fabric, sponge, film, and gel forms. The blending of two or more polymers has increasingly become an important technique to develop new biomaterials exhibiting combinations of properties that could not be obtained from individual polymers. Chitosan film has been investigated as a potential biomedical material because of its good biocompatibility[25]. Interestingly and in according with published reports, films obtained from high molecular weight chitosan with low levels of deacetylation degree will show better water resistance but poor biocide properties. Thus, as the solubility of chitosan film diminishes, antimicrobial capacity is also reduced. For instance, as the number of protonated amine groups are reduced in the chitosonium acetate film chemistry by for instance alkaline neutralization, the film becomes water insoluble but no longer exhibits antimicrobial performance. Thus, by means of physical or chemical treatments, film disintegration in water could be prevented, but, at the same time, dissolution of the protonated glucosamine groups in water would be blocked o deactivated, hence reducing the biocide character of the formulation[5]. Poly (ethylene terephthalate) (PET) is widely used for barriers, fibers, sheets, packaging and films because of its excellent characteristics such as transparency and outstanding process ability. It is light in weight, and

has good impact resistance. It is one of the candidate matrices since it is inexpensive, inert, nontoxic, and readily available [26]. The aim of this work was to evaluate the anti-bacterial and mechanical properties of chitosan based solvent-cast blends with synthetic PET.

2. Materials and methods

2.1. *Materials*

Chitosan (CS) polysaccharide with low molecular weight (90.0% degree of deacetylation and viscosity of 185 cps at 1% in 1% acetic acid as stated by the manufacturer) was purchased from Sigma–Aldrich (Spain). *Staphylococcus aureus* (*S. aureus*) (CCUG 15195), *Escherichia coli* (*E. coli*) (CCUG 17620) and *Klebsiella pneumoniae* (*K. pneumoniae*) (CCUG 225) were obtained from the Swedish Type Culture Collection (Gutenberg, Sweden). PET granules (0.82±0.02 intrinsic viscosity as stated by the manufacturer) were obtained from Tondgooyan petrochemical, Iran. Ninhydrin (2,2-Dihydroxyindane-1,3-dione) and hydrindantin (2,2'-dihydroxy-1H,1' H-2,2'-biindene-1,1',3,3'(2H,2' H)-tetrone) were purchased from Sigma-Aldrich (Spain). Trifluoroacetic acid (TFA), chloroform and acetic acid were purchased from Merck (Germany).

2.2. *Film preparation*

The PET/chitosan gradient polymer films were prepared by casting method. Films of PET, chitosan and their homogenous blends were prepared at 40 °C by a solution-cast technique using trifluoroacetic acid (TFA), chloroform, and acetic acid as the solvents. The concentration of PET and chitosan in the original solutions were 10 and 2 (w/v %), respectively. For this work, 2gr of chitosan was dissolved in a ternary solvent system containing TFA, acetic acid and distilled water with 45%, 40% and 15% (v/v), respectively. Afterward that 10gr of PET was dissolved in a mixture of solvents, composed of chloroform and trifluoroacetic acid with 30% to 70% (v/v) respectively. Chitosan and PET were mixed in

1:99, 3:97 , 6:94 and 9:91 mass ratios, and were denoted as 99% PET , 97% PET , 94% PET and 91% PET respectively. They were stirred at room temperature for 24 hours to obtain homogenous solutions. There was no phase separation even after one day at room temperature. To prevent the deformation of the cast films, the PET, chitosan solutions, and their blends were cast on a glass substrate. To prepare the PET/chitosan homogenous blend films, in order to avoiding gelation of the chitosan, PET and chitosan were firstly dissolved separately in their solvents. When the chitosan solution was well homogenized, it was mixed with the PET solution.

2.3. *Methodology of the bacterial culturing, and antimicrobial tests*

The following steps were performed to determine the antimicrobial properties of chitosan:

1. Culturing and purifying bacteria

2. Activation of bacteria or Inoculum preparation and bringing its population to the certain number

3. The transfer of bacteria to the environment samples containing chitosan And the control sample.

4. The measurement of the living bacteria concentration by growing them on plate and obtaining CFU that is described in the following steps.

