Introduction Poly(3-hydroxybutyrate) (PHB), the simplest and most common member of the group of polyhydroxyalkanoates (PHA) can be considered as a polymer with high potential for applications as a degradable biomedical material [1,2,3]. This polymer has been extensively studied in view of its wide application in many fields as engineering, packaging, medical diagnostics, tissue engineering, drug delivery therapy and others [4-7]. Despite considerable attention of researchers toward the investigation of PHB, there are still many open questions concerning, in particular, features in molecular dynamics of this polymer in relationship with its structure [8,9] and transport properties [10,11]. The search for this relationship is of importance for the creation of new materials with controlled transport characteristics. The purpose of this work was to study some features of the segmental momility of PHB membranes, possessing special structural organization, by the EPR spin probe technique [12]. Another task was to determine changes in the molecular mobility of PHB upon sorption of the polymer with water vapor. Experimental The experiments were performed using two series of PHB samples differing by their structural organization (morphology)–textured and isotropic. The textured PHB samples were prepared by extracting the initial polymer powder (Biomer trade mark) with boiling chloroform (solubility 10–2 g/ml). The extracted soluble fraction was used to prepare a 3% PHB solution in chloroform. The solution was poured into a Petri dish, tightly covered with glass, and allowed to stand at 20°C until complete evaporation of the solvent. The isotropic PHB samples were prepared by dissolving the initial powder in dioxane (5%) and heating the solution to boiling. Then dioxane was completely evaporated and the residue was used to prepare a 3% PHB solution in chloroform (on heating). The solution was filtered through a Schott filter (pore size, 160), poured into a Petri dish, tightly covered with glass, and allowed to stand at 20°C until complete evaporation of the solvent. In order to remove the residual solvent from PHB films, the samples were kept for 2–3 h in vacuum at 80°C. The completeness of solvent removal was checked by monitoring a decrease in intensity of the corresponding absorption bands of dioxane (873–876, and 2855 cm–1) and chloroform (756, 3012–3040, 2976–2992 cm–1) in the IR spectra [13]. The degree of polymer crystallinity in the samples of both types was about 70%, as evidenced by the X-ray diffraction data [8,9]. The weight-average molecular mass determined by viscosimetry were was (310± 26) x103 for the textured PHB samples and (293± 32)x103 for the isotropic ones. The nitroxyl radicals, 2,2,6,6-tetramethyl-1-piperidinyloxy (Tempo) and 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (Tempol), used as spin probes were introduced into PHB samples prior to pouring the polymer solutions into Petri dishes. The radical concentration in solid polymer samples was ~1017 spin/cm3. The solvent-free PHB films prepared as described above were cut into 2-cm-long 2-mm-wide strips. The strip thickness varied from 60± 5 to 40± 4 µm. The EPR spectra were measured using a stack of plates placed into 4-mm-diam glass ampules. The effect of moisture on the PHB films was studied upon exposure the film samples to a saturated water vapor at 20°C for 15–17

h. Preliminary experiments showed that this time was sufficient to provide for the equilibrium saturation of the samples with water [10]. The stacks of water-saturated samples were placed into ampules, sealed, and used to measure the EPR spectra. The water content in both isotropic and textured films under these sample preparation conditions was ~5´10-3 g/cm3 (calculated for amorphous component), as estimated according to preliminary data on the water sorption [10,11]. The EPR spectra were measured at the conditions far from saturation on a Radiopan SE/X-2544 spectrometer (Poland). The measurements were performed in the 20–90 °C temperature range under heating or cooling the samples at a rate of 2 K/min. Results and Discussion The highly crystalline PHB films have formed lamellar crystalles composed of macromolecules in a folded chain conformation [14]. The PHB crystals exhibit an orthorhombic unit cell with the parameters a = 0.58 nm, b = 1.3 nm, c = 0.60 nm [8,9], containing two helical macromolecules with antiparallel mutual orientation [15]. In the textured PHB samples, the crystallites are oriented with their unit cell axis c along the normal to the film plane and stacked by their wide faces to form ordered domains. In the isotropic samples, the crystallites have no preferred orientation and are arranged in a random manner [8,9]. The stable nitroxyl radicals (Tempo and Tempol) used as spin probes possess linear dimensions exceeding 0.5 nm [16-18] and cannot penetrate between the folded chains of PHB molecules forming crystallites. Thus, the radicals are most likely located in the intercrystallite layers with an average thickness of 1.8 nm [8,9]. Indeed, the probes in crystalline