

*Dedicated to Professor Alexandru T. Balaban
on the occasion of his 85th anniversary*

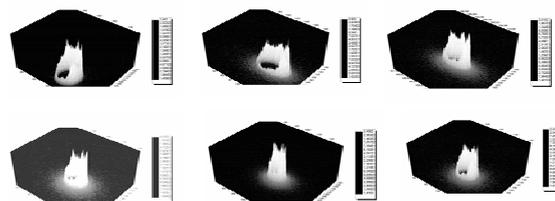
EMBEDDING SILVER NANOPARTICLES AT PHB SURFACES BY MEANS OF COMBINED PLASMA AND CHEMICAL TREATMENTS

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Polyhydroxybutyrate (PHB) has a high biocompatibility and is component of many medical devices like surgical meshes, screws and plates for bone fixation, periodontal membranes, and wound dressing. Nowadays, the development of silver containing polymer systems with antimicrobial properties is a new and promising trend in the modern biotechnology and is intensively in progress. In this context, herein the combined method of plasma and chemical treatments applied on PHB films surfaces is studied by means of Fourier-transformed spectroscopy (FTIR) and small-angle X-ray scattering (SAXS) measurements.



INTRODUCTION

The amount of plastic waste increases every year and the exact time needed for its biodegradation is unknown. Nowadays plastics and synthetic polymers are mainly produced using petrochemical materials that cannot be decomposed. Therefore, they contribute to environmental pollution and are a danger to many animals. During the last decade, much attention has been focused on the production of bacterial polyesters. Polyhydroxyalkanoates (PHAs) are bacterial polymers that are formed as naturally occurring storage polyesters by a wide range of microorganisms. Biodegradable and biocompatible poly(3-hydroxybutyrate) is a homo polymer of 3-hydroxybutyrate and is the most widespread and

best characterized member of the polyhydroxy-alkanoate family. Poly-3-hydroxybutyrate (PHB) is a linear polyester of D (-)-3-hydroxybutyric acid which was first discovered in bacteria by Lemoigne in 1925 from *Bacillus megaterium*. The polymer possesses the important properties of thermoplasticity and biodegradability in compost and different environments comprising marine water, and, in consequence, has attracted considerable commercial interest.¹⁻³ This range of interesting properties and processability opens up a bright future for PHB in applications ranging from surgical sutures, tissue engineering and agricultural foils to packaging for the storage of food products.⁴ Like PVC and PET, it exhibits good barrier properties⁴, and can be used in the packaging industry as a biodegradable plastic for

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contributing to solving environmental pollution problems. In medicine, PHB is compatible with the blood and tissues of mammals. The monomer of PHB is a normal metabolic in the human blood. As the body reabsorbs PHB it could be used as a surgical implant, in surgery, as seam threads for the healing of wounds and blood vessels. PHB does not contain any residues of catalysts like other synthetic polymers. PHB is perfectly isotactic and does not include any chain branching like, for instance, PE. Therefore during processing it flows easily.⁵

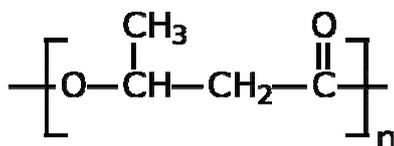
The Ag₂S nanoparticles enhanced the degradation rate and acted as effective catalysts for the degradation of PHB in Ag₂S/PHB nanocomposites.⁶

The aim of the present study was to obtain silver-treated PHB films in order to reduce bacteria formation in case of using PHB as medical devices or packaging. PHB films were processed using a method in two steps: helium plasma treatments followed by silver nitrate chemical modification.⁷⁻⁸

EXPERIMENTAL RESULTS

Materials and methods

The PHB films of 0.025 mm thickness (from Goodfellow) with chemical structure presented in scheme 1 were introduced in an EMITECH RF plasma device at a power of 40 W for 6 and 10 min.



Scheme 1 – Polyhydroxybutyrate formula.

As background gas was used helium at a pressure $p = 4 \times 10^{-1}$ mbar, the plasma fills the gas vessel by diffusion. The

plasma-treated PHB film was then quickly immersed in a 0.1 M solution of AgNO₃ for 2 days at room temperature, protected from light.⁸ When 2-days period expired the samples were rinsed with deionized water and subsequently analyzed by the different characterization techniques.

