ADSORPTION BEHAVIOR OF ALBUMIN AND β-LACTAMASE IN ALGINATE-ENCAPSULATED DEAE-TRISACRYL BEADS

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Introduction

In the previous work[2,3], the separation of β-lactamase from albumin was studied using encapsulated DEAE-trisacryl adsorbent beads. The experimental results[2] showed that the concentration of a desired product in the broth decreased with time at the beginning but went up again for the small-capsule system, while the concentration of desired product decreased monotonously for the large-capsule system. However, a model study[3] could not systematically explain these results. We developed a new model to investigate the effect of capsule size on the product concentration profile in the bulk solution. We considered the capsule core to be a

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Table 1. Variables for the governing equation

Fraction of capsule in broth solution	0.2
Porosity: Hydrogel membrane	0.2
Capsule core	0.5
Adsorbent bead	0.5
Capsule radius: Large	6.5×10^{-4}m
Small	2.5×10^{-4}m
Capsule wall thickness	5.0×10^{-5}m
Adsorbent bead radius	5.0×10^{-5}m
Initial concentration of β-lactamase in broth solution	1.35×10^{-5} mol m^{-3}
Initial concentration of albumin in broth solution	1.50×10^{-1} mol m^{-3}
Ligand concentration in adsorbent bead	1.833 mol m^{-3}

nonhomogeneous system composed of adsorbent beads and core liquid, and the adsorption mode of adsorbates of β-lactamase and albumin on the adsorbents to be competitive binding or noncompetitive binding.

1. Modeling

1.1 Governing equations

The governing equations for the adsorption of each component on the ligand site in the capsule core are described as follows.

In the capsule core containing n adsorbent particles,

$$\varepsilon_c D_{ci}\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_i}{\partial R}\right) = \varepsilon_c\frac{\partial C_i}{\partial t} + 4\pi n r_0^2 D_{ai}\left(\frac{\partial C_{ai}}{\partial r}\right)_{r=r_0} \quad (1)$$

Boundary condition: $$\left(\frac{\partial C_i}{\partial R}\right)_{R=0} = 0 \quad (2)$$

$$D_{ai}\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{ai}}{\partial r}\right) = \varepsilon_a\frac{\partial C_{ai}}{\partial t} + \varepsilon_a r_{ads} \quad (3)$$

$$(C_{ai})_{r=r_0} = C_i \quad (4)$$

1.2 Adsorption kinetics

What determines the transient and final selectivities of β-lactamase and albumin are their adsorption kinetics to DEAE-trisacryl beads. The kinetic study of a single-component adsorption suggests the following equations:

competitive binding model

$$L + P_1 \underset{k_{-1}}{\overset{k_1}{=}} LP_1, \qquad K_1 = \frac{[LP_1]}{[L]\,[P_1]} \quad (5)$$

$$L + P_2 \underset{k_{-2}}{\overset{k_2}{=}} LP_2, \qquad K_2 = \frac{[LP_2]}{[L]\,[P_2]} \quad (6)$$

$$-\frac{dP_1}{dt} = k_1[L]\,[P_1] - k_{-1}[LP_1] \quad (7)$$

$$-\frac{dP_2}{dt} = k_2[L]\,[P_2] - k_{-2}[LP_2] \quad (8)$$

where L, P_1, P_2 are unoccupied ligand, desired product and contaminant respectively. The complexes between the biomolecules are given as LP_1, LP_2, LP_1P_2 respectively.

