FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY FOR CHARACTERIZATION OF ANTIMICROBIAL FILMS CONTAINING CHITOSAN

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Abstract

Biopolymer films containing chitosan as antimicrobial agent were obtained. As polymer matrix poly(vinyl alcohol) (PVA) and bacterial cellulose (BC) were used. The films were characterized using FTIR spectroscopy. FTIR spectra of the pure components and of composites films reveal strong interactions between chitosan and PVA and chitosan and bacterial cellulose. These composite films could be used as antimicrobial food packaging materials.

Key words: bacterial cellulose, food packaging, chitosan, antimicrobial, FTIR

INTRODUCTION

Antimicrobial food packaging technologies are intensively studied because they can offer an alternative to the traditionally methods for food preservation. The purpose of the 'active packaging' is the extension of the shelf-life of the food and the maintenance or even improvement of its quality (Dainelli et al. 2008).

An antimicrobial material can deliver the antimicrobial agent at a desired rate and for a desired time. As antimicrobial agents several substances have been tested, for example: organic acid such as benzoic acids, parabens, sorbates and their mixture, enzymes such as lysozyme, fungicides such as benomyl, imazalil and also antimicrobial natural bioactive substances like spices and essential oils (rosemary, oregano, garlic, etc.) (Seydim and Sarikus, 2006; Rodríguez et al. 2007).

Chitosan, a linear β-1,4-D-glucosamine, is a biocompatible, nontoxic compound mainly obtained by deacetylation of chitin, a natural structural component present for instance in crustaceans. Chitosan is a very good candidate to design novel antimicrobial active packaging technologies to improve the quality and safety and to extend the shelf-life of perishable foods. Chitosan can be blended with other polymers, especially PVA and cellulose. (Liang et al. 2009; Dutta et al. 2009).

Bacterial cellulose has the same structure as plant cellulose and has superior mechanical strength, crystallinity and hydrophilicity. Several medical applications have been reported, the most important being as artificial skin for humans with extensive burns (Czaja et al. 2006). Bacterial

cellulose-chitosan films were obtained by biosynthesis (Phisalaphong and Jatupaiboon, 2008). The possibility to obtain antimicrobial food packaging materials containing PVA and chitosan is already investigated (Tripathi et al. 2009). The presence of bacterial cellulose in these composite films was not investigated till now.

The aim of this paper is to present the obtaining of composite films with PVA - chitosan and BC – chitosan, as a first step for preparing ternary composites films PVA-BC-chitosan. The composite films were characterized using Fourier transform infrared (FTIR) spectroscopy.

MATERIAL AND METHODS

Chemicals and reagents

Chitosan from crab shells, with the degree of deacetylation >75% was purchased from Sigma-Aldrich. Poly(vinyl alcohol) (PVA), average molecular weight (Mw) 85,000–124,000 g/mol, >99% hydrolyzed, was purchased from Sigma–Aldrich and used without further treatment or purification.

Microbial strains and culture condition

The *Acetobacter* sp. strain used in this study was isolated from the traditionally fermented vinegar in Microbiology Laborator of Chemical Engineering Department of "Politehnica" University of Bucharest. Stock culture was inoculated into 50-ml Hestrin & Shramm (HS) medium in a 250-ml conical flask and incubated for 72 h under static conditions. The resulting seed culture was shaken vigorously to release cells from the pellicle. Bacterial cellulose (BC) membranes were obtained from the seed culture in a statically incubation at 30°C on a modified Hestrin-Shramm medium containing 2% glucose. The gel-like BC pellicles, obtained after 14 days, were purified by boiling in a 0.5 M aqueous solution of NaOH for 30 min. The BC thin sheets were then washed with deionized water several times until pH of water became neutral. BC pellicles were dried and used as BC membrane.

Film forming conditions

Chitosan dispersions were prepared in 0.5% (v/v) acetic acid to a final concentration of 1.5% (w/v) and stirred at 37°C for approximately 3 h. The resulted solution was filtered through polyester cloth to remove residues of insoluble particles. To obtain a BC-chitosan membrane a BC dried membrane was impregnated with a chitosan acetic solution for 48 h at room temperature. After this, the BC-chitosan membrane was dried at 60°C.

A casting PVA solution was made by dissolving PVA in water at 90°C. In the resulting solution, chitosan acetic solution was dispersed under vigorous stirring. The resulting mixture was cast onto a Perspex plate with the aid of a casting knife and dried at room temperature for 24 hours. PVA

and chitosan were used in different proportions. The obtained composite films had the following PVA-chitosan ratios (w/w): film 1-1/0.05, film 2-1/0.15 and film 3-1/0.45.

