Analysis of structure and dynamics of superfine polyhydroxybutyrate fibers for targeted drug delivery

A Olkhov\textsuperscript{1,3}, E Kucherenko\textsuperscript{3}, P Pantyukhov\textsuperscript{1,2}, A Zykova\textsuperscript{1,2}, S Karpova\textsuperscript{2}, A Iordanskii\textsuperscript{3}

\textsuperscript{1}Plekhanov Russian University of Economics, 113054 Moscow, Russia
\textsuperscript{2}Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, 119334 Moscow, Russia
\textsuperscript{3}Semenov Institute of Chemical Physics of Russian Academy of Sciences, 119334 Moscow, Russia

E-mail: zykovaak@yandex.ru

Abstract. Creation of polymer matrix systems for targeted drug delivery into a living organism is a challenging problem of modern treatment of various diseases and injuries. Poly-3-hydroxybutyrate (PHB) is commonly used for development of therapeutic systems. The aim of this article is to examine the changes in structure and morphology of fibers in presence of dipyridamole (DPD) as model drug for controlled release. It was found that addition of dipyridamole led to disappearance of spindle-shaped nodules on fibers of PHB in comparison with pure PHB. The research of thermophysical parameters showed that specific melting enthalpy (and the degree of crystallinity) of PHB fibers increased with the addition of DPD. With the increasing of DPD content in PHB fibers, more perfect and equilibrium crystal structure was formed. According to analysis of intercrystalline regions of PHB fibers, it was found that as the crystallinity of PHB in intergranular regions rose, the corresponding decrease of radical rotation speed was observed. It was concluded that fibers of PHB can be used for creating therapeutic systems for targeted and prolonged drug delivery.

1. Introduction
Creation of polymer matrix systems for targeted drug delivery into a living organism is an actual problem of modern treatment of various diseases and injuries [1]. Poly-3-hydroxybutyrate (PHB) is used for creating these therapeutic systems in most cases. High biocompatibility, optimum time of biodegradation and the mission-related physical and mechanical properties of films and PHB fibers allow to consider this polymer as one of the most promising medical polymers [2].

2. Experimental part

2.1. Materials and Preparation of Samples
In this work, polyhydroxybutyrate of the 16F series ((BIOMER®, Germany) with a molecular weight Mw = 2.06x10^5 g/mol was used to produce investigated fibers. As a model drug for controlled release was used dipyridamole \((2,2',2'',2'''-(4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,6-diyl)bis(azanetriyl))tetraethanol)\). Pharmacological class of dipyridamole (DPD): antiplatelet, angioprotectors and proofreaders of microcirculation, adenosynergic tools. Superfine PHB fibers were obtained by electrospinning. Spin dopes including PHB and PHB with 1\%, 3\% and 5\% DPD in chloroform.
2.2. Measurements

The electron paramagnetic resonance spectra (X-band) were recorded on an EPR-V automated spectrometer (Semenov Institute of Chemical Physics, RAS, Moscow) at microwave power of 7 mW and modulation amplitude of 0.5 gauss. As a probe, the stable nitroxyl radical 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) was used. The radical was incorporated into fibers from vapor at a temperature of 50°C to a concentration not exceeding 10^-3 mol/l. The DSC measurements were performed on a Netzsch DSC 204 F1 calorimeter in argon atmosphere at a heating rate of 10 K/min. The morphology of the fibers was studied via electron microscopy on Hitachi TM-1000 scanning electron microscope (Japan).

3. Results and discussion

As can be seen from Fig. 1, in the PHB fibers there is a significant number of spindle-shaped nodules of 5-7 μm in diameter and 15-20 μm in length.

![Figure 1](image_url)

**Figure 1.** (a) Electron micrographs of the PHB electrospun fibers, (b) Electron micrographs of the PHB + 1% DPD electrospun fibers, (c) Electron micrographs of the PHB + 3% DPD electrospun fibers, (d) Electron micrographs of the PHB + 5% DPD electrospun fibers

Number of nodules decreases with increasing of concentration of drug substance (DS) and absolutely is not observed for PHB fibers with the highest concentration (5%) of DPD. The presence of nodules may be associated with insufficient conductivity of a spinning solution or with its sub-optimal viscosity.

Thermophysical and structural characteristics have been investigated by DSC method. The values
of melting enthalpy (AH) and the melting temperature (Tm) of superfine PHB fibers with DPD are presented in Table 1.

Table 1. Thermophysical Parameters of Superfine PHB Fibers with DPD Investigated by DSC

| Thermophysical Parameters | PHB   | PHB+1% DPD | PHB +3% DPD | PHB +5% DPD |
|---------------------------|-------|------------|-------------|-------------|
| AH, J/g                   | 42,9  | 53,7       | 72,0        | 79,7        |
| Tm, °C                    | 168,70| 168,50     | 166,70      | 169,30      |

It follows from the Table 1 that the specific melting enthalpy (and the degree of crystallinity) of PHB fibers increases with the addition of DPD. With the increasing of DPD content in PHB fibers, more perfect and equilibrium crystal structure was formed.

The structure and molecular dynamics of intercrystalline regions were investigated by the electron-spin resonance (ESR) method. It was found that as the crystallinity of PHB in intergranular regions rose, the corresponding decrease of radical rotation speed was observed. That caused the slowdown of molecular mobility of segments. With the increasing content of DPD the correlation time increases in the sequence: 6,6x10^{-9} (from 0%) > 7,1x10^{-9} (1%) > 8,8x10^{-9} (3%) > 9,0x10^{-9} (5%). This effect may be explained by the increase of crystallinity of PHB fibers.

![Graph](image)

**Figure 2.** Ratio of absorbances excluding the destructive component (A), linear areas of kinetic curves in diffusion coordinates (B)

The kinetic curves of drug release are shown in Fig. 2, characterized by an initial non-linear time area and the final area, where the concentration of desorbed DPD increases linearly. A superposition of diffusion and kinetic processes was observed for all kinetic curves of drug release from the PHB fibers with DPD.

Nonlinear area of DS release kinetic curve is defined by the diffusion process. The abrupt change of diffusion coefficient corresponds to structural transition from spindle-shaped particles to cylindrical ones. The rate of destruction process exponentially decreases with increasing DS, what has a good agreement with the previous data. The presence of two release processes (diffusion and kinetic) suggests that DPD exists in PHB fibers in two forms. First form is the DS in “free form”, and...
just this form is able to desorb DS from PHB fibers in accordance with the diffusion mechanism. Second form of DS exists in the immobilized state in the polymer and is characterized by an extremely low diffusion mobility, but its release from the polymer is supported by the partial degradation of PHB (weight loss with the inclusion of immobilized DS form) by zero-order equation.

4. Conclusion
In the absence of DPD or its low levels (0% and 1%) fibers have a complex geometry with the alternation of cylindrical fragments about 3.1 microns in diameter, typical for conventional fibers and spindle-shaped nodule fragments with an average diameter of 5.7 microns.

With the increasing of DPD content in PHB fibers up to 5 % these fragments disappear, and the fiber geometry becomes cylindrical only.

It is shown that the addition of DPD to PHB polymer matrix leads to a significant increase in crystallinity and to a slowdown of molecular mobility in the amorphous regions of superfine fibers.

All results, especially the impact of DS concentration on fiber shape and its dynamic characteristics are in good agreement with the thermophysical parameters and should be used for creating therapeutic systems for targeted and prolonged drug delivery.

References
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