

## ELECTROSPINNING OF BIODEGRADABLE POLYMER SCAFFOLDS

**M. A. Bychuk, N. R. Kil'deeva,  
M. A. Kurinova, N. V. Bogdanov,  
M. V. Kalinin, A. V. Novikov,  
and G. A. Vikhoreva**

UDC 547.917.04

*Ultrathin fibers could be prepared by nozzle-less electrospinning from solutions of biodegradable polyhydroxybutyrate (PHB) polyesters and its mixture with polycaprolactone in  $\text{CHCl}_3$ . The formation region of the continuous network of linkages that was required for stable electrospinning of the fibers was established by studying the PHB solution viscosity as a function of concentration. It was found that microscopic amounts of HCl could regulate the viscosity of solutions of high-molecular-weight PHB.*

The principal characteristics determining if biodegradable polymers can be used to fabricate medical implements are the required set of physical, chemical, and mechanical properties; the processing technology; and the lack of irritating and toxic action *in vivo* of the polymers themselves and their degradation products. Aliphatic polymers in which monomers are linked through esters, which are readily degraded and have various physical, biological, and chemical properties, are most interesting. Not only the lack of allergic tissue reactions but also the ability of the polymers to undergo enzymatic destruction to form natural metabolites assure that biodegradable polymers such as the polyester of natural polyhydroxybutyrate (PHB) are biocompatible [1, 2]. PHB exhibits thermal plasticity and solubility in common solvents, which enable it to be worked into fibers and films and fabricated into a broad assortment of implements.

The design of a new generation of medical implements, i.e., porous polymer scaffolds for tissue engineering, is an important area for scientific and industrial development intended to expand the applications of biodegradable polymers [3]. Tissue engineering is based on the cultivation of cells in biodegradable polymer scaffolds with a high specific surface area. The development of methods for preparing such polymeric materials holds great potential for achieving high effectiveness and stability and for expanding their possible medical applications.

We developed previously from biodegradable polyesters [PHB and polycaprolactone (PCL)] a film with a highly porous structure that was formed during phase separation [4]. The present article focused on the development of a biodegradable scaffold in which the porous structure was formed differently, i.e., by creating a fibrous material from ultrathin fibers by nozzle-less electrospinning from solutions of PHB and its mixtures with PCL.

In contrast with capillary devices in which the spinning solution is fed at a given flow rate through a capillary nozzle and an accelerated and thinned jet is formed through the action of electrical forces, liquid jets on the NS-LAB 200 Nanospider nozzle-less electrospinning device are drawn from the free surface of the spinning solution. For this reason, one of the most important electrospinning parameters is the solution viscosity, which plays a special role in forming surface drops, overcoming internal friction forces during jet drawing, and suppressing cavitation, i.e., processes that assure electrospinning can be performed without using metering devices.

PHB is a natural polymer of bacterial origin that is synthesized by several species of prokaryotic microorganisms as a high-molecular-weight crystallized polymer that is soluble in several chlorinated solvents [2]. We used the polymer as a powder of molecular weight (MW) 860 kDa (OAO G. S. Petrov Institute of Plastics). The high MW of the PHB was responsible for the very high viscosity of its  $\text{CHCl}_3$  solutions. Figure 1 shows rheological curves of PHB solutions in

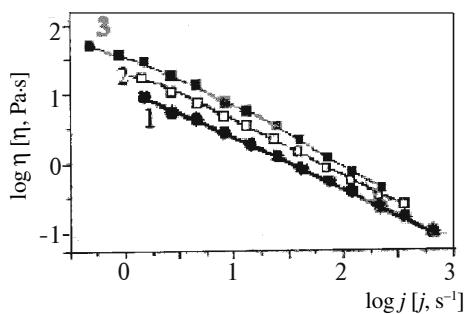


Fig. 1.

Fig. 1. Viscosity as a function of shear rate of PHB solutions in  $\text{CHCl}_3$ . Polymer concentration 3 (1), 4 (2), and 6% (3).

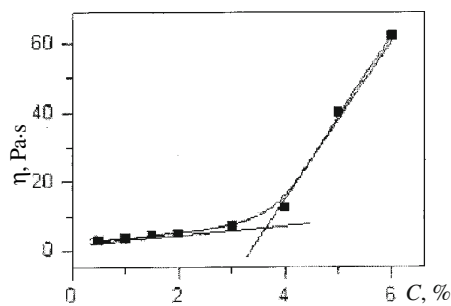


Fig. 2.

Fig. 2. Initial viscosity of PHB solutions in  $\text{CHCl}_3$  as a function of concentration.

$\text{CHCl}_3$  in coordinates of  $\log \dot{\eta} = f(\gamma)$  that were obtained on a Rheotest 2 rotary viscometer (Germany) using a cylinder-in-cylinder measurement cell.