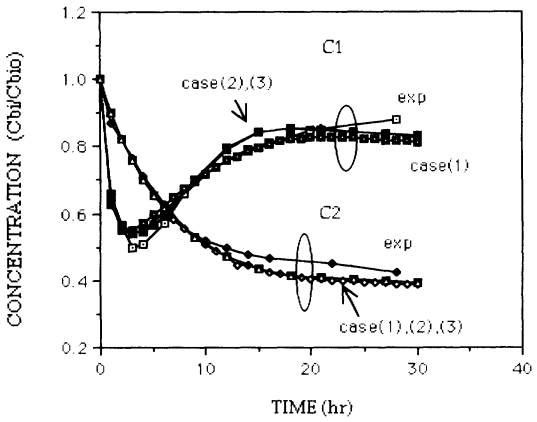

Fig. 1 Concentration profiles of β-lactamase and albumin in the broth solution for the small-capsule system. (case (2): C1 ▲ C2 ◇; case (3): C1 ■ C2 △; case (1): C1 □ C2 □)

noncompetitive binding model

$$L + P_1 \underset{k_{-1}}{\overset{k_1}{=}} LP_1, \qquad L + P_2 \underset{k_{-2}}{\overset{k_2}{=}} LP_2 \quad (9)$$

$$LP_1 + P_2 \underset{k_{-3}'}{\overset{k_3'}{=}} LP_1P_2, \qquad LP_2 + P_1 \underset{k_{-4}'}{\overset{k_4'}{=}} LP_2P_1 \quad (10)$$

From the concentration profiles of β-lactamase obtained by Nigam's experimental work[2] we can conclude that β-lactamase is eventually desorbed from the adsorbent. With no ligand present, the concentration of any adsorbate should decrease to 89% of the initial broth concentration for the encapsulated-bead system because of the void space of the capsule. We can assume that the ideally transitional complex (LP_1P_2) is spontaneously decomposed to small complexes (LP_1 or LP_2) and adsorbates (P_2 or P_1). Thus Eq (10) for the noncompetitive binding mode can be combined into one equation as

$$LP_1 + P_2 \underset{k_{-3}}{\overset{k_3}{=}} LP_2 + P_1 \quad (11)$$

1.3 Method of numerical solution

The parabolic governing equations are solved by a computer using the finite difference method[1]. The fixed variables[2] for the governing equations such as porosity, capsule size and fraction of the capsule in the broth solution are shown in **Table 1**.

2. Results and Discussion

Generally in a multi-variable simulation system not all variables have equal sensitivities to the final concentration profile and mass flux. We have selected the sum of the squares of differences as the basis of the fitness.

$$E_{1,t} = \int_0^t \left(\frac{C_{b1,m}(t)}{C_{b10}} - \frac{C_{bl,exp}(t)}{C_{b10}}\right)^2 dt,$$

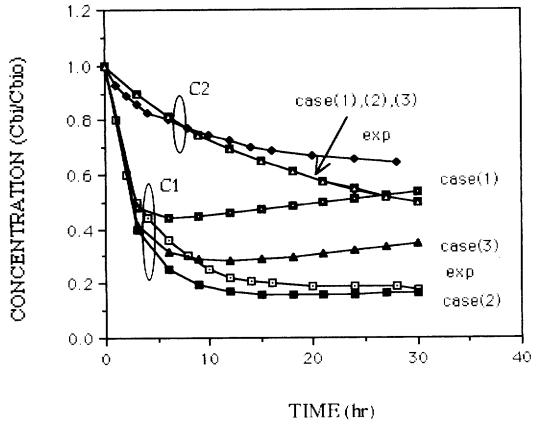

Fig. 2 Concentration Profiles of β-lactamase and albumin in the broth solution for the large-capsule system. (case (2): C1 ■ C2 ◇; case (3): C1 ▲ C2 △ ; case (1): C1 □ C2 □)

Table 2. Data obtained by the trial-and-error method for the small capsule system