1. Culturing and purifying bacteria

In order to purification of bacteria, culture method was used on the plate containing solid culture medium. In this method the bacteria were cultured sequentially on a solid culture medium up not to see any contamination in the medium. The composition of the culture medium plate is agar (12 g per litter), sodium chloride (8 grams per litter), pythons (5 grams per litter), and meat extracts (3 grams per litter).).

2. Activation of bacteria or Inoculum preparation and bringing its population to the certain number

After the creation of single colony on the plate culture method on the plate, under sterile conditions (under the hood microbial) a colony of bacteria by a loop in 5 ml medium containing pythons (5 grams per litter), meat extracts (3 grams per litter), and sodium chloride (8 grams per litter) inoculated and was placed in the incubator for 24 hours at 37 $^{\circ}$ C and 100 rpm speed. The resulting solution is named Inoculum and is used as a substance for inoculation. By using CFU method the population of bacteria in the Inoculum solution was measured. This method for each type of Gram-positive bacteria and gram-negative were used in this project separately. The results showed existence of bacterial population to be approximately 10^{10} CFU / ml in the Inoculum solution.

3. The bacteria was transferred to the environment samples containing chitosan And the control sample.

This section is divided into two parts:

A) Preparation of the buffer solution

The conditions which are very important when measuring the antimicrobial properties are maintained at a specific pH of the solution at a certain level for all of tested samples. In experiments conducted on samples with different pH test solutions, the buffer solution was made in the amount of 2.7 consolidations. For almost all microorganisms tested in this work, the pH was at optimal growth conditions. For the preparation of the solution, initially a solution with a concentration of 0.25 M of the KH_2PO_4 (Merck, Germany) was prepared and during the testing of the solutions preparation of 0.3 mM a certain period solution was kept.

B) The transfer of bacteria to the environment samples containing chitosan and the control sample

At this stage, 50 ml of buffer solution sterilized in an autoclave was poured into the Erlenmeyer flask by 100 ml and then by micro-pipette 0.05 ml of bacterial solution was added to each of the Erlenmeyer. By doing this, concentration of bacteria obtained from the

activation stage was raised to the 108 CFU / ml. To determine the percentage reduction of bacteria, control samples, and samples containing chitosan was used. The control sample is a sample without anti-bacterial substance (chitosan). In the following, samples of pure poly (ethylene terephthalate) which is for a control sample and poly (ethylene terephthalate) containing chitosan were transferred to the Erlenmeyer flask containing the bacteria. Erlenmeyer flask was kept in the incubator at 37 ° C and speed 100 rpm for a certain period of 30 days (in this project).

4. The measurement of the living bacteria concentration by method of growing on plate and obtaining CFU

In this method only living bacteria which are able to divide are measured. Initially 1 mL of sample in a test tube containing 9 ml of each Ringer's solution (solution 9 gr / lit of the Nacl) was diluted step by step, and After reaching the desired dilution, 0.1 ml of the final test tube with a certain degree of dilution was placed in the middle of a plate containing solid medium and thoroughly released by the loop on the surface of the plate. The number of dilution steps by consecutive testing was determined experimentally. To increase accuracy and reduce the percentage of errors in the tests from each sample (control samples and samples containing chitosan) two sample plates were prepared. Plates are in the incubator at 37 ° C for 72 h and then each colony was counted. The number of colonies per plate was multiplied by the dilution factor, the concentration of living bacteria in CFU / ml was determined. (According to ASTM-E2149)

According to ASTM- E2149 microbial population reduction is calculated by this equation:

Reduction of microbial population (Degree of growth inhibition) % = (C-T)/C *100

Where:

C = CFU per millilitre (or mean $\log_{10}$ density of bacteria) for the number of bacterial colonies on plates containing without chitosan samples, and T = CFU per millilitre (or mean $\log_{10}$

density of bacteria) for the number of bacterial colonies on the plate containing the samples that are containing chitosan. Therefore all of our calculations were carried out based on the comparison with the pure PET (C).

2.4. Characterization

The ATR-FTIR spectrum of the PET, Chitosan and their blend films were recorded using a Bruker Tensor 27 ATR-FTIR spectrometer. The spectra obtained within the wave number range of 4000-600 cm^{-1} at a resolution of 4 cm^{-1}.

Small pure and blend samples were coated with gold-palladium, and the cross-sectional areas of the samples were viewed for their morphologies using scanning electron microscope (SEM, Philips, XL-30). Fractured cross sections of the samples were obtained by breaking the samples thoroughly immersed in liquid nitrogen for 30 min.

