# Desorption of Human Serum Albumin and Human Fibrinogen from the Poly(3-hydroxybutyrate) Surface

**A. L. IORDANSKII, E. V. DMITRIEV, P. P. KAMAEV, G. E. ZAIKOV**

Institute of Chemical Physics, RAS. 4 Kosygin str. Moscow, 117334, B-334, Russia

*Received 12 June 1998; accepted 12 January 1999*

**ABSTRACT:** The protein desorption of human serum albumin and human fibrinogen from the surface of poly(3-hydroxybutyrate) films was studied using ATR–FTIR spectroscopy. The diffusion model for reversible and irreversible sandwiched layers was confirmed. The reversible ratio (ratio of reversible adsorbed concentration to irreversible adsorbed concentration as a function of time allows for a suggestion as to a kinetic model of the initial stage of thromb formation. The parameters of adsorption/desorption for both proteins are compared. The reversible ratio of plasma protein adsorption is proposed as a quantitative criterion of thromb-resistance behavior for polymers in biomedicine; namely, controlled drug release vehicles, artificial vessels, magistrals, reservoirs for blood storage, and surgical threads, especially. The mechanism of interaction of protein molecules with poly(3hydroxybutyrate (PHB) macromolecules is discussed. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 595–600, 1999

**Key words:** HSA; HFb; protein; poly(3-hydroxybutyrate)

## INTRODUCTION

Poly(3-hydroxybutyrate) [PHB] and its derivatives have found a special range of biomedical and friendly environmental applications because of their combination of biocompatibility[1,2] and sorption-diffusion properties[3,4] coupled with their biodegradability.[5] The base materials of PHB have been in use as biodegradable plastics[6] for a few years. Many researchers wordwide have investigated how biocompatible materials should be defined, both *in vitro* and *in vivo.* However there are few data that describe the behavior of biodegradable materials in contact with biologically active media; namely, with plasma and blood.

Polymer–blood interaction leads to changes of physicochemical and biochemical properties of polymer materials under service conditions. Protein adsorption rate, quantitative composition of adsorbed protein layer, spatial architecture, and structure of macromolecules on the polymer surfaces define the intensity of the subsequent adhesion and aggregation of platelets or other blood cells.[7–9] This paper presents the first data on the desorption kinetics of typical plasma proteins to model buffer solution from the surface of PHB films.

## EXPERIMENTAL

Adsorption of human serum albumin (HSA) and human fibrinogen (HFb) is performed using a two-plane cell. Proteins were purchased from Sigma, and other reagents were commercially available. The flow of protein solutions (phosphate buffer pH = 7.1, $T$ = 36.5°C, and $I_{NaCl}$ = 0.1) circulated between polymer plane surfaces inserted in the adsorption cell.[10] Distance between planes was 1.1 cm to prevent turbidity. Protein concentrations in phosphate buffer solutions were from 5 to 50 mg/cm$^3$ for HSA and from

*Correspondence to:* G. E. Zaikov.

Journal of Applied Polymer Science, Vol. 74, 595–600 (1999)
 CCC 0021-8995/99/030595-06

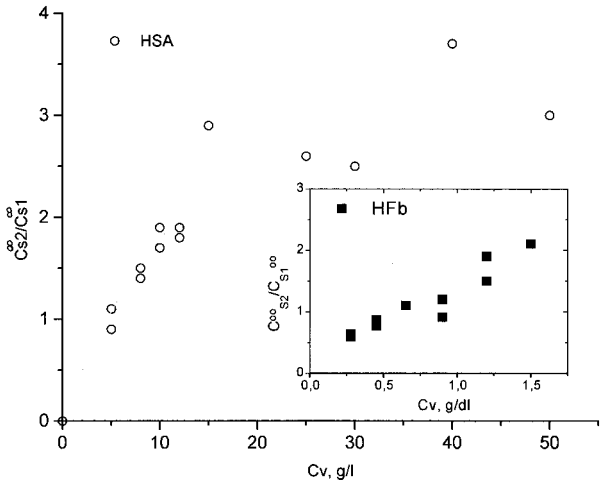


**Figure 1** Adsorption isotherms for HSA and HFb from buffer solution on PHB surface.

0.28 to 1.5 mg/cm$^3$ for HFb. The rates of flow through the experimental cell were between 0.1 and 0.40 cm/s. The surface concentrations of proteins were determined by ATR FTIR spectroscopy (Brucker IFS48 Fourier transform spectrometer) after drying in a vacuum camera for 24 h at room temperature.