Characterization methods

The spectra were recorded on a Bruker Vertex 70 FTIR spectrophotometer, equipped with a diamond ATR device (Golden Gate, Bruker). ATR-FTIR (Attenuated Total Reflection - Fourier Transform Infrared) analysis was performed in the range 600-4000 cm⁻¹, at a resolution of 2 cm⁻¹ at incidence angle of 45°. For a spectrum 128 scans were taken, with a baseline correction.

The small-angle X-ray scattering experiments (SAXS) were performed on a Nanostar U-Bruker system equipped with a Vantec 3000 detector (diameter of 200 mm) and an X-ray I μ S microsource. The wavelength of the incident X-ray beam was $\lambda = 1.54 \text{ \AA}$ (Cu K α) and the beam was collimated by three pinholes. The sample-to-detector distance was 107 cm allowing measurements with the q values between 0.01 \AA^{-1} and 0.15 \AA^{-1} . An area integration was employed to reduce the data to a one-dimensional q ($4\pi\sin\theta/\lambda$) versus $\ln(\text{Intensity})$ trace.

RESULTS AND DISCUSSION

Significant changes in the surface topography induced by plasma followed by silver nitrate treatments on catheters were evidenced by using FTIR and SAXS experiments.

The results obtained for the FTIR spectra of treated and untreated PHB film are presented in Fig. 1 a) and b). The characteristic FTIR peaks of PHB are summarised in Table 1 and were obtained from a commercial sample of PHB (Goodfellow).

The FTIR spectra for PHB plasma treated samples and plasma followed by chemical treatment samples were compared with the spectrum obtained from commercial PHB in Fig. 1a.

Table 1

FTIR characteristic peaks of PHB

Characteristic peaks cm ⁻¹	Assignment
2900	C-H vibration
1720	C=O stretching
1457	CH ₃ asymmetric deformation
1379	CH symmetric deformation
1280	C-O-C stretching
1263	C-O-C stretching; CH deformation
1228	C-O-C stretching
1178	C-O-C stretching
1130	CH ₃ rocking
1097	C-O-C stretching
1054	C-O stretching
1042	C-CH ₃ stretching