Tensile strength (TS) and percent of elongation at break (% ε) of the films were determined using a Universal Testing Machine (Zwick1446-60), according to ASTM D882.

2.5. *Glucosamine release tests*

The release of chitosan was quantified using the ninhydrin test in order to establish a potential correlation between chitosan release and the antimicrobial performance of the materials. The first observation is that there is a different relation between the fractions of released chitosan depending on the amount of used chitosan. A higher amount leads to proportionally higher migration of polysaccharide to the solution[5]. The ninhydrin reagent was prepared as follows[27]: 2 gr ninhydrin and 0.3 gr hydrindantin were dissolved in 75 ml dimethyl sulfoxide (DMSO). While flushing with nitrogen, 25 ml of 4 M lithium acetate buffer with pH of 5.2 (unless stated otherwise) was added, and the resulted dark red solution was further bubbled with nitrogen. The reagent was stored and refrigerated in a dark bottle with dispenser. Unless stored under a nitrogen atmosphere, the reagent bleached within a week to a yellow colour in exposure to oxygen. However, the slight bleaching occurred in the

first 3 days of storage did not affect the reaction yield. 1 ml of the ninhydrin reagent from the dispenser bottle was added to 1 ml of chitosan solution in deionized water (in duplicate). The tubes were immediately capped, briefly shaken by hand, and heated in a covered boiling water bath for 30 min. The tubes were then cooled below 30°C in a cold water bath and the content diluted with 5 ml of 50% (v/v) ethanol/water. The solutions were then vigorously stirred on a Vortex mixer (15 s) to oxidise the excess of hydrindantin[27]. The absorbance at 570 nm was measured by UV/VIS spectrophotometer (Jenway UV/vis spectrophotometer) and the concentration of chitosan in the sample calculated from a standard calibration curve (Figure1).

3. Results and discussions

3.1. *ATR-FTIR spectroscopy*

The ATR-FTIR spectrum of the pure PET film shows absorption peaks at around 3481 cm^{-1} for the OH stretch end group and at around 1710 cm^{-1} for the C=O stretch carbonyl group (Figure2). In the ATR-FTIR spectrum of chitosan, two characteristic peaks at 3446 and 3448 cm^{-1} appeared and this can be attributed to the $–NH_2$ and –OH groups stretching vibration. Both of characteristic peaks for chitosan at 1654 and 1583 cm^{-1} could be attributed to the C=O stretching (amide I) and amide II vibration, respectively. Compared to the spectra of the PET, CS and PET/CS films, the absorbance intensities at wave numbers 1571, 1583 and 1654 cm^{-1} in the PET/CS blend were decreased.

The intensity of the stretching bands of the PET component at 1710 and 3481 cm^{-1} for carbonyl and ester groups became significantly weaker. All these registered events indicate that there are obvious interactions among the amino, carboxyl, and hydroxyl groups of the two components inside the blend film. These interactions should be attributed to the hydrogen

bonds possibly formed between amino (in chitosan) and carboxyl groups (in PET) or hydroxyl (mainly in chitosan) and carboxyl groups.

3.2. *Scanning Electron Microscopy (SEM)*

The electron-micrographs of the films were obtained by SEM of the fractured cross-section. In general, the SEM examination reveals very little phase details in samples of PET with chitosan, particularly when the latter polymer is a minor phase constituent, suggesting that a good dispersion and/or phase interaction occurred between the two polymers (Fig. 3A and 3D) in the compositional range. Regarding chitosan, when the fraction of this polymer was increased (Fig. 3E), the compatibility of the two materials seemed to be reduced too.

3.3. Film mechanical properties

The mechanical properties of films were mainly evaluated by the tensile test. The results for tensile strength (TS) and elongation at break (% ε) for films with different PET/chitosan blend ratios are shown in table 1. The tensile strength of the films decreased relatively by the chitosan fraction increment. TS values were reduced from 50.01±4.8 MPa for pure PET film to 26.59±2 MPa for PET/chitosan (91/9)(W/W) blend ratio. Compared to the TS values of the widely used plastic films such as LDPE and HDPE with values of 23.6 and 47.4 MPa, respectively[28], the PET/chitosan films retained good tensile strength. Mean values for the elongation at break (% ε) amounts are also presented in Table 1. The results indicated that addition of chitosan to the blend reduced extensibility of the films, creating more brittle products. Compared to the % ε values of LDPE and HDPE of about 205% and 570%, respectively[28], all PET/chitosan films exhibited poor % ε values of 78.46±8% for pure PET film to 21.14±3.8% for 91/9 (W/W) PET/chitosan films. Weak tensile properties of chitosan are the main reason for impaired elongation.