Bacterial PHB samples were obtained from the Biochemical Society of Russia, Institute of Biochemistry RAS (Moscow, Russia). After preliminary filtration, molecular weight characterization of the samples was performed by viscosity measurements in chloroform.[11] The specimens of PHB ($M_w$ = 570,000) with crystallinity ($\alpha$ = 68%) were produced by two-stage microbial synthesis. The $M_w$ of PHB was determined by the following relation: $[\eta] = 7.7810^{-5} M_w^{0.82}$; where $[\eta]$ is the intrinsic viscosity in chloroform at 30°C.

## Modeling

### *Diffusive-Kinetic Model*

In general, the adsorption process involves the transport of protein molecules from the model solution or the liquid biological media (plasma, blood) to the polymer surface, structural rearrangements in the protein macromolecules near or on the polymer surface up to surfacial denaturation, and the proper adsorption of the proteins.[12,13]

The nonpenetrable (moderately) hydrophobic polymer surface is considered an interface dividing liquid biological medium and polymer material. It is matter of common experience,[14] that near the polymer surface, there is a layer of solvent (buffer), where protein transport is preferably determined by diffusion. Thickness of the diffusion layer is related to the Reynolds number, kinematic viscosity, and diffusion coefficient of a protein.[15]

Interpretation of the adsorption results was made without considering the hydrodynamic conditions that could lead to mistaken conclusions about the proper rate of adsorption. Because of this, it should be noted that the time in which a protein concentration on a hydrophobic surface attains a maximal limiting value varies from a few seconds in the arterial blood flow[16] to several minutes in the venous flow,[16] and even to about an hour in the model solution experiments.[17]

The proposed transport-kinetic model of protein adsorption includes: (1) the transport stage in the boundary diffusional layer described by the following set of differential equations

$$D_p \frac{\partial^2 C_v}{\partial x^2} = \frac{\partial C_v}{\partial t} \quad \text{at} \quad t > 0,\ 0 < x < \delta_D \qquad (1)$$

with boundary conditions:

