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MACROMOLECULAR COMPOUNDS AND POLYMERIC MATERIALS

Study of a Possibility of Applying the Films of the Silk Fibroin and Its Mixtures with Synthetic Polymers for Creating the Materials of Contact Lenses

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Abstract—Light and gas permeability, antimicrobic and physico-mechanical properties are studied of the films from the mixtures of fibroin with the synthetic polymers.

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At present more than 85 million people in the world use contact lenses for the correction of sight [1]. The material of contact lenses should satisfy a large set of requirements: the optical transmission, biological inertness, chemical and mechanical stability, wetting ability, strength, elasticity, increased oxygen permeability [2].

Two basic categories of the contact lenses are distinguished: the rigid gas-permeable and soft lenses on the basis of the low cross-linked hydrogels. The materials of rigid lenses are predominantly hydrophobic and can include silicon and fluorine, and methyl methacrylate as the monomers. The contemporary rigid materials of contact lenses is highly oxygen-permeabe and of low adhesion to the bacterial cells [3], but are, as a rule, badly wet with water and have hydration ability not more than 45%. Hydrophilic hydrogel materials consist, for example, of poly -2-hydroxyethylmethacrylate or contain methacrylic acid [1, 2, 4]; known also the use of polyvinyl alcohol [5]. Such materials are swelling in physiological solution to 80% and more and passing well the oxygen dissolved in the water. Their main disadvantages are low strength, tendency toward the deposit of protein and growing of bacteria [3, 6, 7].

For preparing the more qualitative contact lenses are carried out permanent studies of new materials with the high oxygen permeability. The materials with the high oxygen permeability currently used include biopolymers or their derivatives (for example, cellulose acetobutyrate). It is known [8, 9] that the films from the biopolymer of silk fibroin are characterized by the high oxygen permeability, which at 25°C reaches (90–95) × 10^{-11} cm^2 of O_2 s^{-1} mm^{-1} Hg (for the comparison the contemporary fluorosilicone materials of rigid contact lenses have the oxygen permeability index at the level (60–64) × 10^{-11} cm^2 of O_2 s^{-1} mm^{-1} Hg [10]). Fibroin is compatible with the human organism and is used for biotechnological and biomedical purposes [11−13], and high the air permeability of materials on the basis of this polymer makes it promising for creating the contact lenses.

The fibroin films can be obtained from dialyzed water-salt solutions, solutions in hexafluorineisopropanol or *N*-methylmorpholin-*N*-oxide [14, 15]. The fibroin films are characterized by micro-phase separation and contain together with the amorphous phases the phases organized in the form of α-spiral structure (silk I). The equilibrium degree of swelling fibroin films in water in a neutral medium reaches 60%. The treatment of these films, for example, by water-alcohol solutions makes them not dissolved due to increase in the degree of crystallinity and appearance of a crystalline β-plicated supramolecular structure (silk II). However, with an increase in the degree of crystallinity the oxygen permeability of the fibroin films regularly decreases [by 8], the film become more hydrophobic and more fragile.

For the purpose of regulation of the supramolecular structure of the fibroin films and thus of their solubility, hydration ability and physico-mechanical properties it is possible to mix it up with other film-forming polymers in solution. Taking into account the assumed application of films in ophthalomology, their biological compatability and nontoxicity is the criterion of the selection of synthetic polymers for the mixing with the fibroin.

In this work are studied mixtures of fibroin with the polymethylmethacrylate, poly-3-hydroxybutyric acid, poly-L-lactic acid and polyvinyl alcohol. In [16, 17] was shown that in some ratio the fibroin is compatible in solutions and in films with the polyvinyl alcohol and the poly-3-hydroxybutyric acid. The purpose of the work is a study of the influence of synthetic component on the permeability to gas, the light transmission, the bacteriostatic and physico-mechanical properties of the mixed films.