3.4. Antibacterial activity

The bactericidal properties of chitosan against Gram-positive S. aureus and Gram-negative E. coli and K. pneumoniae were tested by suspending the film in bacterial broths and incubating it in appropriate conditions (37 degrees Celsius). The results of the antibacterial activity can be observed in figure 3. It is well recognized that chitosan has good antimicrobial activity and these results suggest that the antibacterial activity is due to the presence of the chitosan. Finally, the protonated NH_3^+ of chitosan in acidic solution can absorb the electronegative substances in the bacteria cell to induce the leakage of nutritious substances, and form a polymeric membrane around bacteria cell, which prevents the transport of essential nutrients into the cell and thus results in the death of cell[29].

3.5. Chitosan release tests

The chitosan migration was quantified for PET/chitosan blend films in an attempt to correlate this with the observed antibacterial effects. To carry out these tests, the ninhydrin method was used. The results, a general trend can be observed in which the chitosan release was in general higher in the samples showing better antibacterial properties. Observation is that there is a different relation between the fractions of released chitosan depending on the amount of chitosan used; a higher amount leads to proportionally higher migration of chitosan to the solution (Figure4). The concentration of chitosan in acetic solutions during 90 days is calculated by ninhydrin test method (Figure5). This finding agrees with previous work in which a direct correlation between the chitosan release and the antibacterial properties was demonstrated when pure chitosan films and composite matrices gliadins/chitosonium- acetate films were evaluated[5].

4. Conclusion

The PET/Chitosan blends presented a significant antimicrobial property, which increased with the increase in the chitosan content of the films. In addition, chitosan was antibacterial

on its blend with poly (ethylene terephthalate) to both Gram-positive and Gram-negative bacteria. The antibacterial activity increases with the amount of chitosan in blends. Also, the tensile strength and elongation at break of PET/chitosan films decreased with the increase in chitosan content. On the basis of our results, PET/chitosan blend films have the potential to be used in the food industry as antibacterial packaging materials.

References

1- Vacheethasanee, K., Temenoff, J. S., Gary, A., "Surfactant polymers designed to suppress bacterial (Staphylococcus epidermidis) adhesion on biomaterials", J. Biomed. Mater. Res. 42, 425 (1998).

2- Baumgartner, J. N., Yang, C. Z., Cooper, S. L., "The influence of thrombus components in mediating bacterial adhesion to biomaterials", J. Biomaterials., 18, 83 (1997).

3- Ouattara, B., Simard, R. E., Piette, G., "Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan", Int. Food. Microbiol., 62, 139-148 (2000).

4- Quintavalla. S., Vicini. L., "Antimicrobial food packaging in meat industry", Meat. Sci., 62, 373-380 (2002).

5- Fernandez-Saiz, F., Lagaron, J. M., "Characterization of antimicrobial properties on the growth of S. aureus of novel renewable blends of gliadins and chitosan of interest in food packaging and coating applications", **Int. J. Food. Microbiol.,** 124, 13-20 (2008).

6- Chillo, S., Suriano, N., Lamacchia, C., "Effects of additives on the rheological and mechanical properties of non-conventional fresh handmade tagliatelle", **J. Cereal. Sci.**, 49, 163–170 (2009).

7- Yoshida, C. M. P., Oliveira, E. N., Franco, T. T., "Chitosan tailor-made films: The effects of additives on barrier and mechanical properties", Packag. Technol. Sci., 22, 161–170 (2009).

8- Di Pierro, P., et al., "Chitosan–whey protein edible films produced in the absence or presence of transglutaminase: Analysis of their mechanical and barrier properties", Biomacromolecules, 7, 744–749 (2006).

9- Jeon, Y. J., Kamil, J. Y. V. A., Shahidi. F., "Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod", **J. Agr. Food. Chem.**, 50, 5167–5178 (2002).

10- Dutta, P. k., et al., "Perspectives for chitosan based antimicrobial films in food applications", Food. Chem., 114, 1173–1182 (2009).