$$C_v = C_V^0 \quad \text{at} \quad x = \delta_D \qquad (2)$$

on the liquid–liquid boundary (diffusional layer/stirred bulk volume) and

$$D_p dC_v/dx = dCs/dt \quad \text{at} \quad x = 0 \qquad (3)$$

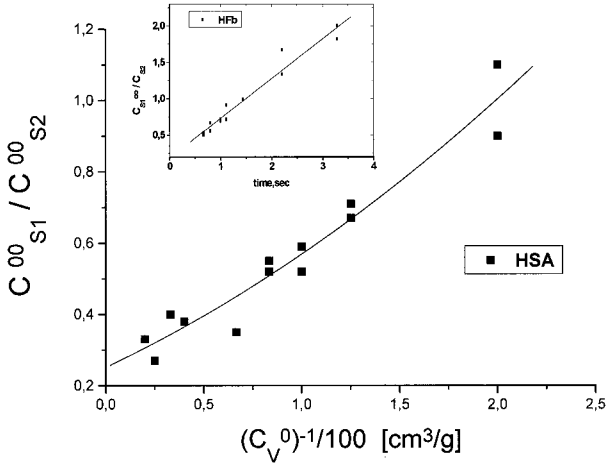


**Figure 2** Replotting of adsorption isotherms for HSA and HFb.

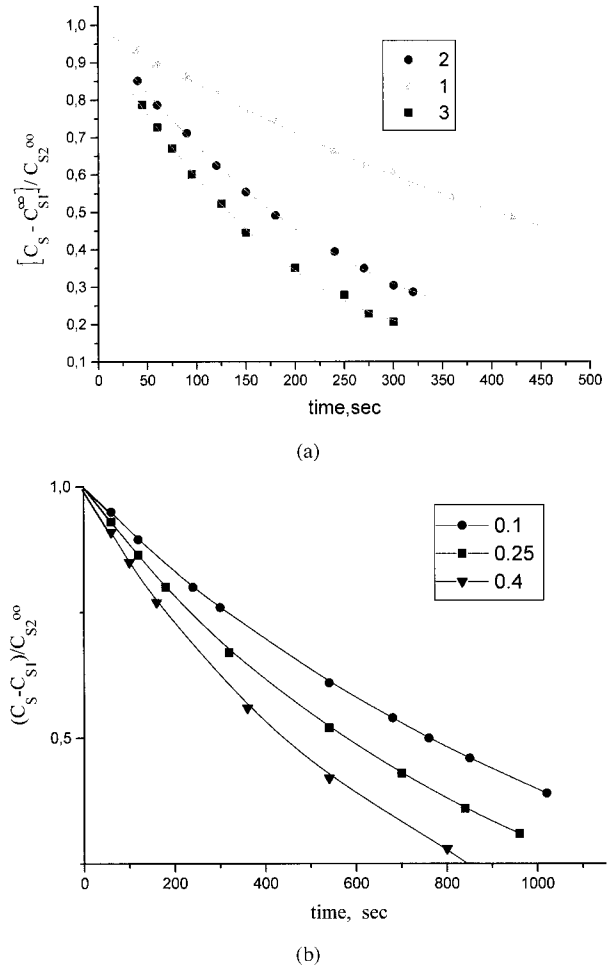


**Figure 3** Reversible ratio as function of time for HSA (a) and HFB (b). Desorption at different rates of buffer flow: 0.1 cm/s (3), 0.25 cm/s (2), 0.4 cm/s (1).

on the polymer/liquid interface (polymer surface/diffusional layer). Here, $C_v$ and $C_s$ are volume protein concentrations in the diffusional layer with thickness $\delta_D$, and on the surface, respectively, $D_p$ is diffusion coefficient of protein molecules in a protein solution, $C_V^0$ is constant volume concentration, and $x$ and $t$ are coordinate and the time of diffusion, respectively; (2) formation of the irreversible fraction of an adsorbed protein corresponds with following equation:

$$C_{S1}/dt = k_1 C_v (C_{S1}^{\infty} - C_{S1}) \qquad (4)$$

where $C_{S1}$ is the surface protein concentration for the irreversibly adsorbed molecules and the molecules undergoing conformational transformations, respectively and, $k_1$ is the constant of irreversible adsorption; (3) formation of the reversible protein layer

$$dC_{S2}/dt = k_{21} C_v (NC_{S1} - C_{S2}) - k_{22} C_{S2} \qquad (5)$$

where $C_{S2}$ is the concentration of reversibly adsorbed protein, $k_{21}$ and $k_{22}$ are the corresponding constants of adsorption and desorption, and $N$ is the average number of adsorptive sites created by irreversible adsorption. At any moment, the total surface concentration of protein is the sum of all protein populations: the reversible and irreversible fractions $C_S = C_{S1} + C_{S2}$.

### *Protein Desorption*

In the case of protein desorption into buffer solution, the boundary condition (2) can be rearranged to the simple equation