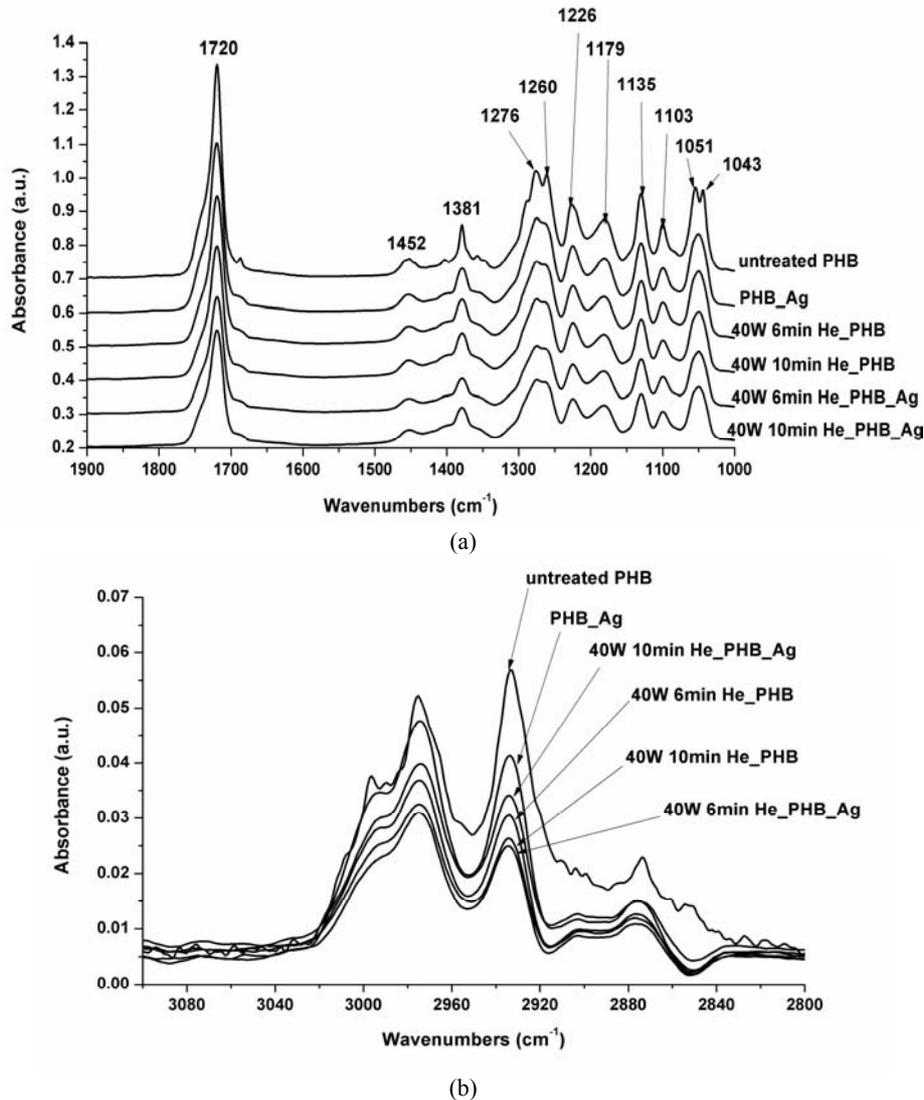


Fig. 1 – FTIR measurements: (a) range 1800-1000 cm^{-1} ; (b) range 3080-2800 cm^{-1} .

The most prominent marker band for the identification of PHB is the ester carbonyl band at 1720-1740 cm^{-1} . The bands present at 1178 cm^{-1} , 1228 cm^{-1} and 1263 cm^{-1} are bands sensitive to crystallinity and are characteristic of C-O-C. The peak intensities observed for commercial PHB were greater than those for treated PHB. A greater absorbance correlates to a higher degree of crystallinity; this observation could lead to the inclusion that the treated PHB have lower degree of crystallinity compared to that of the commercial PHB.⁹⁻¹¹ From Fig. 1a, no major changes in the PHB structure can be identified, while from Fig. 1b the presence of silver nanoparticles is demonstrated by the shift and the change in intensity of the FTIR peaks in 3000-2840 cm^{-1} range.

Randriamahefa *et al.* reported that FTIR can also be used to evaluate the Crystallinity Index

(CI) of PHB quantitatively.¹² A relative measure of the degree of crystallinity can be obtained by calculating the CI and can be used to compare the relative crystallinity obtained from the various FTIR spectra. In the literature, the CI is defined as the absorbance ratio of the reference band at 1724 cm^{-1} which is insensitive to the degree of crystallinity to the crystallinity sensitive band at 1228 cm^{-1} . The equation for calculating the CI is demonstrated in Equation (1) below.

$$CI = A_{1228}/A_{1724} \quad (1)$$

The CI obtained for commercial PHB was greater compared to the values obtained for treated PHB (Fig. 2). Greater CI correlates to higher % crystallinity, but this CI is not to be confused with an absolute degree of crystallinity being useful for comparison between samples.

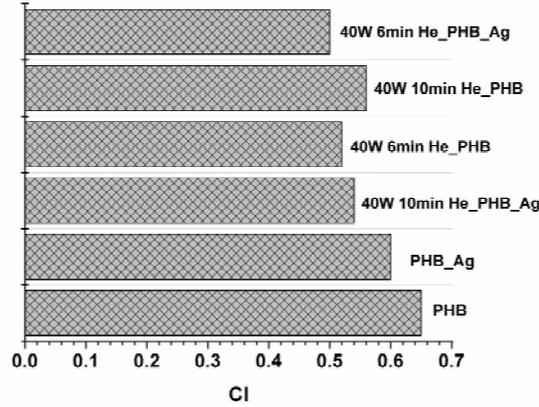


Fig. 2 – Crystallinity Index calculated from FTIR.