11- Durango, A. M., et al., "Development and evaluation of an edible antimicrobial film based on yam starch and chitosan", Packag. Technol. Sci., 19, 55–59 (2006).

12- Ferreira, C. O., Nunes, C. A., Delgadillo, I., "Characterization of chitosan–whey protein films at acid pH", Food Res. Int., 42, 807–813 (2009).

13- Ye, M., Neetoo, H., Chen, H. Q., "Effectiveness of chitosan-coated plastic films incorporating antimicrobials in inhibition of Listeria monocytogenes on cold-smoked salmon", Int. J. Food Microbiol., 127, 235–240 (2008).

14- Babiker, E. E., "Effect of chitosan conjugation on the functional properties and bactericidal activity of gluten peptides", Food. Chem., 79, 367–372 (2002).

15- Alonso, D., et al., "Cross-linking chitosan into UV-irradiated cellulose fibers for the preparation of antimicrobial-finished textiles", Carbohyd. Polym., 77, 536–543 (2009).

16- Cagri, A., Ustunol, Z., Ryser, E. T., "Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing p-aminobenzoic or sorbic acids", J. Food Sci., 66, 865–870 (2001).

17- Fernandez-Saiz, P., Lagaron, J. M., Ocio, M. J., "Optimization of the biocide properties of chitosan for its application in the design of active films of interest in the food area", Food Hydrocolloid, 23, 913–921 (2009a).

18- Fernandez-Saiz, P., Lagaron, J. M., Oci. M. J., "Optimization of the filmforming and storage conditions of chitosan as an antimicrobial agent", **J. Agr. Food. Chem.**, 57, 3298–3307 (2009b).

19- Pranoto, Y., Rakshit, S. K., Salokhe, V. M., "Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin", LWT-Food Sci. Technol., 38, 859–865 (2005).

20- Vasconez, M. B., Flores, S. K., Campos. C. A., "Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings", Food Res. Int., 42, 762–769 (2009).

21- Fernandez-Saiz, P., Ocio, M. J., Lagaron, M., "Antibacterial chitosan-based blends with ethylene–vinyl alcohol copolymer", Carbohyd. Polym., 80, 874-884 (2010).

22- Huang, M., Khor, E., Lim, L. Y., "Uptake and cytotoxicity of chitosan molecules and nanoparticles: Effects of molecular weight and degree of deacetylation", Pharmaceut. Res, 21, 344–353 (2004).

23- Coma, V., Martial-Gros, A., "Edible antimicrobial films based on chitosan matrix", J. Food Sci., 67, 1162-1169 (2002).

24- Fernandez-Saiz, P., Ocio, M. J., "Film-forming process and biocide assessment of high-molecular-weight chitosan as determined by combined ATR-FTIR spectroscopy and antimicrobial assays", Biopolymers, 83(6), 577–583 (2006).

25- Guiping, Ma., et al., "Preparation and properties of water-soluble chitosan and polyvinyl alcohol blend films as potential bone tissue engineering matrix", Polym. Adv. Technol., 21, 189-195 (2010).

26- Moore, S., "Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction", J. Biol. Chem., 243 (23), 6281-6283 (1968).

27- Prochazkovab, S., "Quantitative determination of chitosans by ninhydrin", J. Carbohydrate Polymers., 38, 115-122 (1999).

28- Philadelphia, P. A., " Standard test method for tensile properties of thin plastic sheeting", (2002).

29- Shi, Z. L., Neoh, K. G., "Antibacterial and mechanical properties of bone cement impregnated with chitosan nanoparticles", Biomaterials, 27, 2440-2449 (2006).

Figure captions:

Figure 1. Calibration curve for chitosonium acetate solution using the ninhydrin test method.

Figure 2. ATR-FTIR spectra in the range of 599 to 3899 cm^{-1} taken in PET pure film (dashed line) and PET/Chitosan blend film.

Figure 3. Scanning electron micrographs of the cross section of: (A) PET pure film; (B) PET/CS (99/1) (wt%); (C) EPET/CS (97/3) (wt%); (D) PET/CS (94/6) (wt%); (E) PET/CS (91/9) (wt%), For samples (A)–(E) the scale marker is 5 μm.