Membranes characterization

Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of the composite films and possible interactions between their components. The FTIR spectra of the membranes were measured with a Jasco FT/IR6200 spectrophotometer. The spectra were the average of 50 scans recorded at a resolution of 4 cm^{-1} in the range from 4000 to 500 cm^{-1} with a TGS detector.

Composites swelling

Swelling ability of composite materials was also studied. Membranes were cut into 2 cm × 2 cm square shapes and dried to constant weight. After the moisture content was removed, they were immersed in deionized water at room temperature.

RESULTS AND DISCUSSION

The FTIR spectra of pure PVA film, BC membrane and chitosan film are presented in figure 1. Figure 1a showed the absorption peaks of PVA at about 3247.5 cm^{-1} (–OH stretching) and at about 1082 and 1414.5 cm^{-1} for the –C–O group. Because the PVA film was not so transparent in figure 1a is presented an ATR–FTIR spectrum (Rodrigues et al. 2007). Characteristic absorption peaks of bacterial cellulose are at 3350 cm^{-1} due to O-H stretching and at 2916.81 cm^{-1} due to CH stretching. The band at 1649.8 cm^{-1} is due to deformation vibration of the absorbed water molecules (Wonga et al. 2009). The characteristic absorption of the chitosan is the band at 1559.17 cm^{-1}, which is assigned to the stretching vibration of amino group of chitosan and 1333.5 cm^{-1} assigned to vibration of C-H. Another band at 3367.1 is due to amine NH symmetric vibration. The peak of 2927.41 cm^{-1} is typical C-H vibration. The peaks around 896.73 and 1154.19 cm^{-1} correspond to saccharide structure of chitosan. The broad peak at 1080.91 indicates C-O stretching vibration (de Souza Costa-Júnior et al. 2009, Krishna Rao et al. 2006).

In figure 2 are presented the spectrum of BC in comparison with the spectrum of BC membrane impregnated with chitosan solution. Even the bacterial cellulose membrane is only impregnated with chitosan, some differences are visible in the spectrum of the composite material. The absorption band at 3350.71 cm^{-1} shifted to 3349.75 cm^{-1} and became border, indicating a possible overlapping stretching of hydrogen bounded –OH and NH_2. Characteristic bands at 2916.81cm^{-1} for BC and at 2927.1 cm^{-1} for chitosan, typical for CH stretching, shifted to 2895.59 cm^{-1}. The peaks at

1559.7 cm^{-1} and 896.73, which correspond to saccharide structure of chitosan, are also present in the composite spectrum.

In figure 3 are presented the spectra of all the composite films containing PVA and chitosan in different proportions. Film 1 has the lower content of chitosan, the second has three times higher chitosan and the third has nine times higher chitosan content. In figure 3a,b,c one can observe characteristic peaks of PVA and chitosan in the composite films spectra.

