Nanofiber-bonded cloth materials based on poly-3-hydroxybutyrate with antibacterial properties for medical purposes

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Abstract. Different transdermal systems based on solid polymer matrices or gels containing functional substances with antiseptic (antibacterial) properties have application to the therapy of many infectious diseases and cancer. Today the most promising type of matrices with antiseptic characteristics are the nano- and microfiber nonwoven materials. Fibers on the biopolymer (poly(3-hydroxybutyrate)) basis were obtained using the electrospinning method. In the present work, the effects of iron (III) complex with tetraphenylporphyrin and its influence on bactericidal and antibacterial properties of the ultrathin PHB fibers were investigated.

1. Introduction
The previous experimental studies of biopolymer materials revealed the necessity of development of special materials with specific structure. The aim of the research work was to develop the method of preparation of nanostructured fibrous material based on the biopolymer poly(3-hydroxybutyrate) (PHB), which would be suitable for manufacturing of innovative drugs, overpouches, capsules with specific properties, and as well as for bandages, insulants and barrier materials.

Much attention is paid to the development of systems based on biopolymers with such characteristics as biocompatibility, thromboresistance and bioresorption [1]. Nowadays one of the most commonly used biopolymers is PHB, which is frequently applied to such therapeutic systems. One of the most perspective matrices with antiseptic properties are the nano- and microfiber nonwoven materials, obtained by electrospinning. The electrospinning method is based on the orientation of a polymer solution as a thin viscous jet in the field of mechanical and electrostatic forces with the subsequent formation of fibers with a nanoscale diameter [2].

2. Materials and Methods
Natural biodegradable polymer PHB 16F series, which was obtained by microbiological synthesis from company BIOMER® (Germany), was used as the polymeric matrix for the formation of the ultrathin fibers. The PHB’s average molecular weight was 2.06x10^5. Meso-tetraphenylporphyrin iron (III) chloride complex (FeCl(TPP)) was used as a modifying additive for the development of fibrous matrices with antiseptic properties. The forming solutions of PHB and PHB with FeCl(TPP) was prepared in chloroform. The PHB concentration in the solution was 7% wt. The mass content of FeCl(TPP) in the forming solution was 1, 3 and 5% wt. respectively in ratio to PHB mass. The forming solutions of PHB with FeCl(TPP) were prepared at a temperature of 60 °C with the use of automatic magnetic stirrer. Fibers were obtained by the electrospinning method with the use of monocular laboratory setup with the diameter of capillary 0.1 mm, the current electric voltage was 12 kV, the distance between the electrodes was 18 cm, and the electrical conductivity of the solution was 10 mmhos/cm.

It is possible to vary the main technological parameters, for instance, voltage, diameter of capillary or distance between the electrodes in dependence to characteristics of forming biopolymer solution and content of additional components. Such possibilities show that the details of the process of electrospinning method provide the potential of producing different kinds of non-woven materials for medical purposes with specific properties (depending on the certain parameters of the process and the electrical conductivity, viscosity and concentration of the forming solution of PHB) [3].

In the current research, the conceptual approach was applied to the development of biopolymer fibrous matrices with a wide range of properties for medical purpose. Moreover, such matrices are based on the biopolymer with the specified structure obtained by the spinning in an electrostatic field from a melt of a natural biopolymer poly-3-hydroxybutyrate (PHB), a bactericidal agent - porphyrin complex (Fe-3-CITPhP) and they have antimicrobial and microbial activity.

The main goal of this study is to obtain such specific nanostructured materials for medical purposes, which can provide the maximum surface density in minimum area contact with the tissues of the body, in accordance with the requirements of physical and mechanical performance and hygiene standards.

3. Results and Discussion
Various parameters were studied to characterize the structure of the nonwoven material. Physical, mechanical, chemical properties of the material were determined. Assessment and comparison of some technological parameters allowed classifying the materials, which were obtained by electrospinning method, in dependence to their morphology and location of ultrathin fibers. Among basic indicators of fiber organization in the material some properties were identified as the determinant, such as density of fiber structure (\( \gamma \)), the index of orientation of the fibers (\( \phi \)), material intensity (function of superficial filling, expressed as the mass per unit volume) (\( \delta \)). The average values for these parameters are given in Table 1.