Figure 4. Degree of growth inhibition of PET/chitosan with different mass ratio during 30 days against S. aureus (■), E. coli( ) and K. pneu ( ).

Figure 5. The concentration of chitosan in acetic solutions during 90 days.

Table caption:

Table 1. Tensile properties of the PET/chitosan blend films as a function of chitosan content

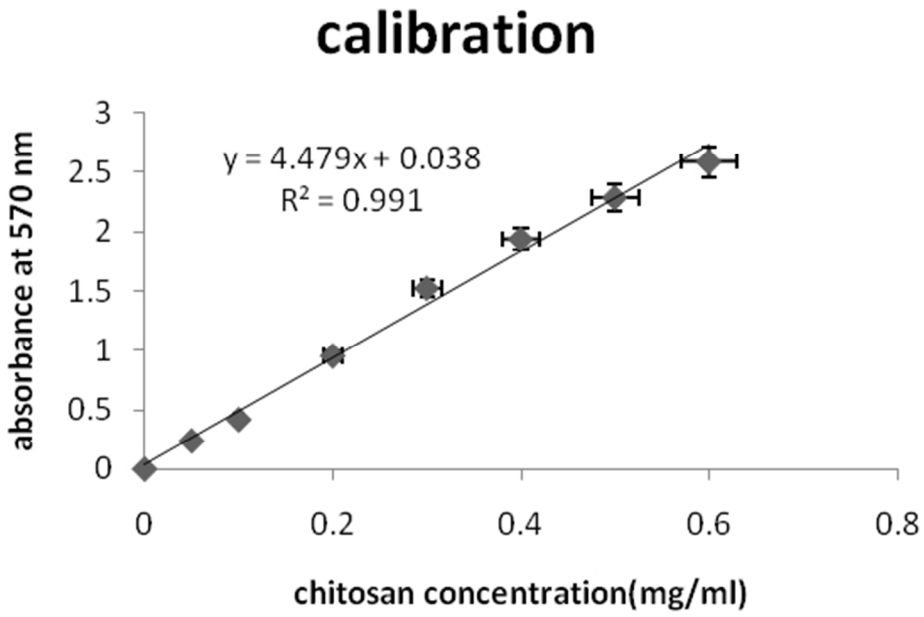

Figure 1. Calibration curve for chitosonium acetate solution using the ninhydrin test method.
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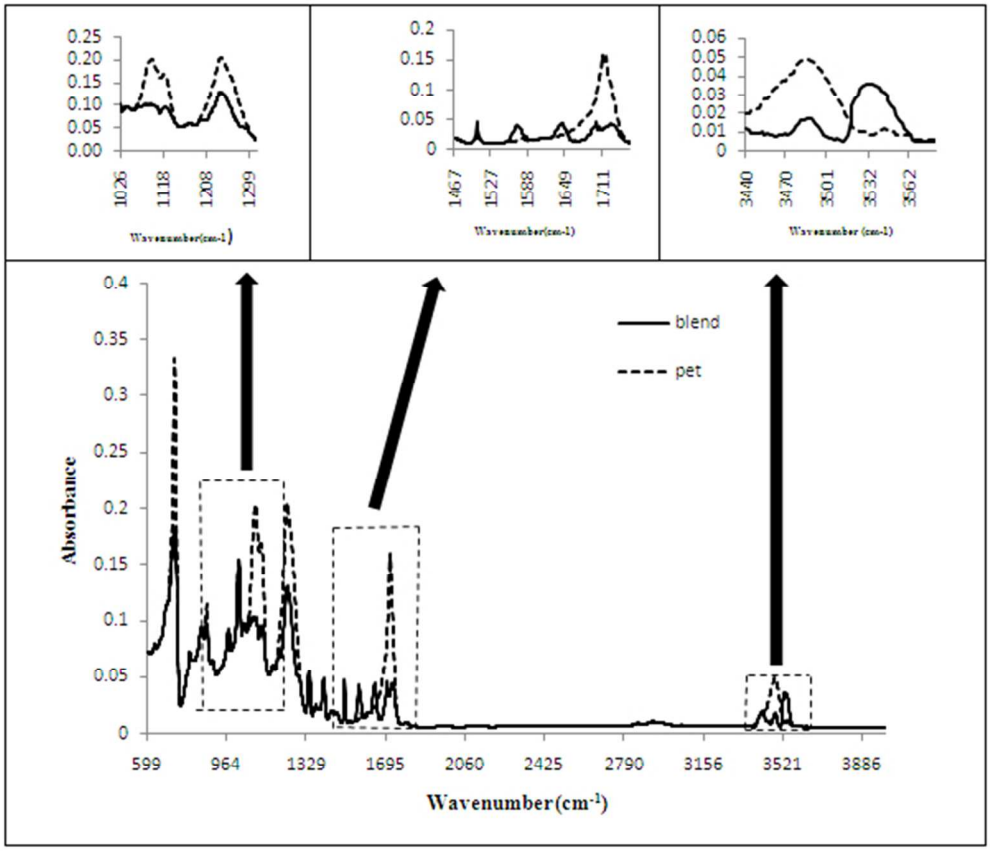

