METHODS

Mathematical Model of Binding of Albumin—Bilirubin Complex to the Surface of Carbon Pyropolymer

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 9, pp. 352-356, September, 2005
Original article submitted January 28, 2004

We proposed a mathematical model and estimated the parameters of adsorption of albumin—bilirubin complex to the surface of carbon pyropolymer. Design data corresponded to the results of experimental studies. Our findings indicate that modeling of this process should take into account fractal properties of the surface of carbon pyropolymer.

Key Words: *albumin; bilirubin; carbon pyropolymer; mathematical model*

Liver diseases rank 8th-9th as a major cause of adult mortality in developed countries [5]. Progression of fatal changes in liver function includes the phase of acute and chronic liver failure. Modern systems for extracorporeal liver support are unreliable [9]. They consist of a bioartificial synthetic unit (immobilized hepatocytes) and artificial unit (membranes and adsorbents) to remove hepatotoxins [10]. A key problem in constructing an artificial unit is the absence of potent preparations to extract protein-bound substances (fatty acids, bile acids, conjugated and unconjugated bilirubin, phenols, mercaptans, *etc*.) from the patient's blood.

Unconjugated bilirubin (UB) is a typical hepatotoxin that not only possesses high affinity for the protein transport molecule of albumin, but also modifies its conformation and inhibits transport function [7]. Although bilirubin exhibits relatively low toxicity, the concentration of UB serves as a criterion of the effectiveness of extracorporeal devices to remove protein-bound substances [11].

It was hypothesized that effective system for UB removal would effectively eliminate other protein-bound hepatotoxins [8]. The methods to remove UB from blood plasma and whole blood are based on the use of new-generation carbon pyropolymers (CPP). They have a large-area sorption surface and are characterized by rapid adsorption. These properties of CPP are associated with physicochemical characteristics of the product formed after heat activation of synthetic copolymer vinyl pyridine styrene divinyl benzene (VP—SDVB). As distinct from activated carbons from other sources, CPP obtained by pyrolysis and heat activation of VP—SDVB retain mechanical strength and surface smoothness of granules typical of a precursor polymer. Several methods of medicinal treatment are based on the removal of protein-bound substances from ascitic fluid, blood plasma, and whole blood by using these adsorbents. However, specific features of interactions in the system of albumin, UB and CPP remain unclear. It hinders or excludes the possibility to predict the removal of protein-bound toxins from the patient's organism, which makes difficult to select an individual approach to therapeutic treatment. An adequate mathematical model for this

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physicochemical system should be developed. Modern physicochemical models for elimination of UB from the organism do not consider the presence of a strong liquid competitive adsorbent (albumin). Albumin has 2 binding sites for bilirubin (association constants 9.5×10^7 and 0.3×10^7 M^{-1}). Moreover, specific characteristics of adsorbent pores are not taken into account. It is necessary to perform an accurate and consecutive experimental study and computational modeling of the process accompanying extraction of UB from albumin solutions and biological fluids.

Physical model. Adsorption of UB to CPP in a special mass-transfer device is a complex physicochemical process proceeding in a heterophasic medium (fluid/solid). Hydrodynamic processes should be taken into account. The phase of CPP has significant fractal properties. Modeling of the so-called "shuttle experiments" (stir-bath) includes shuttling of CPP granules in a reservoir with albumin and UB. This model suggest the use of dissolved (*i.e.*, regularly distributed) CPP. Simple kinetic rules can be applied to adsorption sites that serve as one of the reagents.

Let us consider the simplest mathematical model for a complete mixing system. Heterophasic characteristics of the system are disregarded in setting up an equation of the model. To a first approximation, this system is considered as homogenous (liquid).

Kinetic model. Let us introduce the following designations:

Al, albumin;

B, unconjugated bilirubin;

C, CPP adsorption sites for bilirubin;

AlB, albumin—bilirubin complex, bilirubin bound to albumin via the primary site ($K_{AlB}=9.5\times107$ M^{-1});

AlB_2, albumin--bilirubin complex, bilirubin bound to albumin via the primary and secondary sites ($K_{AlB2}=0.3\times10^7$ M^{-1});

AlC_n, albumin—CPP complex;

BC, bilirubin—CPP complex.

Albumin has 2 binding sites for UB. For simplicity, assume that binding to a "weak" site proceeds when a "strong" site is occupied. The reaction rate constants are shown in parentheses:

1. $Al+B\rightarrow AlB$ (k_1).
2. $AlB+B\rightarrow AlB_2$ (k_2).
3. $AlB\rightarrow Al+B$ (k_3).
4. $AlB_2\rightarrow AlB_2+B$ (k_4).

