Modification via Preparation for Poly(3-hydroxybutyrate) Films: Water-Transport Phenomena and Sorption

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ABSTRACT: The effect of the preparation technique on the sorption–diffusion parameters of poly(3-hydroxybutyrate) (PHB) films was studied. The films formed by a single-stage technique have an axial texture of the crystalline phase, with the polymer chain oriented predominantly perpendicularly to the film plane. Moreover, the crystallites in PHB are preferably ordered in stacks in the course of diffusion; the alignment of the crystallites noticeably decreases the "sites" of PHB (polar groups) which are accessible to water molecules. As a result, the sorption capacity decreases but the rate of diffusion increases. On the contrary, PHB films prepared by a two-stage technique are characterized by a poor ordering of crystallites without texture organization. Here, the sorption/immobilization on the polar groups of PHB is increased, but diffusivities are decreased. Concentration dependencies of the water-diffusion coefficient are discussed. Additional information on the existence of protein impurities in the PHB samples obtained by the above two preparation techniques was obtained by FTIR spectroscopy and H–D exchange methods. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 475–480, 2000

Key words: poly(3-hydroxybutyrate); preparation technique; sorption; diffusion; water

INTRODUCTION

The family of novel biopolymers, polyhydroxyalkanoates,[1] and its main representative, poly(3-hydroxybutyrate) (PHB), have been extensively applied in agriculture, in biotechnology, and, especially, in medicine owing to a relevant combination of physicochemical properties,[2,3] an adjustable ability to biodegradation,[2,4] and environmental compatibility.[5,6] Under natural/landscape conditions, the polymer degrades to carbon dioxide and water. In addition, a favorable compatibility with biological tissues and blood as well as biodegradation properties with PHB has led to its

investigation as matrices for controlled drug delivery.[7–10]

Controlled design of the transport characteristics of the polymer matrix becomes a rather important task for the applications of PHB-based materials as surgical sutures and systems for prolonged drug release or as an environmentally safe packaging material.[11] Even though the number of works devoted to the microbiological synthesis of this polymer and characterization of its physicochemical properties has been increasing, the diffusion transport of water and solutions in PHB[12–14] still remains quite scarce. The transport behavior of PHB films and matrices is controlled by structural/morphological organization of the polymer, which, in turn, depends on the crystallization history[15,16] and the film-prepara-

tion procedure. The main objective of this work was to study the effect of the preparation procedure on the transport characteristics of PHB-based films.

EXPERIMENTAL

Materials and Processing

PHB films with a thickness of 40 ± 5 μm were cast from different solvents. The solubility of the initial polymer powder (biomer) in boiling chloroform was about 10^{-2} g/mL. To obtain a soluble fraction of the polymer, the solution was filtered using a Schott glass filter. Then, the solvent was evaporated, and the polymer film was cast from the chloroform-soluble polymer; 3% solutions in the chloroform were used. This solution was poured into a Petri dish, tightly closed with a glass plate, and allowed to stand at 293 K until the solvent was evaporated.

The second type of test samples was prepared by dissolution of the initial polymer powder in dioxane by heating the solution to the boiling point; a 5% solution of the polymer was prepared. After evaporation of dioxane, the polymer was dissolved in chloroform on heating, and a 3% solution was obtained. This solution was filtered through a Schott glass filter, poured into a Petri dish, closed tightly with a glass plate, and allowed to stand at 293 K until the solvent was evaporated.

According to viscozimetric measurements,[17] the M_w was equal to $(110 \pm 26) \times 10^3$ and $(193 \pm 32) \times 10^3$ g/mol for the samples prepared by the chloroform dissolution and dioxane–chloroform dissolution, respectively. As estimated by X-ray measurements, the crystallinity of the above polymer films was the same, 70%,[18,19] for both the one-stage and the two-stage preparation methods.

Sorption and Diffusion

Sorption measurements were conducted using the method of vacuum gravimetry. We used a McBain microbalance with a sensitivity of 0.67 mg/mm. The details concerning the procedure of sorption experiments were reported in ref. 20.