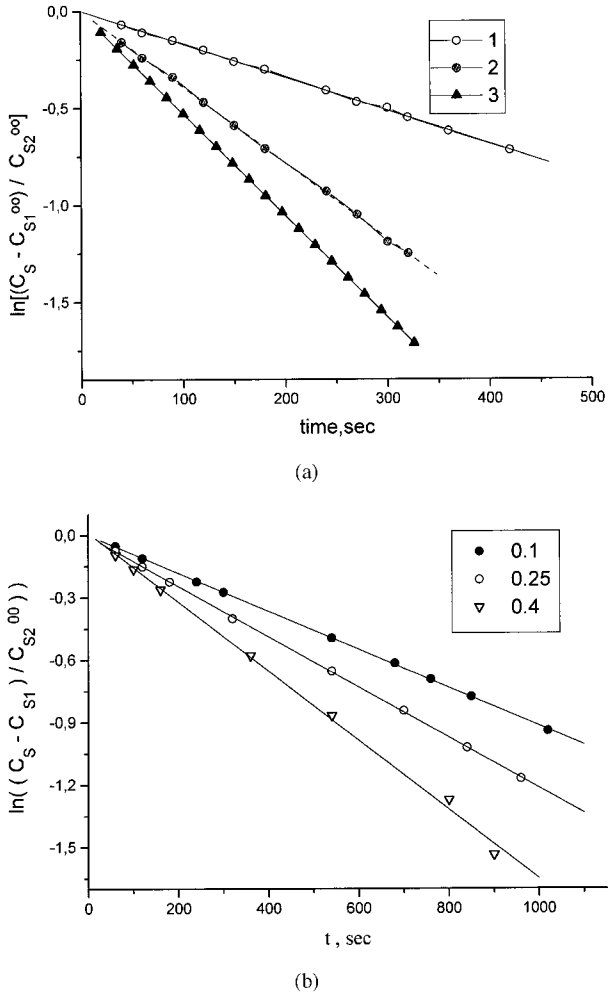


**Figure 4** Replotting of desorption profiles for HSA (a), and HFb (b) in semilogarithmic coordinates. The rates of buffer flow are 0.1 cm/s (1), 0.25 cm/s (2), 0.4 cm/s (3).

**Table I Parameters of Desorption from PHB Surface for HSA and HFb**

| Protein | $k_{21}$ $cm^3/g*s$ | $k_{22}$ $10^3$ $s^{-1}$ | $N$ | $C^{\infty}_{S1}$ $10^6$ $g/cm^2$ | $\frac{C^{\infty}_{S2}}{NC_{S1}}$ | $\frac{k_{21}C^0_{V_a}}{k_{22}}$ |
|---|---|---|---|---|---|---|
| HSA | 1.05 | 16.0 | 4.4 | 1.15 | 0.747 | 2.35 |
| HFb | 0.762 | 2.83 | 6.8 | 0.34 | 0.447 | 0.81 |

[a] The values for $C^0_V$ are take as physiological concentration of proteins in blood.[14]

$$C_V = \bar{C}_V \approx 0 \quad \text{at} \quad x = \delta_D \tag{6}$$

where $\bar{C}_V$ is averaged volume concentration of protein.

For $C_{S2}$, solution of the system of the diffusion eqs. (1,3,6) and differential adsorption eqs. (4,5) in the time range $t > \delta^2_D/D_p$ is adequately described by the equation

$$C_{S2} = \frac{Nk_{21}C^o_V C^{\infty}_{S1}}{k_{21}C^o_V + k_{22}} \exp\left(-\frac{k_{22}k_D t}{k_{22} + k_D}\right) \tag{7}$$

where $k_D = 2D_P/\delta^2_D$, and the other symbols are the same as in eq. (6).[13]

## RESULTS AND DISCUSSION

Results of our adsorption/desorption experiments on the interaction of proteins with a number of hydrophobic polymers have been reported elsewhere.[19–22] Both kinetic data[18] and structural methods (transmission electron microscopy, ESR)[20,21] have provided evidence of the existence of reversible and irreversible protein adsorption on the surfaces of polyethylene,[23] polysiloxane,[19] and segmented polyetherurethanes.[24] A similar situation is seen for the PHB films.

Figures 1 and 2 show the HSA and Hfb adsorption isotherms obtained at 36.5°C under model conditions (phosphate buffer, $I_{\mathrm{NaCl}} = 0.1$) on PHB surfaces. The relative surface concentrations of reversibly adsorbed protein mode ($C^{\infty}_{S2}/C^{\infty}_{S1}$) increases with volume concentration of the proteins and depend markedly upon the nature of the protein involved. Here and henceforeward $C^{\infty}_{S2}$ ($= C^{\infty}_S - C^{\infty}_{S1}$), $C^{\infty}_{S1}$, and $C^{\infty}_S$ are reversible, irreversible, and total surface protein concentrations.

Replotting of the isotherms in a framework of suggested transport-kinetic model, $C^{\infty}_{S1}/C^{\infty}_{S2} - 1/C^o_v$, enables the parameters of adsorption to be determined. The linearities for HSA and HFb are presented in Figures 1b and 2b, respectively.

In Figures 3 and 4, all curves describe desorption experiment (points) for the same systems PHB–HSA and PHB–HFb, respectively. These curves have no special points, such as inflections, or kness. However, the rates of desorption and the time it takes to establish the limiting values of surface concentration $C^{\infty}_{S1}$ depends upon the hydrodynamic conditions, specifically, on the rate of the steady-state flow washing the polymer surface in an adsorption cell, $v$, cm/s (Table II).