Binding of albumin and UB to CPP sites is of considerable importance. First, the molecule of albumin has a greater molecular weight than bilirubin (~65 kDa and ~570 Da, respectively). And second, albumin has a three-domain structure with flexible bonds between domains. Therefore, albumin can exist in various spatial conformations. Adsorption of albumin and bilirubin to CPP proceeds by a variety of pathways (Fig. 1).

The classical method of Langmuir is applied to model kinetic characteristics of binding to CPP. However, this method is inapplicable due to fractal surface properties of pyropolymer. Because of a considerable difference in the size of molecules, the area accessible to bilirubin is probably several times greater compared to that of albumin. It is appropriate to estimate the mean number of sites accessible to bilirubin and overlapped by albumin molecules. The kinetic scheme was constructed taking into account the mean number of overlapped sites. This process is described by equations 5-8, where *C* is the concentration of CPP sites for bilirubin; and *n* is the mean number of sites covered with 1 molecule of albumin:

5. $Al+nC\rightarrow AlC_n$ (k_5).
6. $B+C\rightarrow BC$ (k_6).
7. $AlC_n\rightarrow Al+nC$ (k_7).
8. $BC\rightarrow B+C$ (k_8).

We could develop kinetic models for various variants of binding. However, this approach will result in an increase in the number of unknown variables (constants for the rate of chemical reactions). Thus, it

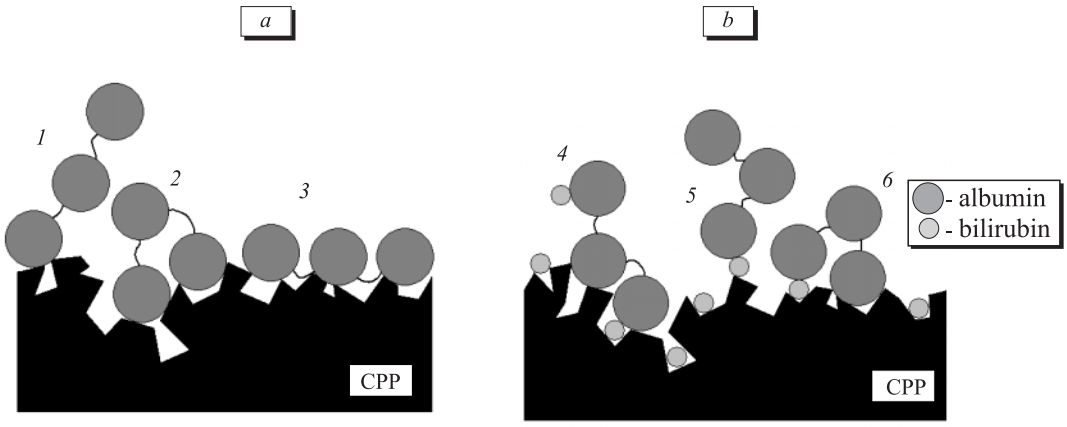

Fig. 1. Variants for adsorption of albumin (*a*, *1-3*) and albumin—bilirubin complex (*b*, *4-6*). CPP, carbon pyropolymer.

will be difficult to derive the chemical reaction rate constant from experimental data. In this case, a laborious complex study will be required to evaluate the sensitivity of a mathematical model to various rate constants.

Albumin was proposed to undergo reversible and partial denaturation on the surface of CPP. This assumption was taken into account to derive kinetic equations for binding of albumin—bilirubin complex to the surface of CPP. It is substantiated by the results of modeling with methods of molecular dynamics [6] and experimental data [2,3]. The proposed model suggests that partial denaturation of albumin on the surface of CPP leads to disintegration of the complex. These changes are followed by consecutive release of bilirubin bound to the weak and strong sites (equations 10 and 9, respectively). Adsorption of bilirubin is taken into account in reactions 9 and 10 (formation of complex *BC*). The mechanism of binding of albumin to the surface of CPP is poorly understood. The mean number of bilirubin-binding sites blocked by 1 molecule of albumin (n) was introduced as a variable of the model. The scheme of these reactions appears as follows:

9. $AlB+(n+1)C \rightarrow AlC_n+BC$ (k_9).
10. $AlB_2+C \rightarrow AlB+BC$ (k_{10}).

The laws of conservation for individual components of the system were applied to develop the system of ordinary differential equations:

$A_0=Al+AlB+AlB_2+AlC_n$;
$B_0=B+AlB+2AlB_2+BC$;
$C_0=C+BC+nAlC_n$,

where A_0, B_0, and C_0 are the total concentrations of albumin, bilirubin, and CPP sites, respectively. The concentrations of A_0 and B_0 are known for each experiment; they do not depend on time. The concentration of sites (C_0) serves as an unknown variable. Let us introduce the following designations: x_1, AlB; x_2, BC; x_3, AlC_n; x_4, AlB_2; x_5, Al; x_6, B; and x_7, C. The laws of conservation appear as follows:

$$x_5=A_0-x_1-x_3-x_4,$$
$$x_6=B_0-x_1-x_2-2x_4,$$
$$x_7=C_0-x_2-nx_3.$$

The following system of ordinary differential equations is used to evaluate kinetic characteristics of this process (according to the above described mechanism):

$$dx_1/dt=K_{AlB}\times k_3(A_0-x_1-x_3-x_4)(B_0-x_1-x_2-2x_4)-K_{AlB2}\times k_4x_1(B_0-x_1-x_2-2x_4)-k_3x_1-k_9x_1(C_0-x_2-nx_3)^{n+1}+k_4x_4+k_{10}x_4(C_0-x_2-nx_3),$$

$$dx_2/dt=k_6(C_0-x_2-nx_3)(B_0-x_1-x_2-2x_4)-k_8x_2+k_9x_1(C_0-x_2-nx_3)^{n+1}+k_{10}x_4(C_0-x_2-nx_3),$$

$$dx_3/dt=k_5(C_0-x_2-nx_3)^n(A_0-x_1-x_3-x_4)-k_7x_3+k_9x_1(C_0-x_2-nx_3)^{n+1},$$

$$dx_4/dt=K_{AlB2}\times k_4x_1(B_0-x_1-x_2-2x_4)-k_4x_4-k_{10}x_4(C_0-x_2-nx_3).$$

The unknown constants of chemical reactions, concentration of CPP sites, and variable n were estimated from experimental data using dBSolve 5 software (Table 1) [4].

A fifth-order L-stable Rosenbrock method was applied to solve a differential algebraic system [1].

A correlation was revealed between 2 initial concentrations of bilirubin (10 and 30%) at an initial albumin concentration of 30 g/liter (Fig. 2). It should be emphasized that the maximum concentration of bound bilirubin was determined by its initial concentration and number of CPP sites. The concentration of bound albumin reached the same level (0.2 mM), which did not depend on the total initial concentration of albumin in the system.

A significant difference in binding of various components from the system can be related to several reasons. Due to a difference in the size of albumin and bilirubin molecules and fractal characteristics of the CPP surface, large molecules of albumin bind to surface sites on CPP granules. Complexes of albumin and CPP can be theoretically formed at the sites localized inside pores. However, these sites are inaccessible to large molecules of albumin and complexes (*Alb* and AlB_2). Molecules of bilirubin with a lower linear size can permeate pores and bind to internal sites of CPP.

If the proposed mechanism of binding is the case, the mean number of CPP sites for albumin molecules

TABLE 1. Chemical Reaction Rate Constants and Total Number of Adsorption Sites for Bilirubin (Estimation from Experimental Data)

Constant	n=1	n=5
k_1, $M^{-1}min^{-1}$	4.84×10^{2}	8.511×10^{1}
k_2, $N^{-1}min^{-1}$	7.958×10^{1}	3.948×10^{4}
k_3, min^{-1}	5.095×10^{-6}	8.959×10^{-7}
k_4, min^{-1}	2.656×10^{-5}	1.316×10^{-3}
k_5, $M^{-1}min^{-1}$	5.489	1.632×10^{11}
k_6, $M^{-1}min^{-1}$	3.226×10^{-4}	3.369
k_7, min^{-1}	3.010×10^{-3}	1.420×10^{-4}
k_8, min^{-1}	1.011×10^{-7}	8.754×10^{-5}
k_9, $M\times min^{-1}$	1.686×10^{4}	3.029×10^{15}
k_{10}, $M^{*}min^{-1}$	1.325×10^{2}	9.742
C, mM	1.174	2.706

Note. *M^{-2} for n=1; and M^{-6} for n=5.