FTIR Spectroscopy

The FTIR spectra of the PHB films were recorded with an IFS-48 Bruker FTIR spectrometer at a resolution of 2 cm^{-1}. To remove traces of the solvents, the films were placed into a thermostated chamber, outgassed, and kept for 2–3 h at 353 K. The completion of solvent evaporation was checked using the FTIR spectra of the test samples. We observed a decrease in the intensity of the following bands: at 873–876, and 2855 cm^{-1} for dioxane and at 756, 3012–3040, and 2976–2992 cm^{-1} for chloroform.[21,22]

Bacterial PHB obtained by fermentation contains residual protein admixtures. The content of protein inclusions may be as high as 1.8%, whereas the content of lipids is about 0.5%.[1,23] These values depend on the procedure and degree of polymer purification after its recovery from the microbial biomass. In addition to the residual protein N—H groups, the polymer may contain end hydroxyl groups, which are typical of most polyesters. In the IR region, amide groups show absorption bands at 3282 and 3209 cm^{-1},[24–26] whereas the absorption bands of hydroxyl groups are observed at 3635 and 3550 cm^{-1}.[24,25] Despite minor amounts of these groups in the polymer, changes in the absorption of these groups after deuteration may provide additional information about the polymer structure. In this connection, the following experiments were conducted: Polymer films without any traces of solvents were kept for 2 h in water at 353 K. Then, water was removed from the film surface by filtration paper, and the corresponding FTIR spectrum was recorded. The films were thermostated for 2 h at 353 K in a vacuum, and the FTIR spectra were recorded once more. The films were kept in heavy water for 2 h at 353 K; the water traces were removed by filtration paper, and the corresponding FTIR spectra were registered. The films were repeatedly outgassed and heated for 2 h at 353 K, and their FTIR spectra were recorded.

## RESULTS AND DISCUSSION

Sorption and Diffusion

The sorption isotherms obtained at 313 and 323 K for the two PHB samples prepared by different casting techniques are presented in Figure 1. As follows from Figure 1, water sorption of the PHB sample cast from chloroform is weaker than that of the sample prepared by the two-stage technique. To explain the sorption isotherms of PHB membranes cast from chloroform, we proposed a model similar to the dual-sorption model and cal-
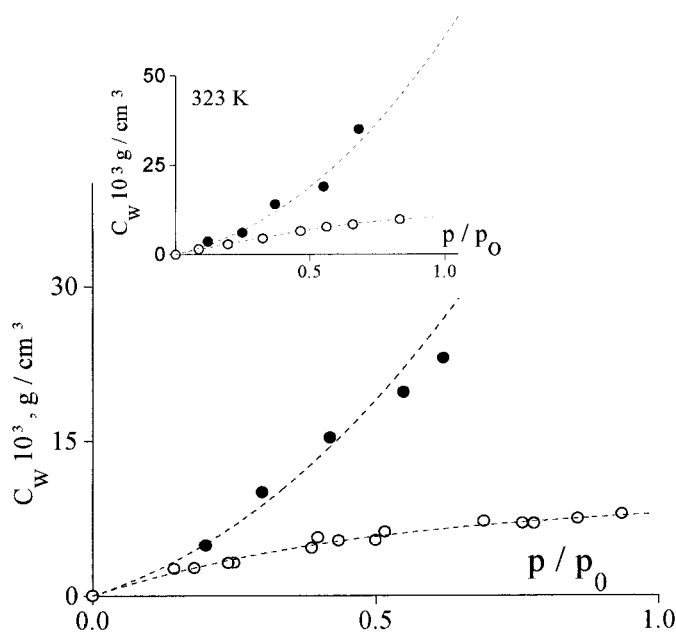
Figure 1 Water-vapor sorption isoterms for PHB films at 313 and 323 K as calculated per amorphous fraction of the polymer. The films were prepared by (open circles) single-stage and (solid circles) two-stage procedures.