For the different values of the buffer flux velocities, $v$, the solution of eq. (7) in semilogarithmic presentation, $\ln[(C_S - C^{\infty}_{S1})/C^{\infty}_{S1}] - t$, enables the estimation of the desorption rate constant values ($k_{\mathrm{des}}$) for HSA and Hfb, respectively. Corresponding data are presented in Table II.

The thickness of the diffusional layer near the polymer surface is a function of the Reynolds number and, thereby, the function of $v$:

$$\delta D = 2.40R(\mathrm{Re})^{-1/2}(D_P/\nu)^{1/3} \tag{8}$$

where Re is the Reynolds number equal to $4abv/[(a + b)\nu]$ for rectangular experimental cell, $\nu$ is kinematic viscosity, $cm^2/s$, $R$, is distance between polymer surfaces in a cell, and $a$ and $b$ are geometrical sizes of an adsorption cell.[10] In Table I, the values of $\delta_D$ are also presented, and now we can calculate the diffusion constant $k_D = 2D_p/\delta^2_D$[13] for determination of the proper desorption constants $k_{22}$. This table summarizes the protein data where the values of ratio $k_{21}/k_{22}$ were obtained from the extrapolation of kinetic data in the above semilogarithmic forms for both HSA and HFb.

The comparison of desorption parameters presented in Table I enables us to make positive conclusions on the interaction of PHB surface with proteins under investigation. In the framework of the above diffusion-kinetic model, there are two important criteria: (1) the degree of shielding for conformationally changed and irreversibly adsorbed molecules of proteins, $C^{\infty}_{S2}/$

**Table II Transport and Kinetics Parameters for Desorption from PHB Surface**

| | $\delta_D$ $10^2$, cm | | $k_D$ $10^3$, $s^{-1}$ | | $k_{des}$ $10^3$, $s^{-1}$ | |
|---|---|---|---|---|---|---|
| $V_0$, cm/s | HSA | HFb | HSA | HFb | HSA | HFb |
| 0.40 | 1.25 | 0.86 | 7.7 | 5.4 | 5.3 | 1.8 |
| 0.25 | 1.58 | 1.41 | 4.8 | 2.0 | 3.9 | 1.2 |
| 0.10 | 2.50 | 1.72 | 1.9 | 1.3 | 1.7 | 0.92 |

$NC^{\infty}_{S1}$; and (2) the effective equilibrium constant of reversible adsorption, $k_{21}C^0_v/k_{22}$, that accounts for the affinity of the native molecules to the proteinated polymer surface.

Moreover, the value $N$ can be considered as an appraisal of acceptor capacity of the irreversibly adsorbed protein molecule.[19] In general, two factors—the conformational stretching (accessibility) on the nonphisiological surface and the chemical structure of protein molecules—affect the value $N$. The first factor depends upon the interaction of native protein globules with the polymer surface and is constant for the nature and morphology of the given polymer surface. For a given type of protein, the second factor is fixed and is inherent in the chemical structure of protein. Consequently, at comparison of adsorption parameters ($k_i$, $C_i$, and $N$) for the different proteins adsorbed on the PHB surfaces, the distinction for the values $N$ is determined by the nature of proteins. Actually, for an extended fibrillary molecule of HFb, the number of contacts among other macromolecules ($N = 6.8$) exceeds the number of contacts for the more compact globules of HSA ($N = 4.4$).

In the range of plasma concentrations, the reversible ratio, $C^{\infty}_{S2}/NC^{\infty}_{S1}$, for HSA (=0.75) is essentially superior to the same ratio for HFb (=0.45), which also supports negative action of the adsorbed fibrinogen on the thromb-resistance behavior of PHB. However, a greater affinity for HSA to the polymer surface, $k_{21}C^0_V/k_{22}$, enables us to treat PHB as the material with good tromboresistance properties.

Along with other hydrophobic polymers, the results above show that the total surface concentration of both HSA and HFb include an irreversibly proteinated layer, the macromolecules of which are incapable of exchanging with a bulk protein volume, and a reversibly adsorbed layer. In accordance with the proposed model, as the ratio $C_{S2}/NC_{S1}$ increases, the thromb-resistance of the PHB surface becomes higher. The reversible fraction of adsorbed protein screens the contacts between platelets and the conformational changed molecules of the irreversible layer. Only the latter are responsible for adhesion of platelets and other blood cells to the proteinated polymer surface.