Figure 1 shows that PHB solutions in  $\text{CHCl}_3$  were structured pseudo-plastic liquids. Sections of Newtonian viscosity were missing in rheological curves in the studied shear-rate range. The rheological curve was a structured branch of the viscosity curve in the selected shear-rate range. Its shape was determined by the system structural change resulting from the destruction of the three-dimensional linkage network by deformation. The initial effective viscosity of PHB solution (3%) in  $\text{CHCl}_3$  reached 9.15 Pa·s. The activation energy for viscous flow of this solution that was calculated using an Arrhenius—Frenkel equation and functions  $\ln \dot{\eta} = f(1/T)$  in the range 20–40°C was low (26.7 kJ/mol). Apparently, structuring in the solution formed large associates of solvated macromolecules, the interactions between which were weaker than those between separate macromolecules. For this reason, shear interactions and increased thermal-motion energy destroyed easily the structure of the polymer solution.

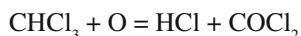
The crossover concentration region of 3.5–4% was determined from the PHB solution viscosity as a function of concentration (Fig. 2). The continuous linkage network that was necessary for stable jet formation during fiber electrospinning began to form in just this concentration region. A study of possible electrospinning from 2–6% solutions of starting PHB in  $\text{CHCl}_3$  showed that a solution of low concentration (2%) was only sprayed. More concentrated solutions hardened too fast and their thin jets broke. Nozzle-less electrospinning from solutions of such low concentration and high viscosity was impossible.

The viscosity of polymer spinning solutions is often reduced by raising the temperature. However, this approach could not be used for a volatile solvent such as  $\text{CHCl}_3$  because it complicated considerably the spinning equipment design and elevated the production hazard. Therefore, use of a polymer with a lower MW was advisable in order to reduce the viscosity of the PHB solutions. In turn, it could be obtained via partial destruction of starting PHB.

Ester bonds are known to undergo acid hydrolysis. Considering that aqueous solutions of acids are insoluble in  $\text{CHCl}_3$ , there was a limited set of acids that could be used as catalysts for PHB destruction. For example, the use of spinning solutions containing formic acid (FA) was proposed for preparing PHB fibers on a capillary electrospinning device [5].

Possible hydrolysis of the polymer and reduction of the solution viscosity in the presence of a stronger acid (HCl) was investigated in addition to the PHB hydrolysis method (10% FA) described in the literature.

Pure  $\text{CHCl}_3$  was slowly altered in air and light. Corrosive acidic vapor consisting of HCl and phosgene was released [6]:



Therefore,  $\text{CHCl}_3$  that was stored for a long time contained HCl.

Spinning solutions were prepared using  $\text{CHCl}_3$  that had been stored for different times. The HCl content was determined by titration in non-aqueous solutions and varied in the range 0.015–0.005%. Destruction of the polymer and

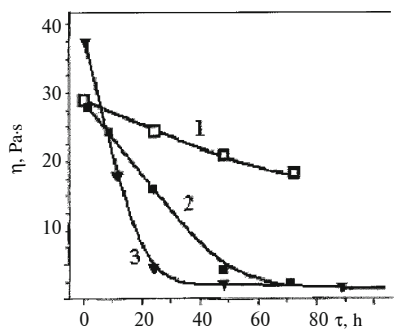


Fig. 3.

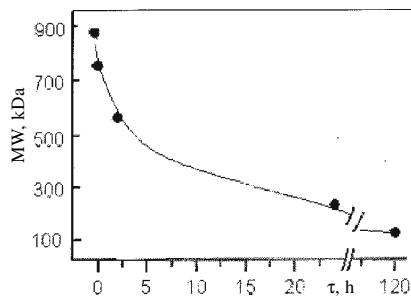


Fig. 4.

Fig. 3. Kinetics of viscosity change of PHB solutions (6%) in  $\text{CHCl}_3$  containing 0.005 (1) and 0.015% HCl (2) and 10% FA (3);  $\tau$  is the solution storage time.

Fig. 4. Change of PHB MW during storage of  $\text{CHCl}_3$  solutions containing 10% FA.

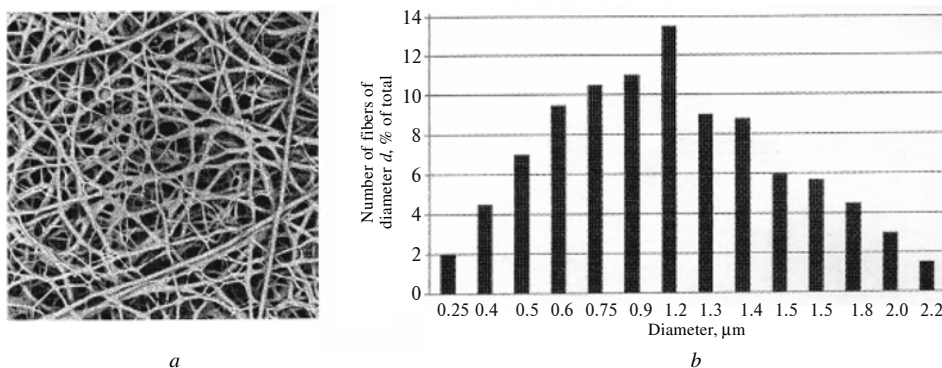


Fig. 5. Photomicrograph (a) and size-distribution histogram (b) of fibers from PHB solution (6%) in  $\text{CHCl}_3$  containing 0.015% HCl.

solution viscosities were monitored using a rotary viscometer with parallel determination of the polymer MW by a viscometric method. Figures 3 and 4 plot the results.