EXPERIMENTAL

For study was used the fibroin of the *Bombyx mori* silk in the form of short fibers, washed off from the fatty, wax and mineral substances; synthetic polymers polymethylmethacrylate with average molecular weight M_w=120 000; poly-3- hydroxybutyric acid, M_w = 45 000; the poly-L-lactic acid M_w = 9000; polyvinyl alcohol, M_w = 41 000 from Aldrich. The films were obtained from the common solvent hexafluoroisopropanol with the boiling point 58°C from Merck-Schuchardt. Fibroin first was dissolved in 6 M aqueous solution of lithium bromide at 60°C with the subsequent dialysis through the semipermeable membrane. The dialyzed solution of fibroin was dried at room temperature to form film and then evacuated at 40°C for 4–6 h. Then a weighted sample of the obtained fibroin film was dissolved in hexafluoroisopropanol at 25°C to the necessary concentration. Synthetic polymers were dissolved in hexafluoroisopropanol at room temperature with stirring to obtain a transparent solution of the required concentration. Mixing the equiconcentrated solutions of polymers in the calculated mass ratio of the components was carried out for 1 h with stirring. The obtained transparent solutions were filtered on the Buechner funnel. Films were obtained by putting solutions on the glass plates with the subsequent solvent elimination by evaporation at room temperature. The thickness of films was 40–50 μ.

The hydration ability of films was defined from the increase in weight of the sample swollen in physiological solution in the equilibrium state. Light transparency of films was characterized as a degree of transmission by the films of light at the wavelength 300–700 nm, which was measured on the spectrophotometer Konica Minolta CM 3600d. The relative of the films permeability to gas was determined on a gas analyzer Ametek from the amount of helium passed for a fixed time interval at the equal conditions through the studied film samples. The physico-mechanical properties of films were determined on a tensile testing machine Instron 1122 according to DIN EN ISO 527-3. The determination of rigidity modulus in the regime creep-elastic restoring was performed on an automatic deformation relaxometer designed in the Material Strength Department of the St. Petersburg State University of Technology and Design.

The rate of microorganisms multiplication on the films was determined by the calculation of the number of bacteria *Staphylococcus aureus*, which were grown from the strains in nutrient medium. 2 ml of the bacterial suspension containing about 10^5 bacteria was applied to the surface of sample under investigation. After putting, the sample was incubated for 24 h at 37°C with the subsequent calculation of the number of cells on the surface using a microscope with the ocular reticulated micrometer in 20 fields of sight.

One of the most important characteristics of the material of contact lenses allowing to assign them to a certain category is their capability toward hydration. In Table 1 are given the values of the equilibrium

Table 1. Equilibrium degree of swelling of the films from the mixtures of fibroin with synthetic polymers

Polymer	Degree of swelling films, wt %, at the content of synthetic polymer, wt %				
	0	10	20	30	50
Polyvinyl alcohol	59	65	80	87	98
Polymethylmethacrylate	59	45	40	30	21
Poly-3-hydroxybutyric acid	59	40	31	21	11
Poly-L-lactic acid	59	44	36	24	15

Table 2. Light transmission by the films from the mixtures of fibroin with the synthetic polymers

Content of polymer in film, wt %	Light transmission, %, at the wavelength, nm						
	UV range			visible spectrum			
	360	370	380	400	500	600	700
Fibroin 100%							
0	90.6	90.1	89.6	88.8	87.9	87.8	87.9
Polymethylmethacrylate							
10	87.6	86.8	86.2	85.4	85.3	86.4	87.1
20	90.6	90.0	89.6	88.8	88.0	88.0	88.0
30	90.8	90.2	89.7	89.0	88.2	88.1	88.2
70	89.1	88.8	88.5	88.4	88.3	88.5	88.6
80	86.8	86.6	86.3	86.3	87.0	87.7	88.2
90	86.9	86.8	86.8	87.0	87.7	88.2	88.5
100	87.9	88.0	88.0	88.0	88.3	88.6	88.7
Poly-3-hydroxybutyric acid							
5	91.2	90.5	89.9	89.1	88.0	88.0	87.9
10	91.2	90.4	89.8	89.2	88.2	88.0	88.0
15	90.5	89.7	89.1	88.4	87.6	87.5	87.6
85	83.4	83.1	83.0	83.1	84.6	85.8	86.5
90	83.8	83.6	83.5	83.5	84.8	85.8	86.3
95	85.1	85.0	85.0	85.0	86.0	86.7	87.0
100	84.0	84.0	84.0	84.0	84.9	85.6	86.0
Poly-L-lactic acid							
5	90.1	89.3	88.5	87.7	86.0	86.0	86.3
10	90.1	89.4	88.7	87.8	86.3	86.0	85.8
15	89.6	88.8	88.1	87.3	86.0	86.0	86.2
85	81.2	80.4	80.0	80.1	82.5	84.6	86.1
90	79.8	79.4	79.3	79.4	82.2	84.2	85.9
95	86.7	86.7	86.7	86.9	88.1	88.2	89.2
100	89.1	89.0	89.0	89.0	89.2	89.4	89.5