polymers are usually located within disordered regions of a crystalline polymer matrix, the rotational mobility of the probes characterizing the dynamics of these regions [17,18]. The EPR spectra of Tempo and Tempol radicals in PHB matrices (Fig. 1) exhibit well resolved triplets with signs of the superposition of signals from radicals characterized by different vales of the rotation correlation time τ. The superposition is manifested by additional extrema in the main triplet signal. The spectra of probes exhibit no angular dependence. As seen from Fig. 1, the signs of superposition of the signals from rapidly and slowly rotating radicals are observed for the Tempol radical. Increase in the temperature is accompanied by growing intensity of the segmental motions in PHB, decreasing rotation correlation time of the spin probes, and the superposition being less pronounced as compared to the pattern observed at lower temperatures. On heating the dry PHB samples to 90 °C and their subsequent cooling, the spectra measured at equal temperatures coincide. Apparently, no irreversible structural changes take place in the polymer in the temperature range below 90°C. Fig. 1 - EPR spectra of (a) Tempo and (b) Tempol radicals in dry textured PHB films at T = 20 (1); 40 (2); 60 (3); 80°C (4) We have performed analysis of the experimental EPR spectra within the framework of the model of isotropic radical rotation. It was suggested that the observed spectra can be considered as superposition of the signals from radicals with the rotation correlation times τ1 and τ2, the corresponding molar fractions being w1 and w2. The EPR spectra of Tempo and Tempol radicals were modeled using the following spin parameters: Azz = 34.3 G, Ayy = 6,2 G, Axx = 6.8 G; gzz =2.00241, gyy = 2.00601, gxx = 2.00901 [18]. The theoretical spectra were calculated using a variant of the Freed programme [17] modified by Timofeev and Samariznov [19]. Figure 2 shows the experimental and theoretical EPR spectra of spin probes in dry and water-saturated of both isotropic and textured morphology. A comparison shows that the theoretical spectra of both Tempo and Tempol spin probes qualitatively describe the main features observed in the corresponding experimental spectra. This coincidence indicates that the spectra actually represent a superposition of the EPR signals from radical molecules with different rotation correlation times. The existence of different rotation correlation times for the molecules of spin probes in the PHB matrix can be explained assuming that noncrystalline component of the polymer contains microscopic regions differing both in density and in the segmental mobility. One factor responsible for such a difference can be the distance from the surface of crystallites. In the vicinity of this boundary, the segmental mobility of macromolecules must be strongly hindered and the structural organization of segments can be more ordered as compared to that at a sufficiently large distance from the crystal surface. Fig. 2 - EPR spectra of Tempo (1, 2) and Tempol (3–6) radicals in textured (1, 3, 5) and isotropic (2, 4, 6) PHB samples in the (1–4) dry and water-saturated (5, 6) state. Dashed curves show the calculated spectra, solid lines present the experimental spectra measured at T = 20°C. The curves for Tempo (1, 2) were calculated using the following parameters: t1 = 1x10–9 s; w1 = 0.4 (1), 0.25 (2); t2 = 8x10– 9 s (1), 6x10–9 s (2); w2 = 0.6 (1), 0.75 (2). The parameters used in calculating the curves for Tempol (3–6) are listed in the table Probably, these microscopic regions are also characterized by greater density as compared to the average density of polymer in the intercrystallite layers. For brevity, these ordered regions will be referred to as the “dense” regions. On the contrary, the segments occurring at a sufficiently large distance from crystallites would possess a maximum segmental mobility and a minimum density (approaching that of the amorphous polymer). These regions will be conventionally referred to as “loose” (or amorphous). Another factor leading to a difference in behavior of the spin probe in PHB can be the presence of polar microscopic regions, formed with participation of the functional ester groups of the polymer, and less polar regions with dominating dispersion interactions. Note that the theoretical spectra of Tempol radical show a better coincidence with experiment than do the spectra calculated for the Tempo spin probe. Probably, the unsatisfactory agreement between theory and experiment for Tempo is related to the anisotropic rotation of this radical known to take place in many polymer matrices [17 p. 139] but neglected in our calculation. In what follows, the consideration will be restricted to data obtained for the Tempol radical. Data on the rotation correlation time obtained by comparison of the experimental and theoretical spectra of Tempol in PHB matrices are presented in the table 1 together with molar fractions of spin probes rotating with different correlation times, assigned to the microscopic regions of different types.