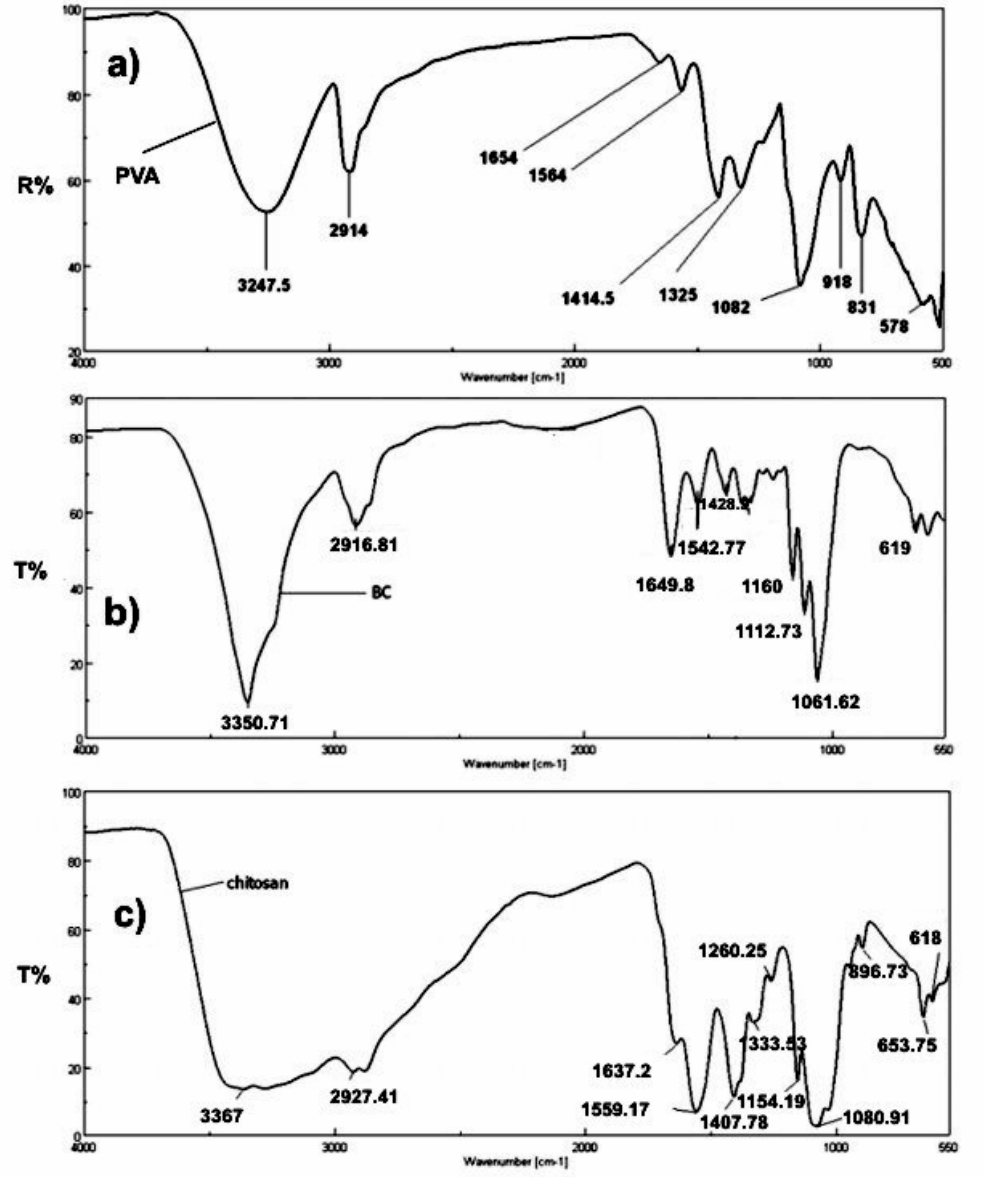

Fig. 1 Initial spectra for all the components used to obtain composites film: a) pure PVA film, b) BC membrane and c) chitosan film.

The peak at 3247.5 in the PVA spectrum shifted to 3326.61 in film 1 spectrum and to 3340 in film 2 and 3 spectra, indicating as in the case of BC-impregnated chitosan membrane, possible overlapping stretching of hydrogen bounded –OH and NH_2. The band at 1654 cm^{-1}, due to water absorption in the initial PVA spectrum (fig. 1a), shifted to 1653.66 in the film 1 spectrum and then disappeared in the film 3 spectrum. The chitosan characteristic band at 1559.17 cm^{-1}, which is assigned to the stretching vibration of amino group of chitosan, shifted to other values: 1566.88 cm^{-1}, 1565.92 cm^{-1} and 1562 cm^{-1}in the films spectra. It is interesting to underline that the peak at 1142.6 cm^{-1} which is sensitive to PVA crystallinity, decreased in the film 1 spectrum and disappeared in film 3 spectrum. This can be an indication that the compounding between PVA and chitosan destroy PVA crystallinity.
The broad peak at 1080.91, which indicates C-O stretching vibration in the spectrum of chitosan, shifted to 1092.5 in the composite film spectra and became more intense which the increasing of chitosan content.

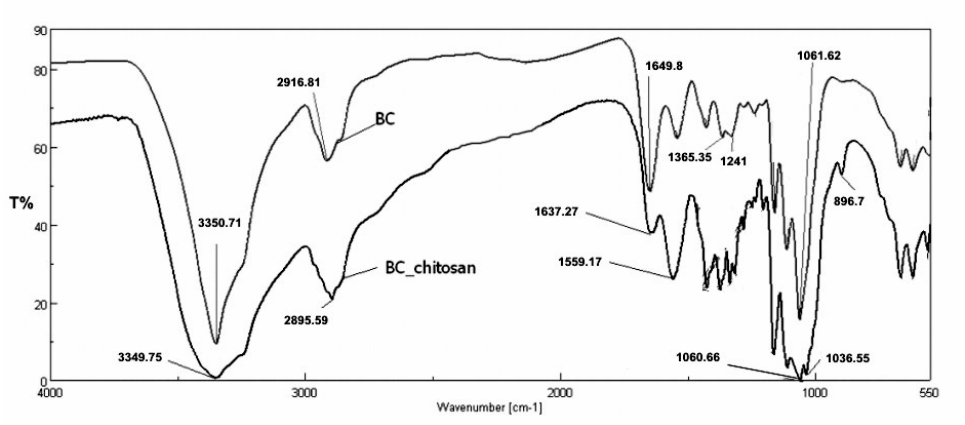

Fig. 2 Comparison between FTIR spectra of pure BC and BC impregnated with chitosan acetic solution.