Diffusivity			Competitive adsorption Case (1)	Noncompetitive adsorption Case (2)	Case (3)
D_{g1}	2.8×10^{-11}	k_1	2.7×10^{-3}	2.7×10^{-3}	2.7×10^{-3}
D_{g2}	6.7×10^{-13}	k_{-1}	2.0×10^{-7}	0	5.0×10^{-8}
D_{c1}	6.7×10^{-11}	K_1	1.35×10^{4}	∞	5.4×10^{4}
D_{c2}	2.8×10^{-12}				
D_{a1}	4.7×10^{-12}	k_2	4.0×10^{-2}	4.0×10^{-2}	4.0×10^{-2}
D_{a2}	5.0×10^{-13}	k_{-2}	6.0×10^{-8}	6.0×10^{-8}	6.0×10^{-8}
		K_2	6.67×10^{5}	6.67×10^{5}	6.67×10^{5}
		k_3	0	6.0×10^{-5}	2.0×10^{-1}
		k_{-3}	0	0	0
		K_3	0	∞	∞

$$E_{2,t} = \int_0^t \left(\frac{C_{b2,m}(t)}{C_{b20}} - \frac{C_{b2,exp}(t)}{C_{b20}} \right)^2 dt$$

$$Sum,_t = E_{1,t} + E_{2,t}$$

By the criterion of $sum,_t$ we selected case (1) for the competitive adsorption and case (2) and case (3) for the noncompetitive adsorption. The best fittable computational concentration profiles and experimental results are shown in **Fig.2.** Diffusivities of albumin and β-lactamase were easily obtained by trial and error because the time for the minimum concentration of β-lactamase in the small capsule system (**Fig.1**) depended primarily on the diffusivities. The adsorption and desorption rate constants and diffusivities obtained by this trial-and-error approach are shown in **Table2.**

In one of the noncompetitive adsorption modes (case (2)), the β-lactamase adsorption reaction is irreversible (k_{-1} = 0) and the reaction for replacement of albumin from β-lactamase complex is also irreversible (k_{-3} = 0, $k_3 = 6.0\times10^{-1}$ m^3/mol/sec). In the other noncompetitive adsorption mode (case (3), the β-lactamase adsorption reaction is reversible ($k_{-1} = 5.0\times10^{-5}$ sec^{-1}) and the albumin replacement reaction is irreversible (k_{-3} = 0, $k_3 = 2.0\times10^{-1}$ m^3/mol/sec).

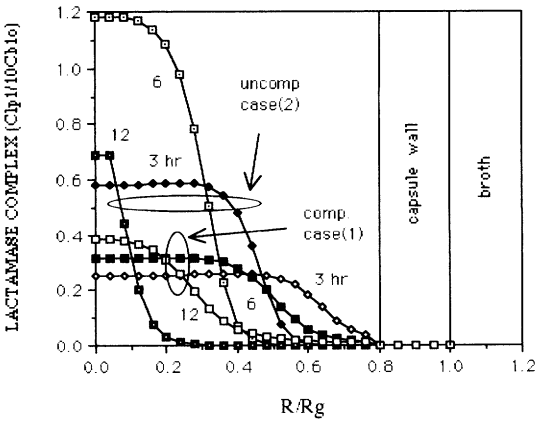

Fig. 3 Concentration distribution of the β-lactamase-ligand complex with time in the capsule core for the small-capsule core system. (case (3): 3hr ◆, 6hr ⊡, 12hr □; case(1): 3hr ◇, 6hr ■ 12hr □)

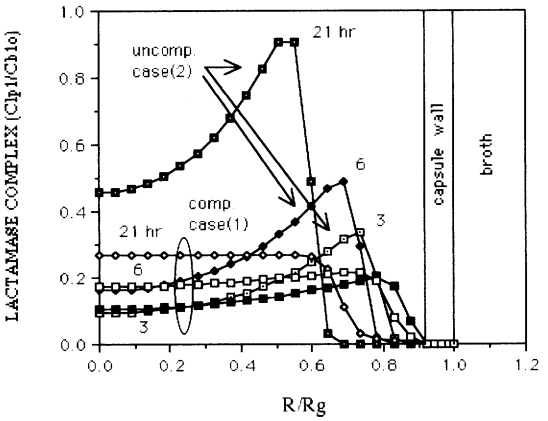

Fig. 4 Concentration distribution of the β-lactamase-ligand complex with time in the capsule core for the large-capsule system. (case (3): 3hr ⊡, 6hr ◆, 21hr □; case (1): 3hr ■, 6hr □, 21hr ◇)