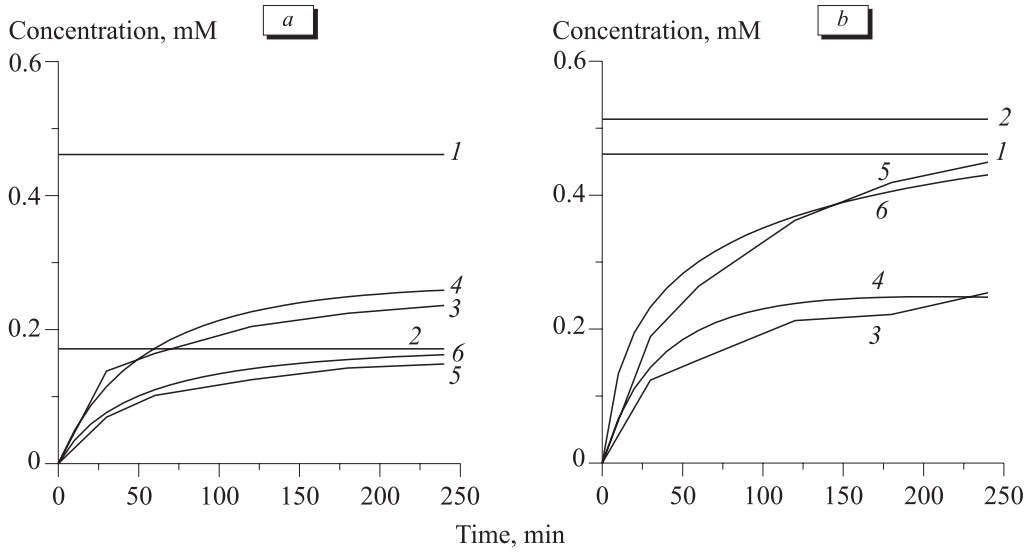

Fig. 2. Experimental and design data on adsorption of albumin—bilirubin complex. The mean number of sites occupied with 1 albumin molecule is 1. The initial concentrations of albumin and bilirubin are 30 g/liter and 10 mg%, respectively (*a*). The initial concentrations of albumin and bilirubin are 30 g/liter and 30 mg%, respectively (*b*). Here and in Fig. 3: total albumin concentration in the system (*1*); total bilirubin concentration in the system (*2*); adsorbed albumin, experimental data (*3*); adsorbed albumin, design data (*4*); adsorbed bilirubin, experimental data (*5*); adsorbed bilirubin, design data (*6*).

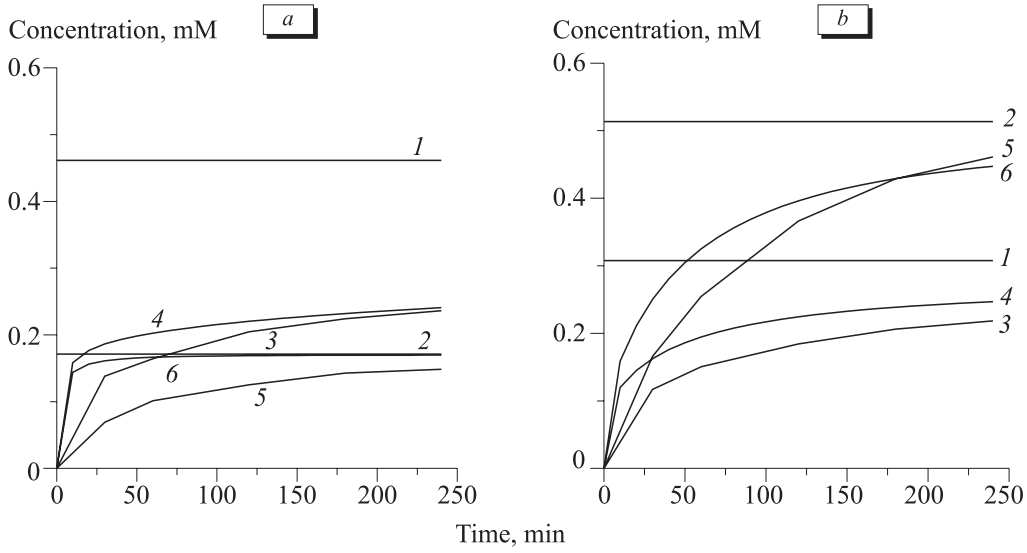

Fig. 3. Experimental and design data on adsorption of albumin—bilirubin complex. The mean number of sites occupied with 1 albumin molecule is 5. The initial concentrations of albumin and bilirubin are 30 g/liter and 10 mg%, respectively (*a*). The initial concentrations of albumin and bilirubin are 20 g/liter and 30 mg%, respectively (*b*).

would be small. To test this hypothesis, computations were performed with the mean number of sites for binding of 1 albumin molecule (n=5, Fig. 3). We observed less consistency between design and experimental data (as compared to study when the mean number of CPP sites was 1). These computations included a "stiff" parameter corresponding to rapid occupation of CPP sites with albumin molecules. A qualitative pattern of the process was preserved. The concentration of bilirubin—CPP complex rapidly reached the saturation value. However, the sites accessible to albumin were not all occupied. The concentration of albumin—CPP complexes was far from the saturation value.

The proposed mathematical model describes the process of adsorption on CPP. The specific feature of this process is rapid binding of albumin molecules to surface sites on CPP granules. However, the sites located inside CPP pores remain accessible to bilirubin. The process is characterized by a small number of sites required for binding of 1 albumin molecule. This is determined by fractal characteristics of the surface.

The general weakness of this model is an impossibility to describe the mechanism of adsorption taking into account chain reactions.

However, the proposed model forms a basis for other mathematical models describing column experiments.

We are grateful to R. Huges (King's College, London) and Prof. V. G. Nikolaev (Institute of Experimental Pathology, Oncology, and Radiobiology, Ukrainian Academy of Sciences) for their help in this study.

This work was supported by INATS (grant No. 2001-0346).

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