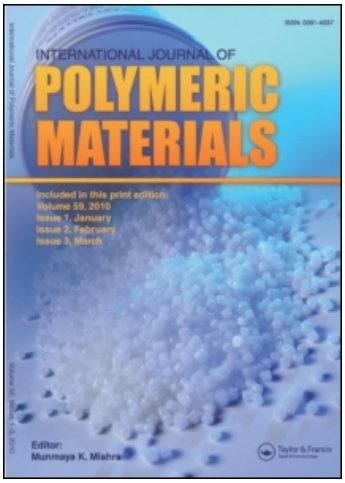

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Desorption of Human Serum Albumin and Human Fibrinogen from the Poly(3-Hydroxybutyrate) Surface

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The protein desorption of human serum albumin and human fibrinogen from the surface of poly(3-hydroxybutyrate) films was studied with ATR-FTIR spectroscopy. The diffusion model for reversible and irreversible sandwiched layers was confirmed. The reversible ratio (ratio of reversible adsorbed concentration to irreversible one) registrated as a function of time allow us to propose the 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 polymer 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 PHB macromolecules is discussed.

Keywords: Human serum albumin; human fibrinogen; desorption; poly(3-hydroxybutyrate)

INTRODUCTION

Poly(3-hydroxybutyrate) [**PHB**] and its derivatives have found a special range of biomedical and friendly environmental applications due to its combination of biocompatibility [1, 2] and sorption-diffusion properties [3, 4] coupled with its biodegradability [5]. The materials based on PHB have been also used as biodegradable plastics [6]. Many scientific teams have been investigating how biocompatible materials

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should be defined both *in vitro* and *in vivo*. But there is shortage of 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 physico-chemical 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 desorption kinetics data 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 as to prevents a turbularity. Protein concentrations in phosphate buffer solutions were from 5 to 50 mg/cm^3 for HSA and from 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 chamber for 24 h at room temperature.

Bacterial PHB samples were obtained from 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 (α = 68%) were produced by two-stage microbial synthesis. The M_w of PHB was determined by the relation [11].

$$[\eta] = 7.78 \times 10^{-5} M_w^{0.82}$$

where $[\eta]$ is the intrinsic viscosity in chloroform at 30°C.

The 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 denaturation on the surface and the proper adsorption of the proteins [12, 13].

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

The interpretation of the adsorption results was made without taking into account the hydrodynamic conditions which could lead to mistaken conclusions about the proper rate of adsorption. In this connection 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 artherial flow of blood [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:

(a) the transport stage in the boundary diffusional layer described by the set of differential equations

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

with boundary conditions:

$$C_v = C_V^0 \quad \text{at } x = \delta_D \tag{2}$$

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

$$D_p \, dC_v/dx = dC_s/dt \quad \text{at } x = 0 \tag{3}$$

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 δ_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; and

(b) the formation of the irreversible fraction of a adsorbed protein corresponds with following equation

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

where C_{S1} is the surface protein concentration for the irreversibly adsorbed molecules and the molecules undergoing conformational transformations respectively, k_1 is the constant of irreversible adsorption;

(c) formation of the reversible protein layer

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

where C_{S2} is the concentration of reversibly adsorbed protein, k_{21} and k_{22} are the corresponding constants of adsorption and desorption, 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 the irreversible ones $C_S = C_{S1} + C_{S2}$.