Experimental values of the ratio of the reversibility ($C_{S2}/C_{S1}$) are maximal for the adsorption system HSA–PHB, as compared with the systems HFb–PHB, HSA–polyethylene HD, or egg albumin–polysulfoamides. We suggest that this reversible ratio of plasma protein adsorption can serve as a quantitative criterion of thromb-resistance behavior for polymers in biomedicine; namely, controlled drug release vehicles, artificial vessels, magistrals, reservoirs for blood storage, and surgical threads, especially.

This research was made possible in part by Award No. RN2-409 of the U.S. Civilian Research and Development Foundation (CRDF) and Grant of Russian Foundation for Basic Research No. 97-03-32156a.

## REFERENCES

1. Poirier, Y.; Nawrath, C.; Somerville, C. Biotechnology 1995, 13, 142.
2. Lafferty, R. M.; Korsatko, B.; Korsatko, W. Biotechnology 1988, 6, 135.
3. Miguel, O.; Fernandez-Berridi, M. J.; Iruin, J. J. J Appl Polym Sci 1997, 64, 1849.
4. Kuboto, M.; Nakano, M.; Juni, K. Chem Pharm Bull 1988, 36, 333–337.
5. Hocking, P. J.; Marchessault, R. H. Macromolecules 1996, 29, 2472.
6. Seebach, D.; Brunner, A.; Bachmann, B. M.; Hoffman, T.; Kuhnle, F. N. M.; Lengweiler, U. D. Biopolymers and Olgomers of (R)-3-Hydroxyalkanoic Acids. Contributions of Synthetic Organic Chemist; Ernst Schering Research Foundation: Berlin, 1995.
7. Bayer, R. E. Ann NY Acad Sci 1977, 283, 1736.
8. Ihleufeld, J. V.; Cooper, S. L. J Biomed Mat Res 1979, 13, 577.

9. Norde, W. Adv Coll Interface Sci 1986, 25, 267–340.
10. Polishchuk, A. Ya.; Iordanskii, A. L.; Zaikov, G. E. Dokl Akad Nauk SSSR 1983, 64, 1431 (in Russian).
11. Marchessault, R. H.; Okamura, K.; Su, C. J. Macromolecules 1970, 6, 735.
12. Beissinger, R. L.; Leonard, E. F. Artif Int Organs 1980, 3, 160.
13. Iordanskii, A. L.; Rudakova, T. E.; Zaikov, G. E. Interaction of Polymers with Bioactive and Corrosive Media; VSP: Utrecht - Tokyo, 1994; Chapter 5.
14. Lighfoot, E. N. Transport Phenomena and Living Systems; John Wiley & Sons; New York, 1974, 496.
15. Back, T. H. Math Bio Sci 1975, 27, 231–285.
16. Brash, J. L.; Lyman, D. J. in The Chemistry of Biosurfaces; Uarir, M. L., Ed., Marcel Dekker: New York, 1971; p 177.
17. Brynda, E.; Houska, M.; Pokorua, Z.; Cepalova, N. A.; Moiseev, Yu. V.; Kalal, J. J Bioeng 1978, 2, 411–418.
18. Iordanskii, A. L.; Polishchuk, A. Ya.; Zaikov, G. E. J Macromol Sci Rev Macromol Chem Phys 1983, C23, 33–60.
19. Zaikov, G. E.; Iordanskii, A. L.; Markin, V. S. Diffusion of Electrolytes in Polymers. New Concepts in Polymer Science; VSP: Utrecht - Tokyo, 1988; 290–314.
20. Iordanskii, A. L.; Ulanov, B. P.; Zimina, L. A.; Zaikov, G. E. Reps Russian Acad Sci 1979, 249, 480–487 (in Russian).
21. Zimina, L. A.; Iordanskii, A. L.; Polistchuk, A. Ya.; Grigorieva, G. L.; et al. Polym Sci 1984, 26 A, 1645–1652.
22. Polistshuk, A. Ya.; Iordanskii, A. L.; Zaikov, G. E. Composite Materials; Naukova Dumka: Kiev, 1985; 36–86 (in Russian).
23. Polishchuk, A. Ya.; Iordanskii, A. L.; Zaikov, G. E. Polymer Sci 1984, 26 A, 1068–1076.
24. Polishchuk, A. Ya.; Vladimirov, A. V.; Iordanskii, A. L.; et al. Polymer Sci 1985, 27, 1327–1334.