degree of swelling the studied films from the mixtures of fibroin with the synthetic components. Analyzing data in Table 1, one can conclude that the films from the mixtures of fibroin with the more hydrophobic polymers (polymethylmethacrylate, poly-3-hydroxybutyric acid, poly-L-lactic acid) can present interest for the study as the material for the rigid contact lenses, the films from the mixtures with the polyvinyl alcohol as the material for the soft contact lenses capable of strong swelling. The properties requirements for these films are different. The materials of rigid contact lenses first of all should be light and gas permeable, while for the soft hydrogel material is important strength characteristics.

Mixtures of fibroin with hydrophobic components. In Table 2 are listed the data on the light transmission in the visible and ultraviolet areas by the films of fibroin and its mixtures with synthetic polymers. Analyzing data in Table 2 with respect to capability of the films of the light transmission in UV and visible region, it is possible to conclude the following. The fibroin films have high transmitting ability reaching 87.8–90.6% toward the light with the wavelength 360–700 nm. At the same level remains transparency of the films of the mixtures when the content of polymethylmethacrylate in the mixture is 20–30 wt %. Attention is drawn to the fact that at the content in the mixture of poly-3-hydroxybutyric acid 5–15 wt % the film transparency increases to 88–91.5%. As has been shown for the films of fibroin with poly-3-hydroxybutyric acid [16], at low content of synthetic component is formed the most homogeneous structure of mixtures with the insignificant micro-phase separation. At the growth of the content of synthetic polymer, the mixture becomes heterogeneous with the macrophase separation. Actually, films become less transparent, their ability of light transmitting falls. This is most noticeable at the mixing fibroin with the poly-L-lactic acid, the minimum transparent ability occurs at the content of poly-L-lactic acid 85–95 wt %.

Thus, the films from the mixtures of fibroin with the polymethylmethacrylate and poly-3-hydroxybutyric acid (content of synthetic component 20–30 and 5–15 wt % respectively) are advantageous owing to their high transparency.

.Adding to the fibroin of hydrophobic component decreases the rate of the development of bacteria on them, as can be judged from the data of Table 3.

Given in Table 3 data make it possible to calculate that on the film of the fibroin through 1 day of thermostating a quantity of bacteria increased 83.6 ($10^{1.8}$) time, and

Table 3. Rate of grow of the bacteria *Staphylococcus aureus* on the samples of the fibroin films

Film composition	Amount of bacteria		
	applied, n_0	after 24 h, n	grow rate $10^{n}0/n$
Fibroin	2.32×10^5	1.94×10^7	$10^{1.8}$
Fibroin–poly-3-hydroxybutyric acid (90:10)	2.32×10^5	Less than 20	$10^{-4.2}$

on the film of the mixture of fibroin with the poly-3-hydroxybutyric acid (ratio of components 0.9 : 0.1) decreased 11 600 ($10^{4.2}$) times, thus, this film possesses antimicrobic properties.

Since the fibroin films with the hydrophobic polymers have low water content, they badly permeate the oxygen dissolved in water. Therefore it is of interest to study the influence of the additions of synthetic polymer on the permeability to gas of the films from the mixtures of fibroin in the dry state. In Fig. 1 are given the dependences of the relative permeability to gas of the fibroin films on the content in them of synthetic component.