The Protein Desorption

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

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

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

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

$$C_{S2} = \frac{N k_{21} C_V^0 C_{S1}^{\infty}}{k_{21} C_V^0 + k_{22}} \exp\left(-\frac{k_{22} k_D t}{k_{22} + k_D}\right) \tag{7}$$

where $k_D = 2D_P/\delta_D^2$ 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 the 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, $I_{NaCl} = 0.1$)

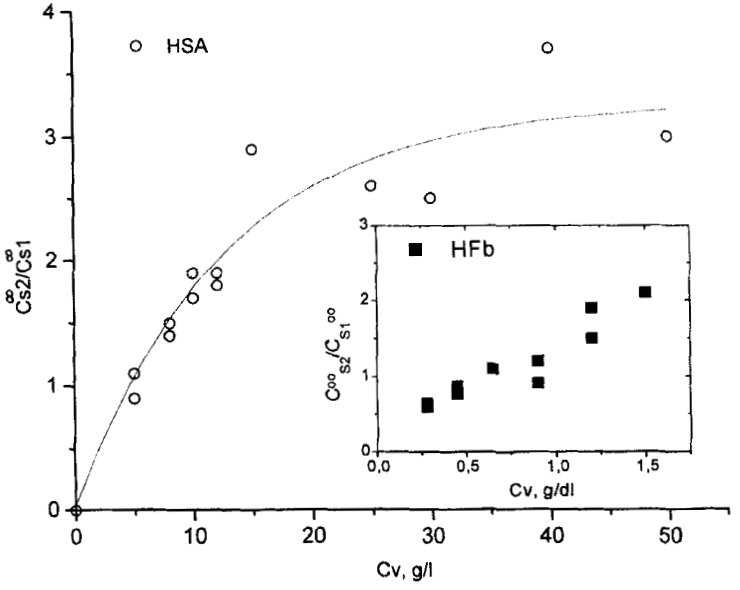

FIGURE 1 Adsorption isotherms for HSA and HFb from buffer solution on PHB surface.

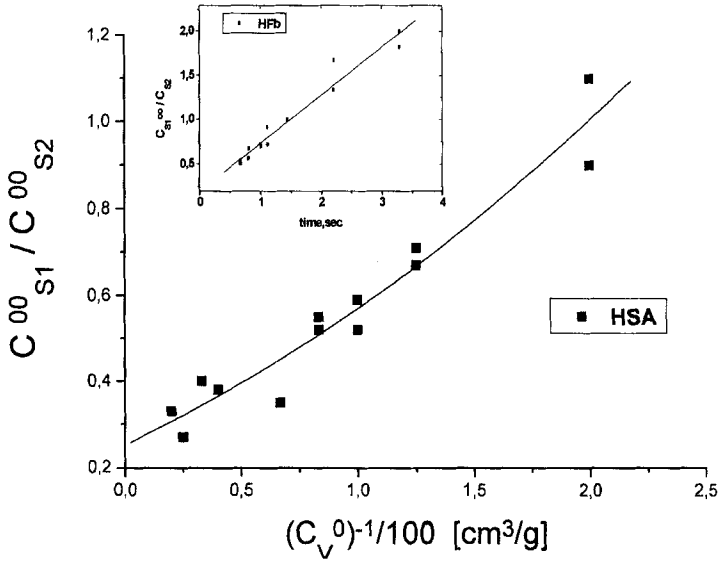

FIGURE 2 Replotting of adsorption isotherms for HSA and HFb.

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 on the nature of the protein involved. Here, $C^{\infty}_{S2}(= C^{\infty}_{S} - C^{\infty}_{S1})$, C^{∞}_{S1}, and C^{∞}_{S} are reversible, irreversible and total surface protein concentrations.

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

In Figures 3 and 4 all curves describe desorption experiment for the same systems PHB-HSA and PHB-HFb respectively. These curves have no special points such as inflections, maxima, *etc.* But the rates of desorption and the time it takes to establish the limiting values of surface concentration, C^{∞}_{S1} depends on the hydrodynamic conditions, namely, on the rate of the steady-state flow washing the polymer surface in adsorption cell, v, cm/s (Tab. 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 us to estimate the desorption rate constant values (k_{des}) for HSA and Hfb respectively. Corresponding data presented in Table II.