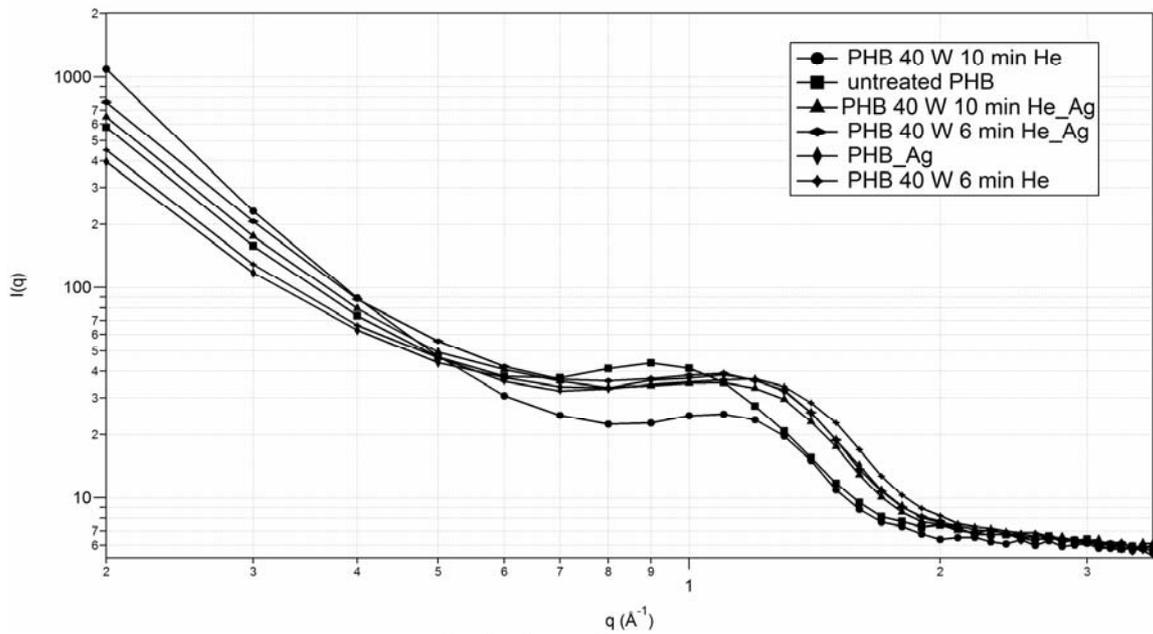


Fig. 3 – Saxs diffraction patterns.

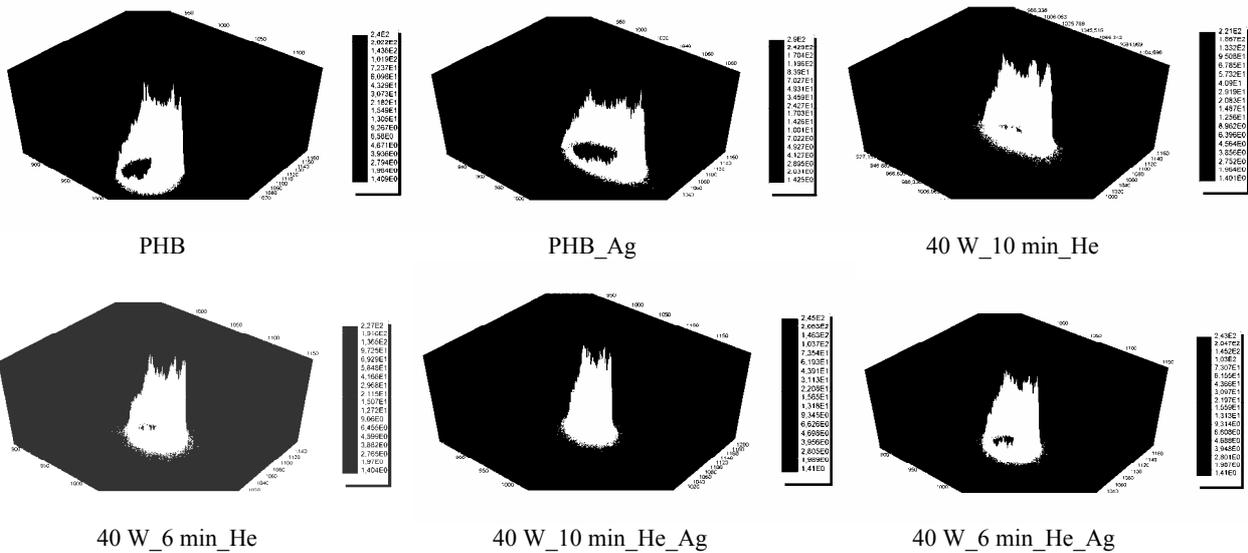


Fig. 4 – 3D SAXS measurements.