It is seen that with an increase in the content of synthetic component the permeability to gas of the investigated films from the mixtures decreases. The change in the permeability to gas of polymer films

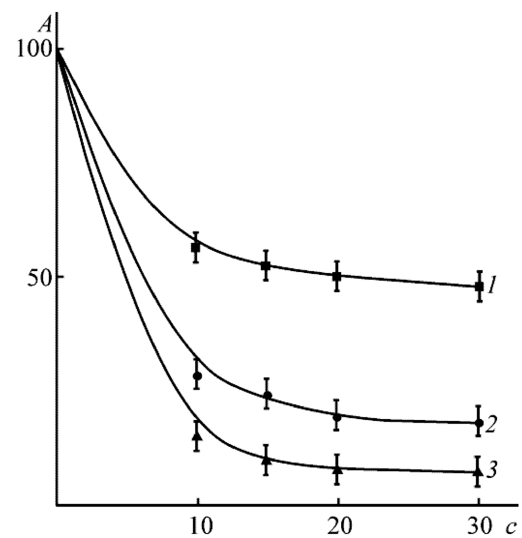

Fig. 1. Dependence of the relative gas permeability *A* (%) of the films from the mixtures of fibroin on the content of synthetic polymer *c* (wt %). (*1*) polymethylmethacrylate, (*2*) poly-3-hydroxybutyric acid, (*3*) poly-L-lactic acid.

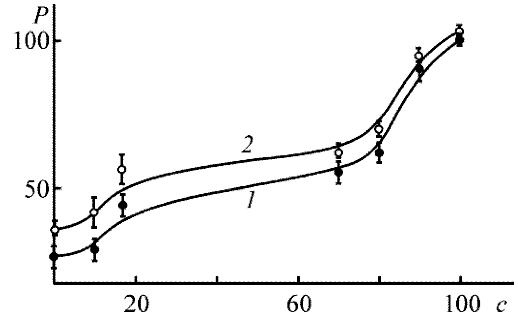

Fig. 2. Dependence of the tensile stress *P* (MPa) of the films from fibroin–PV mixtures on the PV content *c* (wt %). Film: (*1*) not processed, (*2*) processed with ethanol; the same for Fig. 3–5.

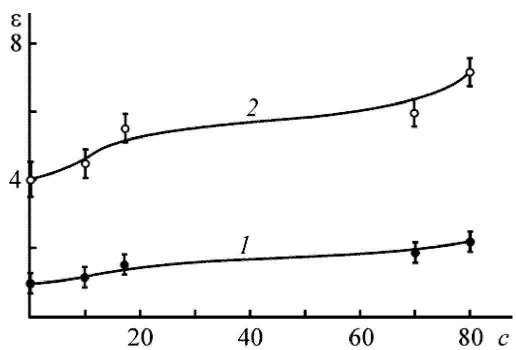

Fig. 3. Dependence of break elongation ε (%) of the films from the fibroin–PV mixtures on the PV content *c* (wt %).

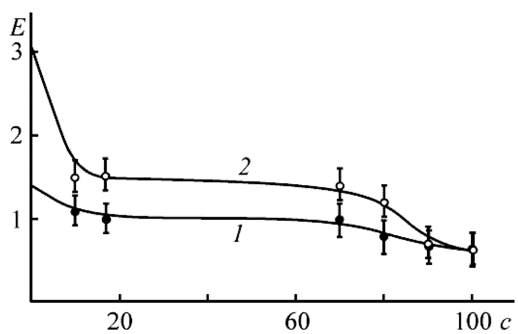

Fig. 4. Dependence of the rigidity modulus *E* (GPa) of the films from fibroin–PV mixtures on the PV content *c* (wt %).

known [8] to be caused by the structure factors (change in the homogeneity, dimensions of phases and interphase free space, degree of crystallinity) or by a change in the moisture content, in this case proportionally change the transport properties of films with respect to the components dissolved in the water, including gases. It is possible to assume that reduction in the permeability to gas at the mixing fibroin with synthetic components is caused by both these factors. It is notable also that the smallest degree of reduction in the permeability to gas occurs at mixing fibroin with polymethylmethacrylate.

Mixture of fibroin with the hydrophilic polyvinyl alcohol. Hydrophilic material that adsorbs a large quantity of water is capable to pass well the oxygen dissolved in water [10]. However, for the hydrophilic materials of contact lenses low strength and multiplication of bacteria are the main disadvantages. For an increase in the strength of the films from the mixtures of fibroin with polyvinyl alcohol (PV) are expedient to regulate the degree of the fibroin crystallinity in the mixture, for example, by the treatment of films with alcohol [8, 15]. This will make it possible to regulate the degree of swelling the films with a high content of synthetic component. Taking into account that the mixtures are investigated as the material of medical designation, ethanol is selected as alcohol.

The dependences of the physico-mechanical properties of the films from the mixtures of fibroin with the polyvinyl alcohol before and after working up with ethanol on the ratio of components in the mixture are given in Figs. 2–4.