culated the thermodynamic parameters of water sorption–diffusion in such samples.[27] Comparing the results of the diffusion experiments allows one to conclude that the preparation procedure has a strong effect on the mechanism of water transport in a polymer. Figure 2 presents the concentration dependencies of the diffusion coefficients for the test PHB samples at 313 and 323 K. It is seen that the diffusion coefficients of the samples cast from chloroform increase with the concentration of the sorbed water according to the Fujita model, the applicability of which was
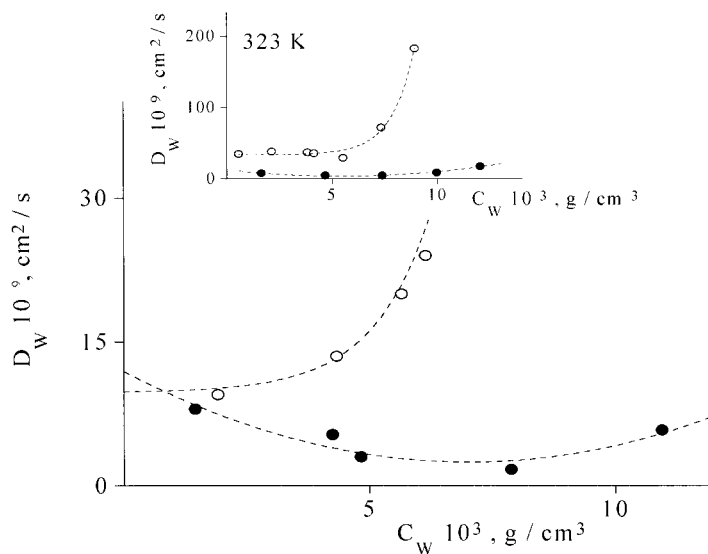
Figure 2 Concentration dependencies for the diffusion coefficients of water at 313 and 323 K in the PHB films prepared by (open circles) single-stage and (solid circles) two-stage procedures.
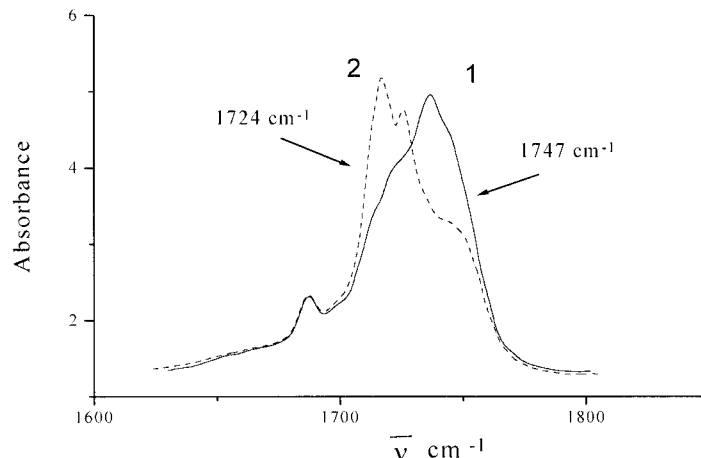
Figure 3 FTIR spectra of (1) dry and (2) wet PHB films in the absorption region corresponding to the carbonyl groups of the polymer.

proved earlier for the PHB samples of this type.[27] In the case of the samples prepared by the two-stage procedure, the diffusion coefficients change slightly with increase in the concentration of the sorbed water, and the concentration dependence shows a minimum: The diffusion coefficients decrease at the early stages of sorption and begin to increase at the final stages.

To analyze the sorption and diffusion experiments, we used the assumption[27] that, at least, two forms of sorbed water occur in a polymer matrix: immobilized water (on polar sorption sites) and free water (with translational mobility). A certain dynamic equilibrium exists between these two forms of water. In the case studied, immobilized water dominates in the polymer, and its sorption capacity may be strongly affected by the degree of accessibility of the polar carbonyl groups of PHB macromolecules for water molecules.

Wide-angle and small-angle X-ray diffraction studies[18,19] showed that, in the PHB membranes cast from chloroform, crystallites are primarily oriented with cylindrical symmetry with respect to the axis perpendicular to the plane of the film. The polymer chains in the crystallites are oriented perpendicular to the film plane. In addition to orientation, the crystallites in the films cast from chloroform are predominantly ordered in the normal direction with respect to the plane of the polymer film. These crystallites are from regions (stacks), where they are piled onto each other by their wide planes. In this case, accessibility of polar sites, on which water molecules are immobilized, decreased with the water-sorption capacity.

PHB films cast by the two-stage technique are characterized by a lowered ordering of crystallites with respect to each other and show no texture. The sorption parameters of such films are higher, and a longer diffusion path of the water molecules in such samples decreases the corresponding diffusion coefficients. The existence of a minimum in the $D(Cw)$ plot is related to the fact that the initial portions of the sorbate are actively bound to a greater number of accessible sorption sites as compared with the samples with anisotropic organization. As the number of the occupied immobilization sites increases, the diffusion coefficients increase according to the Fujita model.

FTIR Spectroscopy

The effect of this immobilization becomes evident by comparing the corresponding FTIR spectra of the dry and wet PHB thin films. In the frequency region of 1600–1800 cm^{-1} associated with the absorption of carbonyl groups, the absorption band at 1745–1750 cm^{-1} corresponds to the absorption of the native carbonyl groups, whereas the absorption band at 1720–1725 cm^{-1} is assigned to the carbonyl groups involved in hydrogen bonding with water molecules.[27–29] Figure 3 presents the corresponding FTIR spectra of the dry and wet PHB films, and one may follow the changes in the ratio between these two types of carbonyl groups.