It can be seen that the solution viscosities decreased to various extents upon storage. Thus, the viscosity decreased from 42 to 2 Pa·s in the presence of FA (10%) for 20 h. An analogous effect was achieved in the presence of HCl (0.015%) for 60 h. However, the HCl concentration in  $\text{CHCl}_3$  was 600 times less than that of FA. Of course, the viscosity decreased more slowly if the content of the acid component was decreased (Fig. 3, curves 1 and 2). The data in Fig. 4, in particular the reduction of the polymer MW from 860 to 115 kDa, indicated that the reduction of the solution viscosity under these conditions was due to polymer destruction. It should also be noted that PHB solutions (6%) in  $\text{CHCl}_3$  containing acid components became more transparent during storage.

As already mentioned, it was impossible to use a 6% solution of starting PHB in  $\text{CHCl}_3$  for electrospinning obviously because of the high viscosity, which hindered deformation of the solution drops in the electric field. PHB fibers, the diameters of which fell in the micrometer range according to Fig. 5, could be spun if less viscous solutions obtained by storage in the presence of HCl (0.015%) were used. Figure 5 shows that the obtained fibers had a broad size distribution and many nodes. This was due to the need to spin from solutions of low concentration, because of which the solvent could not fully evaporate and unhardened fibers were deposited on the substrate. However, such a fiber structure provided a pore volume that was sufficient to cultivate cells when used in tissue engineering.

A mixed solution of PHB and another biodegradable polyester with a lower MW could be used to reduce the PHB solution viscosity. Such a polymer was PCL of MW 45 kDa. On one hand, the use of PCL could reduce the viscosity of the spinning composition; on the other, it affected the structure of the spun non-woven material. Table 1 shows that replacing 50% of the PHB by PCL could reduce the solution viscosity to 2.5 Pa·s.

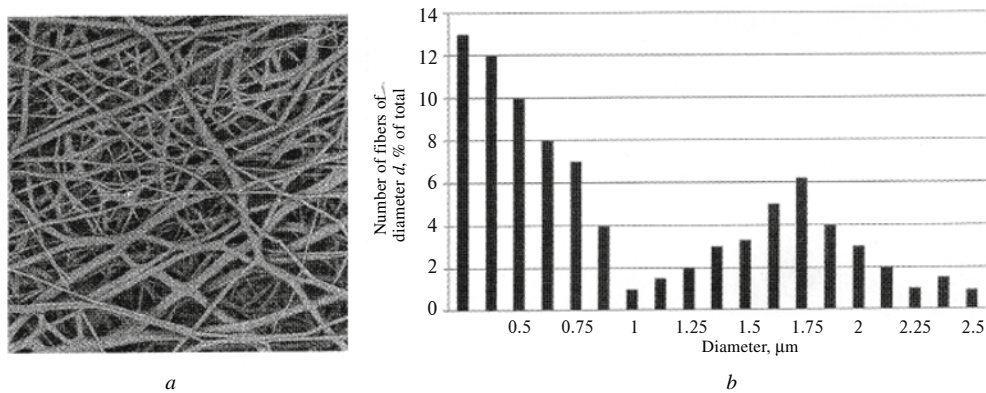


Fig. 6. Photomicrograph (a) and size-distribution histogram (b) of fibers from PHB solution (6%) in  $\text{CHCl}_3$ .

Table 1. Dynamic Viscosity of PHB Solutions (6%, 980 kDa) and Its Mixture with PCL in  $\text{CHCl}_3$

PHB/PCL ratio, %	Initial viscosity, Pa·s
100/-	63.1
50/50	2.5
0/100	0.12

Fibers (Fig. 6a) with a bimodal diameter distribution (Fig. 6b) of thick and thin ones with diameters up to 5  $\mu\text{m}$  and ~500 nm, respectively, were spun from an equal-mass mixture of the polymers (6%) in  $\text{CHCl}_3$  by electrospinning from the free surface at potential  $23 \pm 2$  kV, distance to the substrate 15 cm, 25°C, and cell humidity ~50%. The morphology of the fibrous material was explained by the fact that phase separation occurred in the mixed solution of PHB and PCL and the structure of the isolated phase, the scaffold, formed during solvent evaporation. The action of the electric field on the system containing deformed particles of the isolated phase led to the formation of thin fibers. This structure was optimal for creating biodegradable materials in order to cultivate tissue cells of living organisms. Thin fibers ensured that cells were bound to the polymer scaffold; thick ones, that they could proliferate (multiply and grow) [7, 8].

*The work was sponsored by the RF Ministry of Education and Science under the auspices of base funding for a thematic project.*

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