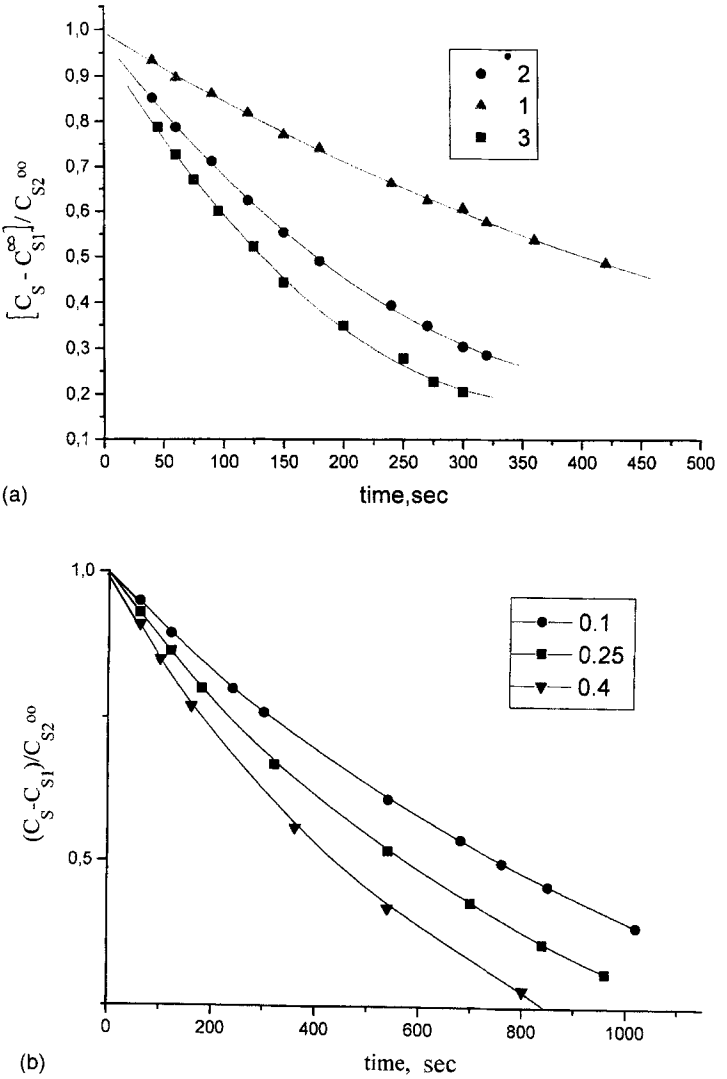

FIGURE 3 Reversible ratio as function of time for HSA (a) and HFb (b). Desorption at different rates of buffer flow: 0.1 cm/s (3), 0.25 cm/s (2), 0.4 cm/s (1).

The thickness of the diffusional layer near the polymer surface is a function of Reynold's number and, thereby, the function of v:

$$\delta_D = 2.40R(\mathrm{Re})^{-1/2}(D_p/v)^{1/3} \tag{8}$$

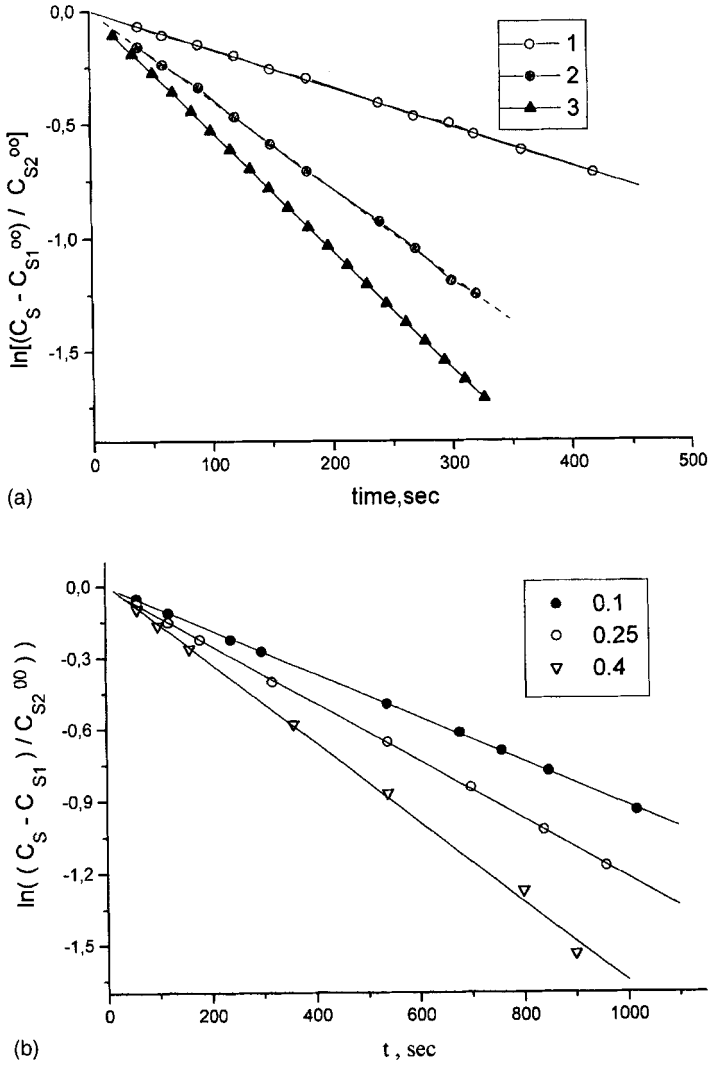

FIGURE 4 Replotting of desorption profiles for HSA (a), and HFb (b) in semilogarithmic coordinates. The rate of buffer flow are 0.1 cm/s (1), 0.25 cm/s (2), 0.4 cm/s (3).

where Re is Reynold's number that is equal to $4abv/[(a+b)v]$ for rectangular experimental cell, v is kinematic viscosity, cm^2/s, R, is distance between polymer surfaces in the cell, a and b are geometrical sizes of adsorption cell [10]. In Table I the values of δ_D are also presented

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	$(C^{\infty}_{S2}/NC_{S1})$	$(k_{21}C^{0*}_{V}/k_{22})$
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

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

TABLE II Transport and kinetics parameters for desorption from PHB surface

V_0, *cm/s*	$\delta_D\,10^2$, *cm*		$k_D\,10^3, s^{-1}$		$k_{des}\,10^3, s^{-1}$	
	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

allowing us to calculate the diffusion constant $k_D = 2D_p/\delta^2_D$ [13] for determination of the proper desorption constants, k_{22}. This Table I summarize the protein data where the values of ratio k_{21}/k_{22} were obtained by the extrapolation of kinetic data in above semilogarithmic forms for both HSA and HFb.

The comparison of desorption parameters presented in Table II enables us to make certain 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: (a) the degree of shielding for conformationally changed and irreversibly adsorbed molecules of proteins, $(C^{\infty}_{S2}/NC^{\infty}_{S1})$; (b) 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.

Besides, the value N may be considered as an index of acceptor capacity of the irreversibly adsorbed protein molecule [19]. In general, two factors: the conformational stretching (accessibility) on the nonphysiological surface and chemical structure of protein molecules affect the value N. The first factor depends on the interaction of native protein globules with polymer surface and is constant for the characteristics of a 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 extended fibrillar 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, $C_{S2}^{\infty}/NC_{S1}^{\infty}$, for HSA ($= 0.75$) is higher than is the same ratio for HFb ($= 0.45$), that supports additionally negative action of adsorbed fibrinogen on thromb resistance behaviour of PHB. A higher affinity of HSA to the polymer surface, $k_{21}C_V^0/k_{22}$, enables us to treat PHB as the material with good thrombo-resistance properties.

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

Experimental values of the ratio of the reversibility (C_{S2}/C_{S1}) are maximal for adsorption system HSA-PHB in comparison 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 polymer in biomedicine, namely, controlled drug release vehicles, artificial vessels, magistrals, reservoirs for blood storage, and surgical threads especially.

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