Table 1 - Rotation correlation times t and molar fractions w of Tempol radicals in dense and loose regions of dry and water-saturated PHB samples with different morphologies* № τ1× 109 s ω1 % τ2× 109 s ω2% τ1× 109 s ω1% τ2× 109 s ω2 % T=20 °C T=40 °C 1** 1,5 16 9,5 84 1,1 17 7,0 83 2 1,5 24 9,5 76 1,1 22 6,0 78 3 1,9 16 9,5 84 1,5 18 7,0 82 4 1,3 16 8,5 84 1,4 30 6,8 70 * - subscripts 1 and 2 refer to the τ and ω values belonging to the loose (amorphous) and dense noncrystalline PHB regions, respectively ** - 1 - textured, dry, 2 - textured, water-saturated, 3 - isotropic, dry, 4 - isotropic, water-saturated An analysis of these data leads to the following conclusions. First, the correlation times of rotation of the Tempol spin probes located in the dense regions are virtually the same for isotropic and textured PHB samples. At the same time, the mobility of this radical in the loose regions is somewhat higher in textured matrices than in the isotropic ones. Second, the distribution of spin probes between dense and loose regions is virtually the same in both textured and isotropic PHB samples. Saturation of the PHB samples with water vapor affects both the mobility of polymer chains (and, hence, the rotation correlation times of stable radicals localized in the microscopic noncrystalline regions of two types) and the molar ratio of the dense and loose regions. Analysis of the results obtained suggests that moisturizing of the PHB samples results in a change of the relative content of loose and dense regions in the polymer. Fig. 3 - The plots of rotation correlation time versus temperature for Tempol radical in the (a) dense and (b) loose regions of (1) textured and (2) isotropic PHB matrices Figure 3 shows the temperature dependence of the rotation correlation time of the Tempol spin probe in PHB samples. Note that the correlation times of spin probe rotation in the dense regions coincide for the textured and isotropic samples virtually in the entire temperature range studied. At the same time, the τ values in loose regions of the textured samples are smaller as compared to those in the analogous regions of the isotropic polymer. The activation energy for the spin probe rotation in all cases was about 10 kJ/mol. The corresponding preexponential factors are 10.5x 10–11 s for the dense regions of both textured and isotropic samples, 1.8 x 10–11 s for the loose regions of textured samples, and 3.2 x 10–11 s for these regions of isotropic samples. Our investigations [11] showed that water molecules exhibit markedly different diffusion mobility in the textured and isotropic PHB samples. The diffusion of water in the isotropic polymer is several times slower compared to that in the textured PHB of the same degree of crystallinity. The difference can be related to at least two factors. The first is a difference in the intensity of segmental motions in the intercrystallite regions of isotropic and textured polymer samples. The second is a possible significant difference in morphology (structural organization of crystallites) between the PHB samples of two types.

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