**Table 1.** Indicators of structure of nonwoven materials based on PHB with different contents of bactericidal additive.

| Sample | Average fiber diameter, \( d \) (\( \mu \text{m} \)) | Density of fiber structure, \( \gamma \) (%) | Index of orientation of the fibers, \( \phi \) | Material intensity, \( \delta \) (g/m\(^2\)) |
|--------|-----------------|-----------------|---------------|-------------------|
| 7% PHB | 2.0-2.5          | 74              | 0.26          | 37.7              |
| 7% PHB + 1% | 93              | 0.34            | 36.6          |
| 7% PHB + 3% | 96              | 0.72            | 36            |
| 7% PHB + 5% | 2.5-3.0         | 89              | 0.67          | 32                |
As it is shown in Figure 1 and Figure 2, the addition of FeCl(TPP) into a solution of PHB leads to significant changes in the morphology of the fiber. At first, original fibers of PHB (Figure 1) the alternation of cylindrical and spindle-shaped thickenings are observed along entire length. The presence of thickenings in the fiber structure can be explained by the low values of electrical conductivity and surface tension of the polymer forming solution. The average diameter of the cylindrical sections of the fibers is 1 - 6 µm, and spindle elements have a maximum diameter ~ 10 µm by length 20 - 30 µm. The addition of 1% FeCl(TPP) leads to the emerging of the uniform fibers in the material’s structure with the average diameter 1.5; 3; 5 µm for each concentration of FeCl(TPP). The increase of FeCl(TPP) concentration of provides the formation of fibers with the average diameter no less than 3 µm (Figure 2).

![Figure 1. Microphotography of fibrous material PHB.](image1)

![Figure 2. Microphotography of fibrous material PHB+3% FeCl(TPP).](image2)

The study of fibrous materials by using differential scanning calorimetry (DSC) showed that the PHB degree of crystallinity increases dramatically with increasing concentration of the FeCl(TPP) in the mixed composition.

The study, which determines the annealing of materials, was carried out to establish the existence of mesomorphic structures in the material, which is capable of further crystallization after annealing. It was postulated that the bactericidal agent fills these regions in the material.

It was confirmed the presence of supramolecular structure of ultrathin fibers based on PHB with FeCl(TPP), which causes a high antimicrobial properties of the resulting material. By the method of DSC was determined thermal transitions and the values of melting and crystallization enthalpies. The obtained data allow to estimate the changes in the speed of crystallization, the nature of the arrangement of polymer units in ultrafine fiber, which determines the specific features of the thermal transitions, which were recorded for samples of the material.

From the obtained data, it was concluded that porphyrine has a plasticizing effect on the crystallization of polymer PHB during the process of electrospinning. The changes in crystallinity degree of polymer are shown in Figure 3.
The method of electron paramagnetic resonance was used to study amorphous regions of the fibers, which allows assessing phenomena detected by DSC and the ratio of polymer phases, providing high accuracy in the evaluation of supramolecular structure of polymer units in the fibers of the material, its location in a germicidal complex.

Moreover, adding of FeCl(TPP) higher content (3-5% wt.) leads to the increase in intermolecular distance, mobility of the chains and resulting in efficiency of the fiber orientation process. As result, the ratio of crystalline and mesomorphic regions in polymer increases.

4. Conclusion
The crystalline and amorphous regions in biodegradable polymers and their compositions show mutual correlation and this is an important issue in modern polymer materials science. An important result of the research was the data from biological tests. The data obtained for the sowing of the test cultures (S. aureus p 209 (Staphylococcus aureus), S. typhimurium (Salmonella typhimurium), E. coli 1257 (Escherichia coli)) point out the prospects of using the FeCl(TPP) for the impregnation of nano- and microfiber nonwoven materials based on the PHB with antiseptic properties for medical properties. During the research, an experimental batch of ultra-fine fibers for medical purposes with different diameter, density and geometry of the spatial position was obtained. The mechanical, chemical-physical, thermal properties, phase distribution in the macromolecular structure were determined. Analysis of these indicators allowed assessment of the possibility to establish the set of characteristics in the fixed range of values, which were taken into account according to requirements to medical materials. The results of the study of ultrafine fibers based on PHB with the bactericidal agent demonstrate the principle of the changes of various properties depending on the component composition of the molding solution.

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