It is seen that the addition of PV to the fibroin leads to an increase both in the strengths (tensile stress) and in the elasticity (increase in the break elongation and reduction in the rigidity modulus) of films in the air-dry state. The dependences on the mixture composition is of nonlinear nature, and it is possible to separate three regions of the components ratio. The most effective relative increase in the films strength and elasticity is observed at the content of polyvinyl alcohol below 20 wt %. In the range of 20–80 wt % of polyvinyl alcohol the growth in strength and elasticity is retarded, while at further increase in the fraction of polyvinyl alcohol it again increases rapidly. This effect can be explained by the fact that at the PV content $c \leq 20$ wt % and $c \geq 80$ wt % the mixture is most homogeneous [17].

At the treatment of the films from mixtures with ethanol the values of tensile stress and elongation of films substantially grow. The observed effect is explained by the appearance of crystalline structural modification of fibroin in the films as a result of their working up with ethanol [8, 16].

The every day use of the material of contact lenses requires their permanent existence in the swollen state. In this connection we carried out the study of the physico-mechanical properties of the films prepared from the mixtures, including those processed with ethanol, in the state of equilibrium swollen in physiological solution. In Fig. 5 are represented the dependences of the break

Table 4. Rate of grow of the bacteria *Staphylococcus aureus* on the samples of the fibroin–polyvinyl alcohol films

Film composition	Number of bacteria		
	applied n_0	after 24 h n	grow rate $10^{n}0^{/n}$
Fibroin	2.32×10^{5}	1.94×10^{7}	$10^{1.8}$
Fibroin – polyvinyl alcohol (90: 10)	2.32×10^{5}	2.3×10^{6}	10^{1}
Fibroin - polyvinyl alcohol (90:10) + 0.5 мас% Ag	2.32×10^{5}	Less than 20	$10^{-4.2}$

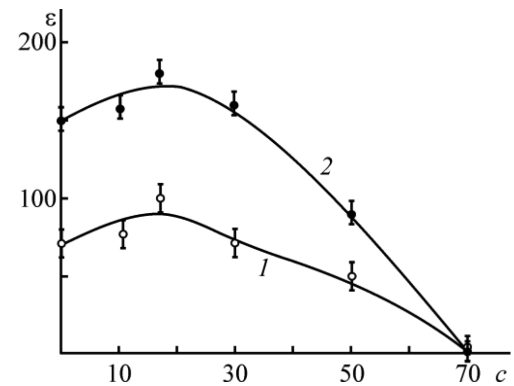

Fig. 5. Dependence of break elongation ε (%) of equilibrium swollen in physiological solution films from fibroin–PV mixtures on the PV content *c* (wt %).

elongation of the swollen films on the composition of polymer mixture.

It is evident that the nature of the dependence of the break elongation on the composition of mixture for the swollen films differs significantly from the same for the films in the equilibrium dry state (Fig. 3). Since polyvinyl alcohol is water-soluble, the degree of swelling the films substantially increases at a high content of this polymer in the film, and grow the indices of break elongation in the comparison with those given in Fig. 3 for the air-dry films. The processing of films with ethanol, leading to structural changes in the fibroin, contributes to the decrease of the degree of their swelling and to reduction in the indices of break elongation (Fig.5, curves *1, 2*). Optimum is the content of PV in the mix-ture 15–20 wt %, which makes it possible to increase the strength and elasticity of films both in the dry and in the swollen state to a sufficient degree.

The high hydrophilic nature of the films from the fibroin–polyvinyl alcohol mixtures can contribute to adhesion and to grow of bacteria on their surface. Therefore it is important to admit to the film bacteriostatic propertie s, for example, by introduction of silver. The introduction of the particles of the metal into the polymeric matrices is achievable by various techniques, one of the known is reduction of metal ions taken as the solutions of a suitable salt [18, 19]. Therefore we studied the possibility of admitting antibacterial properties to the films from the mixtures of fibroin with the polyvinyl alcohol by the introduction of a small quantity of silver by reduction. In Table 4 are given the results of investigation of the rate of bacteria grow on the films. It is evident that putting 0.5 wt % silver on the hydrophilic film from the mixture fibroin-polyvinyl alcohol admits to film the antimicrobic properties: through 1 day of thermostating a quantity of bacteria on it decreases $10^{4.2}$ times.