With these data we simulated the concentration profiles of the biomolecules in a broth solution contaning large capsules with a diameter of 1.3 mm. The results are shown in Fig. 2. The concentration profiles of the experimental results which decreased monotonously fit only one of the noncompetitive adsorption modes (case (2)). The computational concentration profiles of the competitive mode first decrease and then increase. Case (3) of the noncompetitive adsorption mode lie between the above two modes. The phenomenon could be elucidated by examining the adsorption state of bioproduct in the adsorbent bead. The concentration of β-lactamase on the adsorbent bead in the capsule core in shown in **Fig. 3.** The boundary of β-lactamase adsorption region moves into the capsule core as albumin diffuses into the capsule core. The concentration region moves into the capsule core as albumin diffuses into the capsule core. The concentration of the β-lactamase complex inside the adsorption boundary increases with time for the noncompetitive adsorption (case (2)). The concentration inside the boundary edge of the competitive mode, however, does not increase and the rate of adsorp-

tion boundary movement is slower than that in the noncompetitive adsorption mode. On the other hand, the adsorption probability for β-lactamase is infinite for case (2) of the noncompetitive adsorption mode because the β-lactamase adsorption reaction is irreversible as shown in Table 2. Therefore, the total quantities of adsorbed β-lactamase at a certain time can be the same for both modes. The concentration of the β-lactamase in the broth solution increases as the albumin diffuses into the capsule and β-lactamase is desorbed.

For the larger capsule, whose diameter is 1.3 mm, the adsorption state of β-lactamase is shown in **Fig. 4.** The same phenomenon as in the small-capsule system can be found. However, because of the capsule size the adsorption potential inside the adsorption boundary for the noncompetitive adsorption mode is larger than that of the small capsule. Thus we did not find that the concentration profile of case (2) for the small-capsule system first decreases and then increases.

Conclusion

The capsule size of the encapsulated adsorbent system is very important for handling capsules and determining optimum operation time. β-lactamase passes through the hydrogel membrane first and then is adsorbed to the ligand because of its smaller molecular weight. But β-lactamase has less binding affinity than albumin and consequently the bound β-lactamase is displaced irreversibly from the ligand by albumin molecules. The displaced β-lactamase will appear in the bulk solution as time proceeds and will increase in concentration in the bulk solution. However, this phenomenon can be observed only for the small-capsule system which has limiting binding affinity. The noncompetitive adsorption mode of case (2) can adequately describe this behavior of adsorption.

Nomenclature

C_i	= adsorbate concentration in the capsule core	[mol m^{-3}]
C_{ai}	= adsorbate concentration in the adsorbent bead	[mol m^{-3}]
C_{bi}	= adsorbate concentration in bulk	[mol m^{-3}]
C_{bio}	= initial adsorbate concentration in bulk	[mol m^{-3}]
C_{lpi}	= adsorbate-ligand complex concentration	[mol m^{-3}]
D_{ai}	= diffusivity in the adsorbent bead	[m^2 sec^{-1}]
D_{ci}	= diffusivity in the capsule core	[m^2 sec^{-1}]
D_{gi}	= diffusivity in the capsule wall membrane	[m^2 sec^{-1}]
k_i	= adsorption rate constant	[m^3 mol^{-1} sec^{-1}]
k_{-i}	= adsorption rate constant	[m^3 mol^{-1} sec^{-1}]
K_i	= adsorption constant, k_i/k_{-i}	[m^3 mol^{-1}]
L	= concentration of adsorbent ligand	[mol m^{-3}]
R	= radial coordinate for the capsule	[m]
r	= radial coordinate for the adsorbent bead	[m]
R_c	= radius of capsule core	[m]
R_{c+}	= marginally higher than the core radius	[m]
R_{c-}	= marginally smaller than the core radius	[m]
R_g	= radius of capsule	[m]
r_o	= radius of adsorbent bead	[m]
ε_a	= porosity of adsorbent bead	
ε_c	= porosity of capsule core	

<Subscripts>

i	= i'th component

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