Figure 2. ATR-FTIR spectra in the range of 599 to 3899 cm-1 taken in PET pure film (dashed line) and PET/Chitosan blend film.
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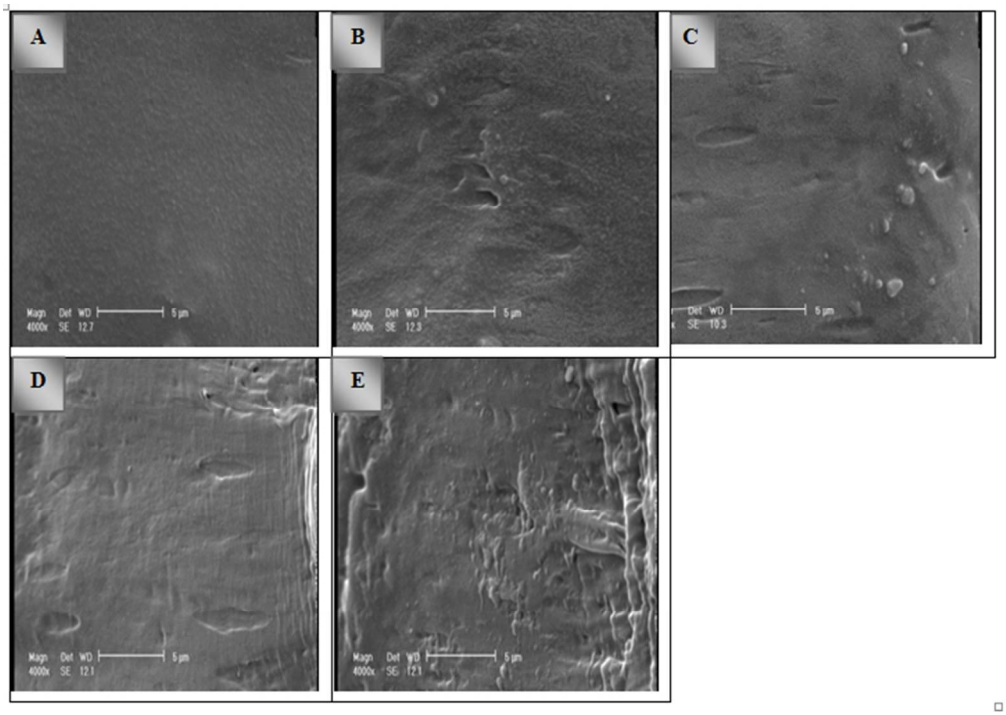

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191x135mm (96 x 96 DPI)