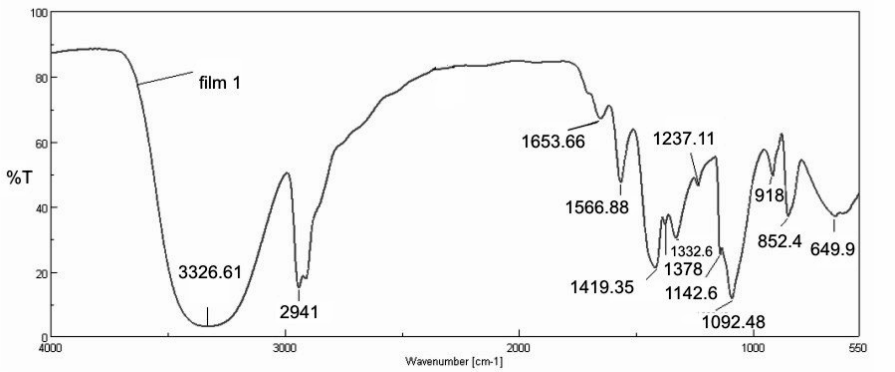

Fig. 3a Spectra of composites film 1 of PVA and chitosan (PVA/chitosan (w/w): 1/0.05)

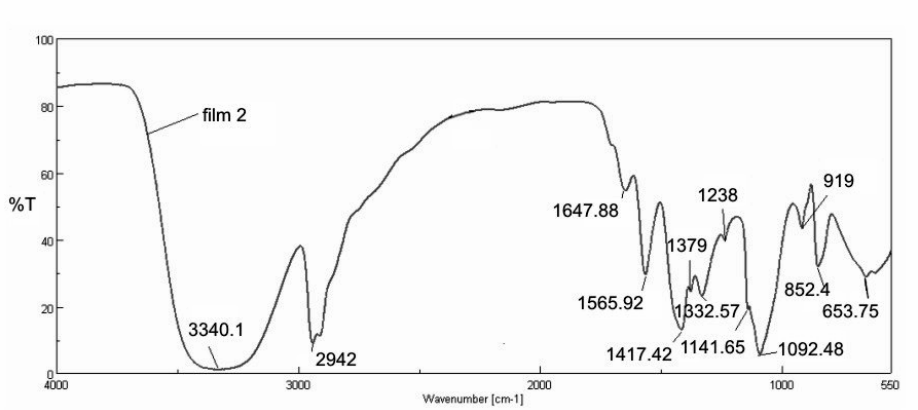

Fig. 3b Spectra of composites film 2 of PVA and chitosan (PVA/chitosan (w/w): 1/0.15)

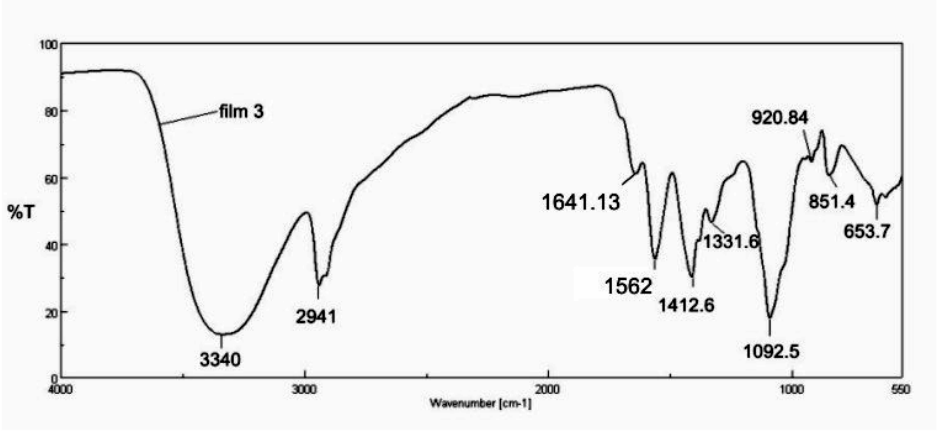

Fig. 3c Spectra of composites film 3 of PVA and chitosan (PVA/chitosan (w/w): 1/0.45)

Swelling results

Swelling results can be presented only for the BC-chitosan membranes, because all the others were dissolved in water after 3 h. Swelling dynamics was obtained by measuring the initial weight (m_i) and the weight of sample in swollen state ($m_{s,\tau}$) using equation (1).

$$Swelling = \left(m_{s,\tau} - m_i\right)/m_i \quad (1)$$

For BC impregnated chitosan the degree of swelling was 46.72%.

CONCLUSION

Chitosan-PVA films and bacterial cellulose membrane impregnated with chitosan were prepared. FTIR investigation of the obtained films reveals that there are possible interaction between the two biopolymers (PVA and BC) and chitosan. For the moment the obtained films PVA-chitosan are not water resistant. The work is in progress because our team wants to develop

biopolymer blending films with antimicrobial properties, which can be used for food packaging.

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