The polymer samples also contain peptide protein groups, which are retained in the polymer after its isolation and purification from the bacterial biomass producer.[2] These groups may also serve as additional sorption sites and may affect the mechanism of water transport in the polymer.

Table I   Absorption of the Groups of Protein Inclusion for Various PHB Samples After Their Treatment with Heavy Water and Its Further Removal (the Thickness of the Test Films Was the Same)

Sample	Integral Absorption at 3700–3500 cm^{-1} and 3400–3130 cm^{-1}		
	Initial Film	After Treatment and Removal of D$_2$O	Change (%)
Cast from chloroform	4.8	4.1	15
Cast by two-stage procedure	13.4	9.1	33

Deuterium exchange between the amide and end hydroxyl groups of PHB decreases the absorption intensity in the region of 3700–3130 cm^{-1} (Table I). The measurements of the absorption intensity at 3700–3100 cm^{-1} show that, in the films cast from chloroform, the content of protein inclusions is twice lower as compared with that in the films prepared by the two-stage procedure. This fact may be explained by considering the effect of the solvent polarity on the solubility of protein conglomerates. Furthermore, a more polar dioxane may decompose native protein aggregations, which bind PHB macromolecules in the initial polymer powder prepared from biomass.

When the initial amide hydrogen in the protein molecules is replaced by deuterium (as can be seen from the FTIR measurements, the accessibility of the polymer sample PHB cast amide groups into the polymer matrix), 33 vol % of the PHB sample prepared by the two-stage procedure and only 15% of amide groups of the PHB sample cast from chloroform are accessible for heavy water. This fact suggests that the latter sample is characterized by a higher degree of crystallinity and a more perfect structural organization. The latter conclusion is proved by the X-ray measurement and by the character of the sorption–diffusion characteristics of the sample. Protein components are known to show the absorption band amide I in the IR region at 1663–1650 cm^{-1}. This absorption band is primarily provided by the stretching vibrations of a carbonyl bond.[24–26] Using the second derivatives of the spectrum, the absorption maxima were determined.[30,31] In this IR region, the above absorption band was separated into the Gaussian components, and the corresponding parameters were selected in such a way that the integral curve should better fit the experimental dependence (the least-squares method) reasonably well.

Figure 4 demonstrates the result of such a treatment, and the absorption band amide I is clearly seen. For the samples prepared by the two-stage procedure, the area under this band was found to be 2.5 times larger than that of the samples cast using only from chloroform. This fact agrees with the results of our calculations in the absorption region of 3700–3100 cm^{-1}. (The values of the integral absorption for different films presented in Table I were 4.8 and 13.4.) Hence, the results of the sorption experiments and analysis of the influence of water on the PHB matrices in the IR region indicate that the preparation conditions have a strong effect on the complex of transport characteristics of polymer membranes.
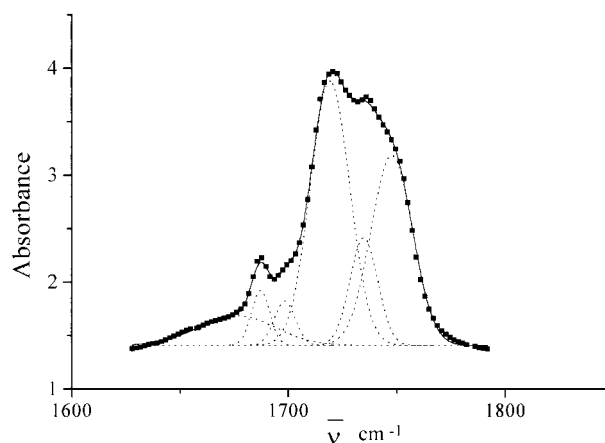
Figure 4   Separation of absorption band in the IR region of 1600–1800 cm^{-1} into components. In the case of the water-containing PHB film, the absorption maximum of the carbonyl groups interacting with water molecules is at 1720–1725 cm^{-1} and the absorption maximum of carbonyl groups not interacting with water is at 1745–1750 cm^{-1}. The absorption band of amide I shows a maximum at 1663–1650 cm^{-1}.

CONCLUSIONS

Application of the solvents with different polarities and varying the conditions of film casting allow one to control the sorption–diffusion parameters of PHB films, and this knowledge is of great importance for the preparation of new polymer medical systems with controlled drug release and for the production of polymer materials with the desired barrier properties.

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