From Figs. 3 and 4 SAXS patterns demonstrated significantly changes after plasma followed by silver nitrate treatments in PHB films, indicating the embedded Ag nanostructures can rearrange themselves depending upon counterionic environment.

The LEPTOS program from Bruker was used for obtaining the 3D pictures from Fig. 4.

In order to obtain information about the embedded Ag the model fitting approach was done by using the NanoFit software. The 3D intensity distribution in reciprocal space is further processed in order to obtain the corresponding real-space structure. Spherical model with a Gaussian distribution for Guinier plot was used to simulate the scattering curves of PHB_Ag_40W_6min_He_Ag, 40W_10min_He_Ag samples, and average values of 65, 70, and 80 nm, respectively, were found for AgNPs dispersed in the polymer matrix.

The availability of silver nanoparticles in PHB films open new applications for those materials. It is thought that silver atoms bind to thiol groups (-SH) in enzymes and subsequently cause the deactivation of bacterial enzymes. Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport.¹³ In addition to their effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They inhibited cell division and damaged the cell envelope and contents of bacteria.¹⁴

Dispersed metal nanoparticles in a polymer matrix provide strong possibilities of fabricating functional materials with unique properties for catalytic, electrical and sensing applications. It is known that metal nanoparticles may enhance or suppress the thermal stability depending on the type of polymer.¹⁵

CONCLUSIONS

PHB is an example of how new technology, derived from nature but not exploited until

recently, can contribute to meeting societal needs for plastics and a clean environment. The addition of metallic silver components PHD films access devices has been treated as a means of preventing bacterial growth. The FTIR and SAXS methods revealed the changes in crystallinity of the treated PHB films and the embedding of silver nanoparticles at polymer surface.

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REFERENCES

1. B. Taidi, D. Mansfield and J. Anderson, *FEMS Microbiol. Lett.*, **1995**, *129*, 201-205.
2. G.-Q. Chen, *Chem. Soc. Rev.*, **2009**, *38*, 2434-2446.
3. M. Drobota, M. Aflori and V. Barboiu, *Digest J. Nanomat. Biostruct.*, **2010**, *5*, 35-42.
4. M. Aflori, M. Drobota, D. Gh. Dimitriu, I. Stoica, B. Simionescu and V. Harabagiu, *Mat. Sci. Eng. B*, **2013**, *178*, 1303.
5. S.Y. Yeo, W.L. Tan, M. Abu Bakar, and J. Ismail, *Polym. Degrad. Stabil.*, **2010**, *95*, 1299-1304.
6. L. E. Macaskie, P. Yong, M. Paterson-Beedle, A. C. Thackray, P. M. Marquis, R. L. Sammons, K. P. Nott, and L. D. Hall., *J. Biotechnol.*, **2005**, *118*, 187-200..
7. R. Handrick, S. Reinhardt, P. Kimmig, and Dieter Jendrossek, *J. Bacteriol.*, **2004**, *186*, 7243-7253.
8. M. Aflori, *Rev. Roum. Chim.*, **2015**, *60*, 817-821.
9. T. Furukawa, *et al.*, *Macromolecules*, **2005**, *38*, 6445-6454.
10. M. Aflori, M. Drobota, *Digest J. Nanomat. Biostruct.*, **2015**, *10*, 587 – 593.
11. N. P. Camacho, P. West, P.A. Torzilli and R. Mendelsohn, *Biopolymers*, **2001**, *62*, 1-8.
12. S. Randriamahefa, E. Renard, P. Guérin and V. Langlois, *Biomacromolecules*, **2003**, *4*, 1092-1097.
13. J. L. R. Arrondo, A. Muga, J. Castresana and F. M. Goni, *Prog. Biophys. Molec. Biol.*, **1993**, *59*, 23-56.
14. C. Petibois, G. Gouspillou, K. Wehbe, J. P. Delage and G. Deleris, *Anal. Bioanal. Chem.*, **2006**, *386*, 1961-1966.
15. P. K. Jain, K. S. Lee, I. H. El-Sayed and M. A. El-Sayed, *J. Phys. Chem. B*, **2006**, *110*, 7238.