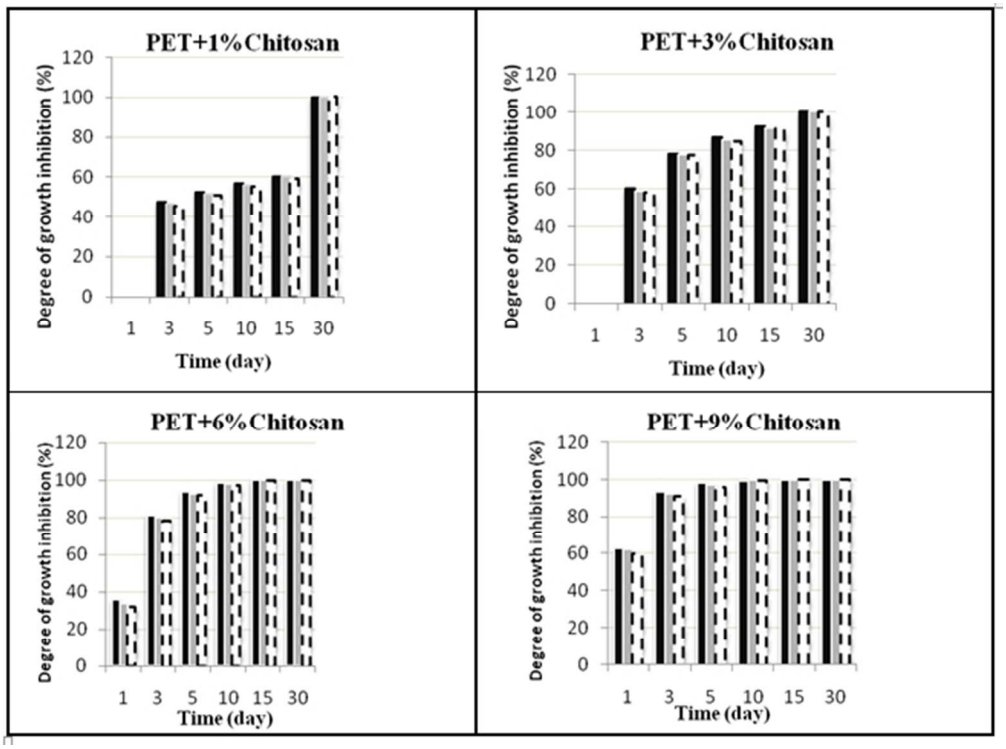

Figure 4. Degree of growth inhibition of PET/chitosan with different mass ratio during 30 days against S. aureus (■), E. coli( ) and K. pneu ( ).
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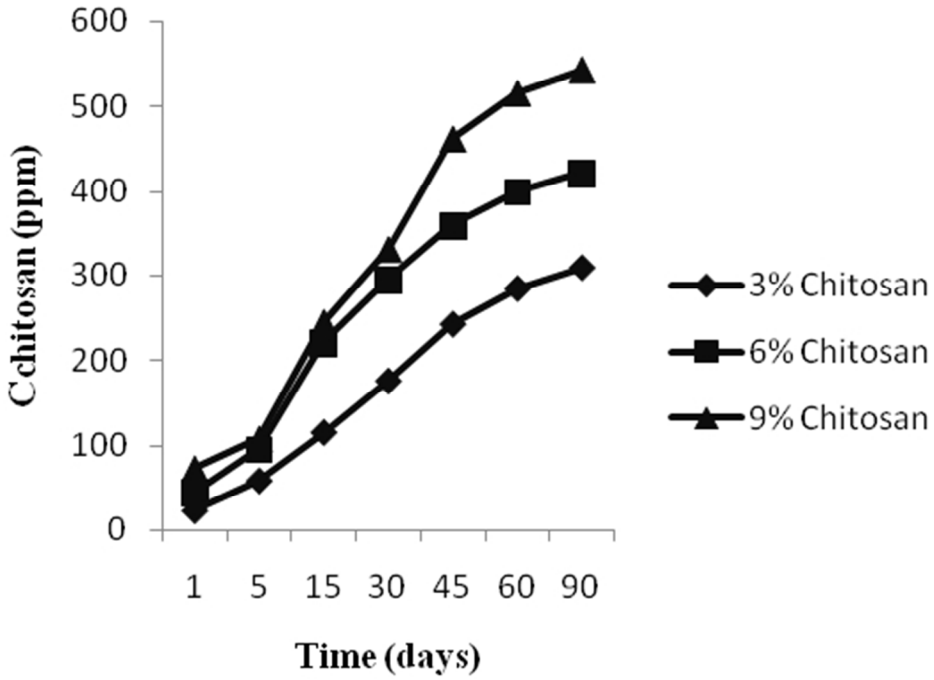

Figure 5. The concentration of chitosan in acetic solutions during 90 days.
150x109mm (96 x 96 DPI)

Table 1
Tensile properties of the PET/chitosan blend films as a function of chitosan content

Chitosan content (wt%)	Tensile strength (MPa)	Elongation at break (%)
0	50.01±4.8	78.46±8.0
1	44.31±3.9	54.47±6.3
3	33.36±3.3	45.46±5.3
6	31.47±2.8	26.62±3.1
9	26.59±2.0	21.14±3.8