CONCLUSIONS

1. It is shown that the mixtures of fibroin with the polymethylmethacrylate and by poly-3-hydroxybutyric acid retain high light and gas permeability at the content of synthetic component 10–20 wt % and can be used as the material for the rigid contact lenses. The hydrophobicity of the fibroin films and their antibacterial properties increase at the introduction of synthetic component.

2. The mixture of fibroin with polyvinyl alcohol is promising as a hydrophilic material for soft contact lenses. The films containing up to 20 wt % of polyvinyl alcohol show the optimum combination of strength and elasticity. The treatment of the films with ethanol makes it possible to regulate the degree of swelling and the physico-mechanical properties of films over a wide range. Introduction into the film from the fibroin–polyvinyl alcohol mixture of a small (0.5 wt %) fraction of silver admits to it antibacterial properties.

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REFERENCES

1. Willcox, M.D.P., Harmis, N., Cowell, B.A. et al., *Biomaterials*, 2001, vol. 22, no. 24, pp. 3235–3247.

2. Nicolson, P.C. and Vogt, J., *Biomaterials*, 2001, vol. 22, no. 24, pp. 3273–3283.
3. Alava, J., Garagorri, N., Briz, N., and Mendikute, J., *J. Mater. Sci.: Mater. Med*., 2005, vol. 16, no. 4, pp. 313–317.
4. Rander, K.J., Quintama, R.P., and Chowhan, M.A., *Encyclopedia of Pharmaceutical Technology,* Swarbrick, J. and Boylan, J.C., Eds., New-York: Dekker Inc., 1993, pp. 361–402.
5. Kuriaki, M., Nakamura, K., Mizutani, J., and Kobunshi, Ronbunshu., 1989, vol. 45, no. 11, pp. 739–743.
6. Fornasiero, F., Prausnitz, J.M., and Radke, S.J., *J. Membrane Sci*., 2006, vol. 275, nos. 1–2, pp. 229–243.
7. Chin-Hu, Ho, Hlady, V., *Biomaterials*, 1995, vol. 16, no. 6, pp. 479–482.
8. Minoura, N., Tsukada, M., Nagura, M., *Polymer*, 1990, vol. 31, no. 2, pp. 265–269.
9. Minoura, N., Tsukada, M., and Nagura, M., *Biomaterials*, 1990, vol. 11, no. 6, pp. 430–434.
10. Lloyd, A.W., Faragher, R.G.A., and Denyer, S.P., *Biomaterials*, 2001, vol. 22, no. 8, pp. 769–785.
11. Asakura, T., Kitaguchi, M., and Demura, M. et al., *J. Appl. Polym. Sci.,* 1992, vol. 46, no. 1, pp. 49–53.
12. Minoura, N., Tsukada, M., and Nagura, M., *Biomaterials*, 1990, vol. 11, no. 3, pp. 430–435.
13. Minoura, N., Tsukada, M., and Nagura, M., *Polymer*., 1990, vol. 31, no. 3, pp. 265–269.
14. Sashina, E.S., Bochek, A.M., Novoselov, N.P., and Kirichenko, D.A., *Zh. Prikl. Khim.*, 2006, vol. 79, no. 6, pp. 881–888.
15. Sashina, E.S., Novoselov, N.P., Vorbach, D., and Meister, F., *Vysokomol. Soedin.*., 2005, vol. 47A, no. 10, pp. 1832–1840.
16. Sashina, E.S., Novoselov, N.P., and Khainemann, K., *Zh. Prikl. Khim.*, 2005, vol. 78, no. 1, pp. 155–160.
17. Sashina, E.S., Novoselov, N.P., Vnuchkin, A.V., and Golubikhin, A.Yu., *Zh. Prikl. Khim*., 2007, vol. 80, no. 3, pp. 468–472.
18. Gubin, S.P., Koksharov, Yu.A., Khomutov, G.B., and Yurkov, G.Yu., *Uspekhi Khimii*, 2005, vol. 74, no. 6, pp. 539–574.
19. Tret'yakov, Yu.D., Lukashin, A.V., and Eliseev, A.A., *Uspekhi Khimii*, 2004, vol. 73, no. 9, pp. 974–998.